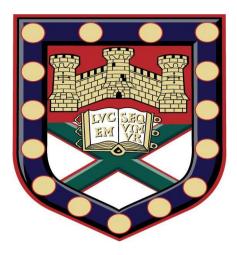
The impact of different loading sports and a jumping intervention on bone health in adolescent males





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Abstract

Adolescence is a crucial period for bone development and exercise can enhance bone acquisition during this period of life. However, there is a lack of scientific evidence on how different loading sports practised during adolescence can affect bone development in males. The present thesis is part of the PRO-BONE study and aimed to investigate the cross-sectional and longitudinal effects of participation in football (osteogenic sport), swimming and cycling (non-osteogenic sports) on bone mass, bone geometry, texture and bone metabolism in adolescent males. An active control group has been included too. Additionally, the thesis examined the effect of a 9-month jumping intervention programme on bone outcomes in adolescent males involved these sports. Cross-sectional findings from Chapter 4 show that footballers have better bone status than swimmers, cyclists and controls (7 to 21 %), and that there are no differences between participants of non-osteogenic sports and controls. Chapter 5 identifies that lean mass is the strongest determinant of bone outcomes, followed by football participation and height in adolescent male athletes, whereas the contribution of the other predictors, such as nutrition, physical activity and fitness, is site specific. Longitudinal evidence in Chapters 6 and 7 show that bone mass (5 to 8 %) and geometry (4 to 10 %) is higher in adolescent male footballers compared to swimmers and cyclists after one year of sport specific training, and that there are no differences in bone development between non-osteogenic sports groups and controls. Chapters 8 and 9 indicate that a 9-month jumping intervention programme can improve bone outcomes only in male adolescents participating in swimming and cycling (4 to 13 %), but not in those engaged in football, while it can improve fitness outcomes in all

groups (4 to 8 %). Collectively, the present thesis contributes to the literature by providing novel evidence in adolescent male athletes on the effects of popular sports such as football, swimming and cycling on bone status and development, and that a jumping intervention programme can improve bone development in those involved in non-osteogenic sports.

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"Have the courage to do what you love and the determination to do it exceptionally well"

Grandfather Dimitrios Vlachos (1931 - 2016, Kastoriá)

Publications, conferences and awards

Publications as leading author directly related to the present thesis

- 1. **Vlachopoulos D,** Barker AR, Ubago-Guisado E, Fatouros IG, Knapp KM, Williams CA, Gracia-Marco L (2017). Longitudinal Adaptations of Bone Mass, Geometry and Metabolism in Adolescent Male Athletes. The PRO-BONE Study. *J Bone Miner Res.* (In press, doi: 10.1002/jbmr.3206).
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Publications as co-author

- 9. Wilkinson K, **Vlachopoulos D,** Klentrou P, Ubago-Guisado E, de Moraes AC, Barker AR, Williams CA, Moreno LA, Gracia-Marco L (2017). Soft tissues, bone mineral density and geometry in active adolescent boys: The PRO-BONE study. *Eur J Appl Physiol.* 117(4):833-842.
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- 12. Avloniti A, Chatzinikolaou A, Delli C, **Vlachopoulos D,** Gracia-Marco L, Draganidis D, George Mastorakos G, Jamurtas AZ, Fatouros IG (2017). Exercise-Induced Oxidative Stress Responses in the Pediatric Population: An Update. *Antioxidants*. 6(1) pii: E6.
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Glossary of terms

20mSRT 20 m shuttle run test

25(OH)D 25-hydroxyvitamin D

ANCOVA One-way analysis of covariance

ANOVA One-way analysis of variance

aBMD Areal bone mineral density

BMC Bone mineral content

BMD Bone mineral density

BMI Body mass index

BUA Broadband ultrasound attenuation

CMJ Counter movement jump

CON Controls

CON-CYC Control -cycling

CON-FOO Control-football

CON-SWI Control -swimming

COVs Coefficient of variations

CSA Cross-sectional area

CSMI Cross-sectional moment of inertia

CTX-I Carboxi-terminal telopeptide of type 1 collagen

CYC Cyclists

DXA Dual energy x-ray absorptiometry

FOO Footballers

HR-QCT High-resolution quantitative computed tomography

HSA Hip structural analysis

IGF-1 Insulin-like growth factor 1

INT-CYC Intervention-cycling

INT-FOO Intervention-football

INT-SWI Intervention-swimming

MVPA Moderate to vigorous physical activity

PBM Peak bone mass

PHV Peak height velocity

PINP Procollagen type I of amino-terminal propeptide

pQCT Peripheral quantitative computed tomography

PTH Parathyroid hormone

QCT Quantitative computed tomography

QUS Quantitative ultrasound

RCT Randomised controlled trial

SLJ Standing long jump

SOS Speed of sound

SWI Swimmers

TBS Trabecular bone score

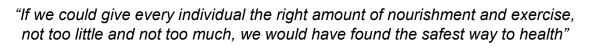
vBMD Volumetric bone mineral density

VO_{2max} Maximal oxygen consumption

VPA Vigorous physical activity

WHO World health organization

Z Section modulus



Hippocrates (460 BC, Kos - 370 BC, Larissa)

1. Introduction

Osteoporosis is a disease characterised by reduced bone mineral content (BMC) and bone mineral density (BMD) and deterioration of bone microarchitecture, resulting in increased risk of fragility fractures (NIH Consensus Development Panel on Osteoporosis Prevention and Therapy, 2001). The most common fragility fractures sites are the distal forearm, spine and hip, and constitute a major public health challenge worldwide (Cooper et al., 2004b, Rennie et al., 2007). Figures estimate osteoporosis affects approximately 22 million women and 5.5 million men between the ages of 50 and 84 years in Europe, and osteoporosis is associated with high rates of morbidity and mortality. In addition, about 3.5 million European men and women suffer an osteoporotic fracture every year, resulting in an economic cost of approximately €98 billion (Kanis and Johnell, 2005a). The scientific literature suggests that prevention is better than treatment and is a key public health strategy to improve osteoporosis-related outcomes.

Bone mass acquisition during growth is not only important for optimal skeletal growth but also is the strongest determinant of osteoporosis risk later in life (Nikander et al., 2010). Bone acquisition occurs throughout childhood and adolescence, with 80-90 % acquired by late adolescence, depending of the sites of the skeleton (Baxter-Jones et al., 2011, Henry et al., 2004). Bone mass reaches its peak between the second and third decade of life, which is called peak bone mass (PBM). PBM serves as an important protective advantage because BMD declines with age, illness or diminished sex steroid production. During the last two decades, increasing evidence suggests that the full potential

of PBM and bone strength are not achieved (Chevalley et al., 2017, Henry et al., 2004). This is alarming given the raised concerns that inadequate bone mass may result in fragile bones and increased fracture risk both in adolescence and in adulthood (Rizzoli et al., 2010, Weaver et al., 2016). In this regard, a 10 % increase in bone mass during adolescence might reduce the risk of fracture later in life by 50 % and delay the onset of osteoporosis by 13 years (Rizzoli et al., 2010). Therefore, maximising bone mass during growth is of great importance in order to reduce the risk of osteoporotic fractures (Bonjour and Chevalley, 2007, Chevalley et al., 2017).

Heredity is a major determinant of PBM, and approximately 60-80 % of the variance in PBM is attributed to genetics (Davies et al., 2005, Stewart and Ralston, 2000, Bachrach, 2001). Environmental factors also influence PBM, including nutritional intake (Pettifor and Prentice, 2011), physical activity (Gracia-Marco, 2016) and smoking (Emaus et al., 2014). Adequate dietary calcium intake is an important component of optimal (full individual potential) attainment of PBM as it influences the development of the skeleton during growth (Huncharek et al., 2008). Increasing dietary calcium to optimal levels through consumption of dairy products (fortified or not) may increase bone mass during growth, but the optimal levels needed and the duration of the consumption to improve bone acquisition remain controversial (Huybrechts et al., 2011, Winzenberg et al., 2006). Another substantial nutrient in bone mineralization is vitamin D, which plays an important role in calcium absorption from the skeleton and in the attainment of PBM. Inadequate vitamin D levels may result in lower attainment of PBM, which could in turn contribute to increased fracture risk in both childhood and older adult life. However, only

subclinical vitamin D deficiency may be detrimental to optimal bone acquisition during growth (Winzenberg and Jones, 2013). Calcium, vitamin D and physical activity can independently influence bone mineral accrual in young people (Vlachopoulos et al., 2016). However, evidence suggests that environmental factors may interact to influence bone status. For example, physical activity interacts with calcium intake (Courteix et al., 2005) and vitamin D levels (Valtuena et al., 2012) to improve bone status across life span (Ward et al., 2007).

Physical activity contributes to the development of bone mass in young people due to its positive association with lean mass. The latter is explained by the mechanostat theory indicating that "bigger muscles exert higher tensile forces on the bones they attach" (Rauch et al., 2004). The importance of physical activity during childhood is dependent on the ability of the skeleton to adapt to mechanical loading and appears to elicit the greatest bone accrual response in the growing skeleton and later in life (Bielemann et al., 2013). The intensity, frequency and the type of physical activity are important factors to consider due to the different ground reaction forces applied on the paediatric skeleton (Vicente-Rodriguez, 2006, Beck, 2009, McKay et al., 2005a). In this regard, exercise is an effective method to improve the development of bone mass during puberty, but not all types of exercise have the same effects on bone outcomes.

Sport participation is a vehicle for children and adolescents to engage in exercise and obtain health benefits, including the skeletal system. Participation

in organised and non-organised sports in 6 to 16 year old males and females ranges from 43 to 92 % with football, swimming and cycling being among the most popular sports practised in UK, EU and US (Sport England, 2016, Aspen Institute, 2013, Scheerder et al., 2011). Evidence suggests that some sports may have a positive or negative impact on bone mass accrual (Vicente-Rodriguez, 2006, Boreham and McKay, 2011). According to their characteristics, sports can be described as osteogenic (weight-bearing exercise) or nonosteogenic (non weight-bearing exercise) (Tenforde and Fredericson, 2011). Football is considered an osteogenic sport due to its positive impact on bone outcomes during childhood and adolescence (Ara et al., 2006, Vicente-Rodriguez et al., 2004a, Krustrup et al., 2010, Calbet et al., 2001). In contrast, sports such as cycling (Rico et al., 1993b, Duncan et al., 2002a, Olmedillas et al., 2011) or swimming (Andreoli et al., 2012, Ferry et al., 2013, Greenway et al., 2012, Ferry et al., 2011) are associated with no changes or even a reduction in bone mass when compared to controls in paediatric groups. Currently, there is no evidence on how different loading sports affect bone development in young males who invest a lot of time in their practice.

Weight-bearing exercise appears to be one of the most beneficial practices to optimise bone development and can enhance bone mineral accrual in children, particularly during the pubertal years. However, the constitution of the optimal exercise intervention programme remains unclear (Hind and Burrows, 2007). Previous evidence suggests that a jumping intervention programme may be a judicious choice for improving bone development in non-athletic children and adolescents, and that short bouts of exercise are more effective than a single longer bout of exercise for improving bone mass and strength (Fuchs et al.,

2001, MacKelvie et al., 2002b, McKay et al., 2005a, Weeks et al., 2008). Despite the beneficial effects of jumping interventions on bone health in the non-athletic paediatric population, there is no evidence in athletic groups that represent a large part of the population and might be in greater need for osteogenic stimulus depending of the sport practised.

Dual energy X-ray Absorptiometry (DXA) is considered the current "gold standard" for osteoporosis diagnosis and fracture risk prediction (Paola Pisani, 2013). However, additional measurements of bone health, such as volumetric BMD measured by quantitative computed tomography (QCT) and bone stiffness measured by Quantitative Ultrasound (QUS) can provide a further insight into the mechanisms implicated in the site specific adaptations of the skeleton to exercise (Hind and Burrows, 2007). In this regard, participation in sport participation might also influence hip geometry outcomes measured by Hip Structural Analysis (HSA) during adolescence (Hind et al., 2012b). There is lack of studies on males and evidence in adolescent female footballers have shown greater hip geometry parameters compared to swimmers and controls, while swimmers showed lower bone tissue at the narrow neck of the femur than footballers and controls (Ferry et al., 2011). A further insight into bone health is that weight-bearing exercise may adapt bone structure and remodelling during growth. A combination of the static measurements provided by DXA and QUS with the assessment of HSA, Trabecular Bone Score (TBS) and bone metabolism markers can improve the understanding of the changes in the skeleton during growth (Jurimae et al., 2010, Prevrhal et al., 2008, Silva et al., 2014).

Therefore, the purpose of the present thesis is to present a series of novel investigations into the effects of osteogenic (football) and non-osteogenic (swimming and cycling) sports on bone development in adolescent males, and to examine for the first time whether a jumping intervention programme can improve bone development in adolescent athletes. The main strength of the present thesis is the inclusion of six studies including cross-sectional, longitudinal and experimental designs and using a combination of bone assessment methods to obtain key data such as bone mass, geometry, texture and metabolism.

2. Literature review

This literature review provides an overview of bone health during growth, the determinants affecting bone health and the methodological approaches to measure bone outcomes during childhood and adolescence. In addition, the role of exercise in relation to bone health will be critically reviewed and insight will be provided on how participation in sports can affect bone health. Finally, the effect of exercise interventions to improve bone heath in children and adolescents will be reviewed. The section will end with a summary of the specific objectives of the present thesis.

2.1 Skeleton: biological role and components

The human skeleton is composed of 305 bones at birth, but this number decreases to 206 bones by adulthood after some bones combine. The role of the skeleton is to support body weight, to provide mechanical support for posture and movements, to protect inner organs, and to serve as metabolically active storage for minerals such as calcium, phosphate, and magnesium (Seeman and Delmas, 2006). The skeleton is the only organ that can be remained after death as nature disposes any other organ that covers the skeletal frame. The axial skeleton (skull, vertebrae, rib cage) forms a protective shell around the brain, spinal cord and inner organs, while the appendicular skeleton (the upper and lower limbs) is crucial for locomotion. Bones serve as attachment sites for muscles and ligaments, thereby enabling the movement of the body (Clarke, 2008).



Figure 2.1. Trabecular microarchitecture of a mediaeval (14th century) paediatric proximal femora remain. The photo was taken at the distal metaphysis of the femora with permission from University of Lincoln as part of an in-vitro diagnostic radiography DXA scan at the University of Exeter.

The skeleton is comprised of long bones (humerus, radius, femur and tibia) and flat bones (such as the skull, sternum, scapula and ileum), and can be divided into cortical bone and trabecular bones (Datta et al., 2008). Cortical bones constitute 80 % of the skeleton and are mainly found around the shaft (diaphysis) of the long bones (Figure 2.1). Trabecular bones represent 20 % of the skeletal mass and account for 80 % of the bone surface (Clarke, 2008). They are found mainly in the vertebral bodies, metaphyseal areas at the end of long bones and in the flat bones. Trabecular bones are metabolically active and maintain bodily mineral homeostasis. The proportion of cortical and trabecular bone vary depending on the skeletal site. For example, the distal forearm and femoral neck constitute 25 % trabecular and 75 % cortical bone, whereas the vertebra contains more than two thirds trabecular bone (Surgeon-Report, 2004).

Deterioration of cortical and trabecular bone predispose to fractures at different locations.

2.2 Bone cells

The skeleton consists of three different kinds of bone cells (osteoblasts, osteocytes and osteoclasts) and of extracellular matrix. Extracellular matrix accounts for 90 % of the total bone volume, which comprises mineralized matrix, organic matrix, lipids and water. The mineralized matrix accounts for 99 % of the body's storage of calcium and 85 % of the storage for phosphorous. The organic matrix contains mainly type 1 collagen, as well as proteoglycans, growth factors, and glycoproteins. The organic matrix is secreted by osteoblasts and is mineralized within 10-15 days (Buck and Dumanian, 2012).

2.2.1 Osteoblasts and osteocytes

Osteoblasts are responsible for bone formation. They originate from mesenchymal stem cells in the bone marrow, and account for 4-6 % of bone cells (Capulli et al., 2014). Osteoblasts build bone by secreting bone proteins and collagen that form the bone matrix. Alongside collagen type 1, osteoblasts also produce osteocalcin, osteonectin, osteopontin and bone sialoprotein (Buck and Dumanian, 2012). The average lifespan of an osteoblast is three months (Manolagas, 2000). The aging osteoblast follows three possible paths: undergo apoptosis, become embedded in the bone as an osteocyte, or transform into a bone lining cell (Rochefort et al., 2010). When bone remodelling should not occur, they prevent direct interaction between osteoclasts and bone matrix

(Capulli et al., 2014). In order to allow osteoclasts to attach to the bone, the collagen matrix must be removed through collagenase secretion (Manolagas, 2000). After the formation of the bone, some osteoblasts become entrapped in the newly formed bone matrix and develop into osteocytes. Osteocytes account for approximately 95 % of the bone cells, they are long-lived and do not divide (Capulli et al., 2014). Osteocytes have the capacity to detect mechanical pressure and load, and thereafter regulate bone remodelling by acting on osteoblast and osteoclast differentiation and function (Rochefort et al., 2010).

2.2.2 Osteoclasts

Osteoclasts are the only cells that can induce bone resorption (Lerner, 2000). They are essential for physiological bone resorption during growth, for remodelling, and for maintaining calcium homeostasis via the endocrine system (Kular et al., 2012). The formation and activation of these cells are dependent of local cytokines from the osteoblasts and other cells, and their main task is to breakdown and remove the extracellular matrix (Compston et al., 2009). Immature osteoclast precursors proliferate and fuse to form giant multinuclear cells. Mature osteoclasts attach to the bone surface, creating an acidic microenvironment that enables bone resorption (Blair and Athanasou, 2004). After bone resorption, osteoclasts undergo apoptosis (Buck and Dumanian, 2012).

2.2.3 Bone modelling and remodelling

Bone modelling and remodelling (Figure 2.2) is the ongoing process where bones are resorbed and formed. During the modelling process, the bones adapt to mechanical forces. The osteocytes are the cells that sense mechanical strain and trigger bone remodelling. This includes changes in shape, mass, and size throughout life (Rauch and Glorieux, 2004). Modelling usually results in a net increase in the amount of bone tissue, due to less active osteoclastic function in the endocortical surface, as compared to osteoblasts working on the periosteal surface without interruption. In addition to cortical thickening, modelling is also important for reshaping the long bones as they grow in length during childhood and adolescence (Parfitt et al., 2000, Rauch, 2005).

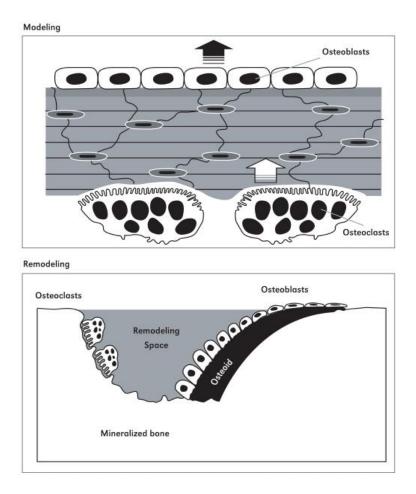


Figure 2.2. In modelling, osteoblast and osteoclast action are not linked and rapid changes can occur in the amount, shape, and position of bone. In remodelling, osteoblast action is coupled to

prior osteoclast action. Net changes in the amount and shape of bone are minimal unless there is a remodelling imbalance. With permission from (Rauch and Glorieux, 2004).

The remodelling cycle begins with recruitment of osteoclasts for bone resorption and ends with bone formation by the osteoblasts, with bone formation lasting approximately three times as long as bone resorption (Kular et al., 2012). The remodelling cycle lasts about 3 to 4 months, where the phase of resorption is 2 to 3 weeks, reversal phase is 4 to 6 weeks, and the final formation phase, where osteoblasts lay down bone until the gap is completely replaced by new, is up to 4 months (Hadjidakis and Androulakis, 2006). During the growth period, about 5 % additional bone is formed in every remodelling cycle as compared to resorption (Parfitt et al., 2000). It has been estimated that the adult skeleton is completely regenerated in 10 years with an estimated turnover of 10 % per year for the entire skeleton. There is constant matrix remodelling with an average 4 % in cortical bone turnover and 28 % in trabecular bone turnover per year (Manolagas, 2000). The balance of the remodelling cycle in a young adult skeleton is close to zero, for as much bone is formed as is removed. After the fifth decade of life, bone formation rate fails to keep pace with resorption activity, and bone loss begins. Thus, a remodelling imbalance causes reduced bone strength and can lead to osteoporosis (Zuo et al., 2012).

2.3 Assessment of bone status

2.3.1 Bone densitometry

Bone health can be assessed by mineral density, which is a radiographic measure of the amount of bone material in a given volume measured by absorption. Single photon absorptiometry was introduced in the 1960s, enabling the non-invasive quantitative assessment of BMC at peripheral sites of the skeleton (Cameron and Sorenson, 1963). Replacement of the radionuclide source with X-ray resulted in better precision, resolution and scanning time (Genant et al., 1994).

The DXA scanner was introduced in the late 1980s, and is the most widely used technique to assess bone density. In order to separate the dense tissue from the soft tissue, the DXA scanner produces two X-ray beams, one with high energy and one with low energy. For each beam, the amount of X-ray that passes through the body is measured, and BMD can be calculated (Dimai, 2016). The radiation dose from a DXA examination is very low, ranging from 1-10 μ Sv for a spine and hip examination, which is negligible and similar to the daily natural background radiation (Damilakis et al., 2013).

DXA is considered the gold standard method for assessing areal BMD (aBMD) by non-invasive means in a clinical setting, and is a valid method to diagnose osteoporosis and to predict the risk of fracture in adults (Cummings and Melton, 2002, Dimai, 2016). In clinical use, the most common sites measured with DXA are the lumbar spine, hip (total hip or proximal femur), and total body (Bachrach

et al., 2016, Lee et al., 2004) (Figure 2.3). In adults, the femur has a special clinical interest for assessment due to the high prevalence of fractures at this location. Lumbar spine measures are considered to reflect trabecular bone health, and the whole body DXA measure reflects bone health of the long bones. Separate measurements can be conducted for other skeletal sites and with a special interest on the distal radius due to the high fracture incidence at this site of the skeleton (Ahmed et al., 2010).

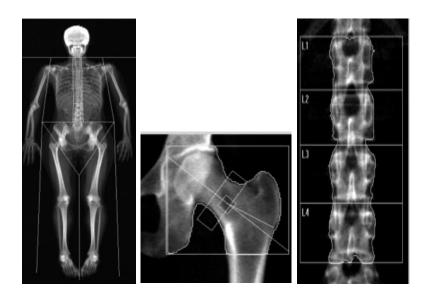


Figure 2.3. Images (2014) obtained by DXA (Lunar) at total body, left femur and lumbar spine as part of the PRO-BONE study.

DXA measures BMC and provides a calculated BMD by measuring BMC and bone area. The projected areal BMD and an estimation of cubic shape is used for clinical practice, and BMD is transformed into T-score for adults. The T-score is the standard deviation of BMD from a healthy 30-year-old of the same sex and ethnicity, representing the peak bone mass. A T-score equal to or less than -1 shows that the BMD is 1.0 SDs or more below the average value in a young adult and indicates osteopenia. A T-score equal to or less than -2.5 shows that the BMD is 2.5 SDs or more below the average value in a young

adult, and indicates osteoporosis according to the WHO classification of osteoporosis (Kanis et al., 2000a).

2.3.2 DXA in children and adolescents

DXA is also widely used in children and adolescents, as it is quick and noninvasive, and the radiation exposure is low. There are certain challenges of using DXA in growing children including the technical issues with acquisition of the data from small bones with low mineral content, and with the interpretation of the results (Gordon et al., 2008). To avoid an overestimation of bone mineral deficits in children, BMD scores are compared to reference data for the same sex and age (Z-score), not to the mean of young adults (T-score). If the bone age, ideally determined with X-ray of the wrist, is delayed or advanced, the BMD can be adjusted to the bone age instead of calendar age. Height adjustments are recommended in paediatric studies, and the whole body BMD is proposed to be assessed relative to height in children (Leonard et al. 2004). DXA machine specific reference data are recommended for Z-score calculations in children (Genant et al., 1994, Zemel, 2012). According to the International Society of Clinical Densitometry, total body less head and lumbar spine (L1-L4) are the two preferred skeletal sites to assess bone health in the paediatric population. Additional regions are recommended depending on the design of the study and the population studied (Bachrach et al., 2016). The terms osteopenia and osteoporosis should not be used to describe densitometry findings in the paediatric population. Instead, BMC or BMD zscores < -2 SDs can be used to indicate low bone status for the specific age. The International Society for Clinical Densitometry stated that one of the

following criteria can indicate osteoporosis in children and adolescents: 1) one or more vertebral fractures occurring in the absence of local disease or high-energy trauma (measuring BMD can add to the assessment of these patients but is not required as a diagnostic criterion); or low bone density (BMC or areal BMD z scores < -2.0) and a significant fracture history (2 or more long bone fractures before 10 years or 3 or more long bone fractures before 19 years of age) (Gordon et al., 2014). Because athletes participating in weight-bearing sports are expected to have higher BMDs than non-athletes, the American College of Sports Medicine recommends that a Z-score a Z-score between -1.0 and -2.0 is considered as "low BMD" and defines "osteoporosis" as secondary clinical risk factors for fracture with BMD Z-scores less than -2.0 (Nattiv et al., 2007). Therefore, a definition of "bone health" in children and adolescents involved in sports could be a BMC or areal BMD z scores > -1.0 in the absence of previous fracture.

2.3.3 Bone geometry estimates

HSA is a technique that uses the properties of DXA images to derive hip geometry estimates that are associated with bone strength (Beck, 2007). The HSA program measures not only the BMD of the hip bone but also structural geometry of cross-sections traversing the proximal femur at specific locations. The bone mass image is used from the DXA scan where pixel values are expressed in areal mass (Hind et al., 2012a). The method employs the principle that a line of pixel values across the bone axis corresponds to a cut plane traversing the bone at that location and contain some of the information about the cross-section. The program analyses the proximal femur at different

locations depending on the DXA used (three locations can be derived from Hologic DXA and one from Lunar) (Figure 2.4). The potential obtained regions are: 1) narrow neck across the narrowest diameter of the femoral neck; 2) intertrochanteric along the bisector of the neck-shaft angle; and 3) the shaft, 2 cm distal to the midpoint of the lesser trochanter. For estimates of endosteal diameter, cortical thickness and buckling ratio, models of the cross-sections using assumed shapes are necessary. The narrow neck is modelled with 60/40 proportion of cortical/trabecular bone, the shaft with 100% of cortical bone and the intertrochanteric regions is modelled with 70/30 proportion cortical/trabecular bone (Beck, 2007).

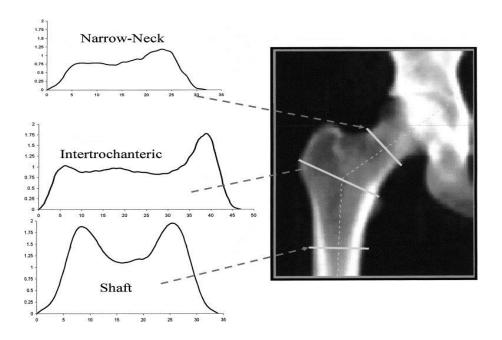


Figure 2.4. Hip structure analysis profile at the narrow neck of the femur, intertrochanteric and femoral shaft sites. Adapted from (Beck, 2007).

Several studies have demonstrated that HSA can predict hip fractures (Faulkner et al., 2006, Leslie et al., 2009). In a study of 7,474 women with 635 incident hip fractures over 13 years, women with fractures (compared with those without fractures) had greater neck-shaft angles, subperiosteal and endosteal

diameters, and buckling ratios and lower hip BMD, CSA, cross-sectional moment of inertia (CSMI), section modulus (Z), and cortical thickness (Kaptoge et al., 2008). Similarly, a study on postmenopausal women (n=76) reported that trochanteric diameter and femoral shaft subperiosteal diameter, as well as buckling ratio, were independent predictors of fracture risk even after controlling for age, body size, clinical risk factors, and BMD (Petit et al., 2004). HSA is being used in studies assessing the impact of exercise and mechanical loading on bone (Hind et al., 2012a, Petit et al., 2004) and has been used in longitudinal studies of children and adolescents (Jackowski et al., 2011b, Alwis et al., 2012). Previously, the coefficient of variations (COVs) of these variables previously found to be between 7.9 % and 11.7 % (Khoo et al., 2005).

2.3.4 Trabecular microarchitecture

TBS is a recently developed technique from which a textural metric can be extracted from the DXA image, providing an indirect index of trabecular microarchitecture (Silva et al., 2014). TBS is not a direct physical measurement of bone microarchitecture, but rather an overall score computed by the projection of the 3D structure onto a 2D plane (Bousson et al., 2012). TBS is calculated as the slope of the log transformed two dimensional variogram, where the slope characterizes the rate of grey pixel variations. A steep variogram slope with a high TBS is associated with better bone structure, whereas low TBS values indicate low bone structure (Figure 2.5). Because the DXA image is usually retrievable, even though it might have been obtained years before, TBS can be readily applied to any available DXA image obtained from GE Lunar and Hologic densitometer models. TBS is typically measured at

the lumbar spine and determined using the same region of interest as the BMD measurement. Although a TBS is given for each vertebra, the average of L1 to L4 is used following DXA lumbar spine reports (Bousson et al., 2015). A recent study investigated the predictors of TBS in adolescents showing that age and pubertal stages were significant determinants of TBS (Shawwa et al., 2016). Normative data do not exist yet, but the COVs of TBS in relation to BMC has been reported to be between 1.1 % to 1.9 % (Silva et al., 2014).

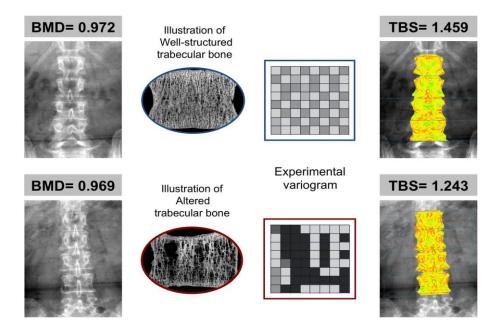


Figure 2.5. Representation of the TBS principles and an example where the TBS appears to be independent from BMD. With permission from (Silva et al., 2014).

2.3.5 Quantitative Computed Tomography

Quantitative Computed Tomography (QCT) is an X-ray based technique that provides three-dimensional information on morphology and composition of the scanned area. Peripheral QCT (pQCT) is a device designed to measure peripheral bones, typically the distal forearm (radius) and the distal tibia (Liu et al., 2010). The radiation dose is low, less than 3 μ Sv for a single slice and

increases in a multiple-slice protocol (Lee et al., 2013). Unlike DXA, pQCT provides cross-sectional images. This enables the separation of cortical and trabecular bone, so that volumetric BMD of the cortical and trabecular bone compartments can be determined separately. It also allows the investigation of the geometrical bone properties. Information about the trabecular bone traits are obtained through a scan at 4 % of the bone length in the distal direction of the proximal end of the bone. While information about cortical bone traits are obtained through a scan at 15-65 % of the bone length in the distal direction of the proximal end of the bone. In addition to volumetric BMD of the trabecular and cortical bone, a measure of cortical thickness, cortical CSA, endosteal and periosteal circumference can be obtained, which enables insight into the size and density of the bone (Wang et al., 2008). Recently, a multislice highresolution pQCT (HR-pQCT) has come into use for research purposes. HRpQCT is a pQCT device that performs several cross-sectional slices and produces a three-dimensional image of the bone. The resolution is high (slice thickness 82 µm) and enables investigation of the microstructure of the trabecular bone (Schipilow et al., 2013).

2.3.6 Ultrasonometry

In addition to DXA and QCT devices, quantitative ultrasound (QUS) was developed in 1984 for the assessments of calcaneal bone status in adults (Langton et al., 1984). QUS uses ultrasound waves characterized by a frequency exceeding the threshold of audibility of the human ear (> 20 kHz). Unlike the usual ultrasound techniques that are based on the reflection of ultrasound waves, QUS involves the transmission of ultrasound pulses through

the investigated bone tissue and the detection of the transmitted pulses once they have passed through the medium. The heel is positioned between two probes as seen in Figure 2.6. The ultrasounds transmitted to the cortex after propagation along the cortical bone layer and received by another ultrasound transducer at a known distance (Paola Pisani, 2013). The QUS parameters obtained are the speed of sound and broadband ultrasound attenuation through the appendicular bone. Bone stiffness (or stiffness index) can be calculated using validated age specific equations (Baroncelli, 2008, Frost et al., 1999). Due to the use of the ultrasound waves, the method has several advantages for the paediatric population. The QUS device is portable and suitable for large school studies, there is no radiation exposure and it is technically simple. It should be noted that if there are decreased values of the output from the QUS it may not be possible to detect the reason for this since the QUS is dependent on the density and the stiffness as well as the micro-structure (Binkley et al., 2008). A study aiming to examine the correlation between bone stiffness measured by QUS and DXA outcomes in children and adolescents indicated that the shortterm precision QUS was 1.8 % for bone stiffness, 2.9 % for BUA, and 0.4 % for SOS. Pearson's correlation coefficients (r) were calculated to assess the correlations between the total body BMC by DXA and bone stiffness calculated by QUS. There were significantly positive correlations between bone stiffness and total body BMD (r=0.693) and BMC (r=0.690), highlighting that QUS method is considered a valid and radiation-free method compared with DXA to assess bone health in children and adolescences (Xu et al., 2014).

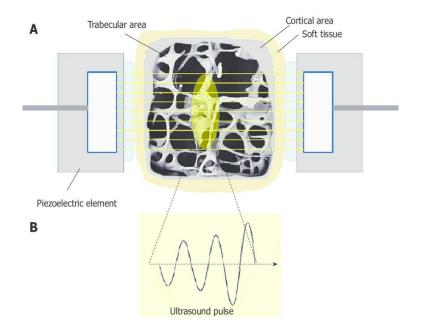


Figure 2.6. Quantitative ultrasound method. A: Ultrasound beam through a bone tissue of phalanx (transmission way) in a typical quantitative ultrasound measurement (section view); B: Ultrasound pulse. With permission from (Paola Pisani, 2013)

2.3.7 Bone formation and resorption markers

Bone turnover markers can provide additional information about bone metabolism, especially during growth, that are not detected by bone devices and can help in the evaluation of osteoporosis treatment (Vasikaran et al., 2011). Bone turnover markers reflect the formation and resorption of the bones and they can be measured in biological samples such as blood or urine, and are determinants of fracture risk (Johansson et al., 2014). The intracellular markers can provide dynamic information about the turnover of osseous tissue and can reflect the cellular activities of bone formation and resorption by monitoring the acute responses on bone remodelling (Nebigh et al., 2017). They can be divided into two categories: 1) bone formation markers, which reflect osteoblast activity, and are by-products of collagen synthesis, matrix proteins or osteoblastic enzymes; and 2) bone resorption markers, which reflect osteoclast

activity and are mainly degradation products of type 1 collagen (Jurimae, 2010). Two common bone formation markers are procollagen type I of amino-terminal propeptide (PINP) and osteocalcin, produced by osteoblasts during osteoid synthesis (Brown et al., 2009). Some bone formation markers are incorporated directly into the bone matrix, but some newly synthesized markers are secreted and circulated in the blood (Szulc and Delmas, 2008). The most common bone resorption marker used is Carboxi-terminal telopeptide of type 1 collagen (CTX-I), a collagen degradation fragment which, when bone is resorbed, is released into the blood and subsequently excreted in urine (Chopin et al., 2012). Several other markers of bone turnover are available but PINP and CTX-I are recommended by International Osteoporosis Foundation (Vasikaran et al., 2011) as markers of formation and resorption, respectively. During puberty, levels of bone turnover markers increase, and have been demonstrated to be higher in early puberty and mid-puberty compared to advanced puberty (Gracia-Marco et al., 2011b). In addition, bone remodelling might be increased due to participation in high intensity weight-bearing activities during puberty (Kambas et al., 2016, Jurimae et al., 2010). Exercise can induce changes in bone remodelling with evidence showing that football training to have substantial effect on bone turnover in boys (Zouch et al., 2008). A different study in pubertal girls did not find significant differences in bone resorption between gymnasts, runners, and controls after 1 year (Lehtonen-Veromaa et al., 2000), however there is no scientific evidence comparing bone remodelling of adolescent male athletes involved in different loading sports.

2.4 Bone development

Bone development starts at 8 weeks as the pattern of the skeleton has been largely formed. At this early skeleton, the initial bone formation starts 6 weeks post fertilization (Wang and Seeman, 2008). The fetal period is characterized by rapid growth in bone size and maintenance of the bone's shape, which is required for maternal nutrition and fetal movements (Steer and Tobias, 2011). After birth, the skeleton maintains a fast rate of growth in length and thickness. The long bones continue their growth during infancy, childhood and adolescence until they reach their adult size. Growth in length is driven by bone formation on the diaphysis side of the epiphyseal plate, while increased thickness is related to net bone formation on the periosteal surface (Clarke, 2008).

2.4.1 Bone mass accrual

PBM attainment typically occurs between the second and third decade of life, with 80-90 % acquired by late adolescence, although this is skeletal site dependent (Baxter-Jones et al., 2011, Henry et al., 2004). Pubertal status is associated with bone growth both in size and density, and evidence shows that boys and girls acquire bone mass at similar rates before puberty, whereas after puberty, males tend to acquire a higher bone mass than females, likely due to the impact of sex-steroid hormones (Davies et al., 2005). During childhood and adolescence longitudinal growth occurs as well as changes in skeletal size and shape (Chevalley et al., 2009). Bones become longer through endochondral ossification at the growth plates, and wider by periosteal apposition. From early childhood to late adolescence, there is an ongoing accumulation of skeletal mass, which increases from approximately 70-95 g at birth to 2,400 to 3,300 g

in young women and men, respectively (Stagi et al., 2013). Skeletal growth requires adequate production of growth hormone, thyroid hormones, growth factors and sex steroids (Bachrach, 2001).

Before puberty, no substantial sex differences are observed in BMD of the lumbar spine, femur and radius, after adjustment for age, physical activity and nutrition (Bonjour and Chevalley, 2014). During puberty, bone size increases while BMD remains almost constant in both sexes (Rizzoli et al., 2010). Bone formation also occurs at the endosteal, inner surface of the cortical bone (Bonjour et al., 1994). Skeletal modelling during growth differs from bone remodelling, since it leads to new bone formation at a different location than the site of resorption, resulting in alterations in bone shape and net accrual of bone tissue (Jurimae, 2010). At the end of puberty, males have higher BMD, mainly due to greater bone size in males compared to females (Chevalley et al., 2011). In males, the prolonged bone maturation period results in larger increases in cortical thickness and bone size. Cortical thickness increases more by periosteal apposition of bone in males, resulting in a stronger bone, whereas in females more bone is deposited at the endosteal, inner surface (Bonjour and Chevalley, 2014).

Findings of a longitudinal study monitoring BMC velocity annually suggest that the highest rate of bone mass accrual, or PBM, in females and males occurs between 12-15 years and 14-16 years of age, respectively (Figure 2.7). In the same study, BMC velocity rate for most skeletal sites decreased by the age of 16-18 years in girls and 17-20 years in boys (Bailey et al., 1999). During the

years of puberty, girls acquired approximately 40 % of their PBM, meaning they had achieved approximately 90 % of PBM by the age of 18 (Theintz et al., 1992). The PBM is relatively stable until the onset of natural loss with aging. At menopause, women experience an accelerated loss for 3-6 years and thereafter a continued loss of bone mineral for both men and women (Faulkner and Bailey, 2007). The PBM is an important predictor for BMD in elderly and hence, maximising the PBM may be essential in the prevention strategy towards osteoporosis.

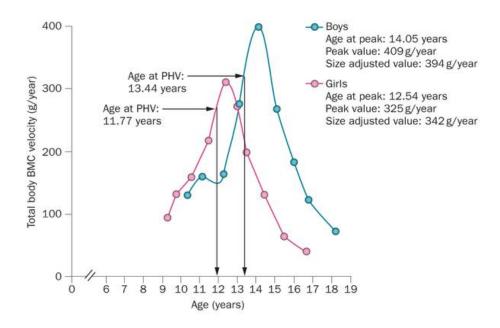


Figure 2.7. Total body peak bone mineral content velocity in boys and girls over a period of 6 years. With permission from (Bailey et al., 1999).

2.4.2 Maturation and bone development

Maturation onset is another important component of bone mass acquisition due to the relationship between the pubertal timing and the PBM accrual. Maturation onset depends on endocrine and hormonal changes on both females and males. Maturity status can be assessed by X-rays to establish biological age, by maturity stages using the Tanner method, by markers of secondary sex

characteristics and by anthropometric measurements to calculate age from peak height velocity (PHV) (Mirwald et al., 2002, Malina et al., 2004, Georgopoulos et al., 2004, Tanner and Whitehouse, 1976).

The Saskatchewan Paediatric Bone Mineral Accrual Study reported that PBM velocity was achieved 6 months later than PHV, where boys and girls obtained 90 % of total height at PHV time but only 57 % of total bone mineral content BMC (Bailey et al., 1999). In addition, a large part of the BMC accrual takes place during the period of adolescent growth surrounding PHV, which is the time of the most rapid longitudinal growth (Figure 2.8). Depending on skeletal site, 33 % to 46 % of the adult BMC has been reported to be accrued in the circumpubertal years (-2 to +2 years from PHV) (Baxter-Jones et al., 2011). The inverse association between PHV and PBM accrual showed that children who reach PHV earlier tend to have higher BMC and BMD than those with a later age of PHV. However, in late adolescence and young adulthood there were no significant differences in BMC in early, middle, and late maturing males, while late maturing females developed less bone mass throughout adolescence than their early and average maturing peers (Jackowski et al., 2011a, Jackowski et al., 2011b).

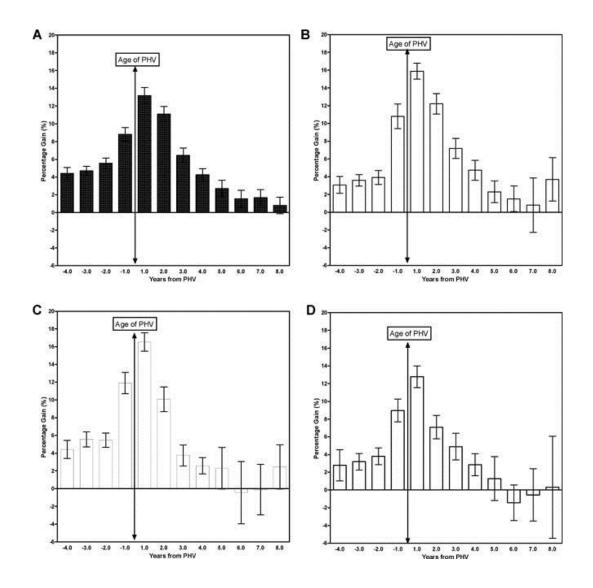


Figure 2.8. Percentage adult bone accrual within 1-year of biological age categories (years from peak height velocity) for four sites in males: (A) total-body BMC (B), lumbar spine BMC, (C) total-hip BMC and (D) femoral neck BMC, Mean and 95% confidence intervals. With permission from (Baxter-Jones et al., 2011).

2.4.3 Body composition and bone development

The attainment of optimal PBM accretion through puberty is accompanied by sex specific alterations in body composition. The effects of lean mass on bone accretion are of particular interest because of the beneficial effects of weight-loading on bone accretion. Muscle contractions generate forces which stimulate bones to adapt their shape and density to such loads (Schoenau and Frost,

2002). A recent systematic review (Sioen et al., 2016) on the associations between body composition and bone health in children and adolescents indicated that the contribution of lean mass to the variance of the different bone parameters is larger than the contribution of fat mass, and that an increase in lean mass is associated with an increase in bone parameters. Most of the 19 studies included in the systematic review indicated that the increase in bone parameters seen in overweight and obese children and adolescents is due to an increase in lean mass and not due to greater fat mass (Sioen et al., 2016).

The contribution of lean tissue to bone accretion has been a topic of numerous investigations. In a study of 363 healthy schoolchildren, aged 10-17 years, the contribution of lean mass to BMC varied by skeletal site, and was greater in boys (5.7-12.3 %) than girls (4.3-10.5 %). Lean mass was most strongly associated to BMC of the femoral neck in both sexes, whereas fat mass was associated to BMC of the total body and lumbar spine (Arabi et al., 2004). Fat mass accounted for only a small percentage of explained variance in BMC, although it had a higher contribution in females than males (up to 6.5 vs. 1.9 % explained variance). In obese children and adolescents (Petit et al., 2005), it has been shown that the greater femoral bone strength in obese children was attributed to their greater lean mass as fat mass did not contribute to measures of femoral strength. As obese and overweight children also have greater lean mass compared with normal-weight individuals, it is of interest to adjust for lean mass when investigating the association between fat mass and bone parameters. Some studies indicate that the association between fat mass and bone parameters are similar with and without adjustment for lean mass (El Hage et al., 2010, Wey et al., 2011, Leonard et al., 2004, Farr et al., 2010). Specifically, it has been reported that the significant associations between fat mass and bone area, BMC and BMD of lumbar spine, are attenuated after adjustment for lean mass (Cole et al., 2012). In addition, Gracia-Marco et al. concluded that adolescents with higher levels of adiposity have greater bone mass (Figure 2.9), but that this association is explained by their higher levels of lean mass (Gracia-Marco et al., 2012). Also Hoy et al. indicated that bone quality of overweight adolescents adapts to lean mass and not to greater fat mass. Therefore, it is relevant to be cautious with the conclusions of studies reporting associations between fat mass and bone parameters without adjusting for lean mass (Hoy et al., 2013).

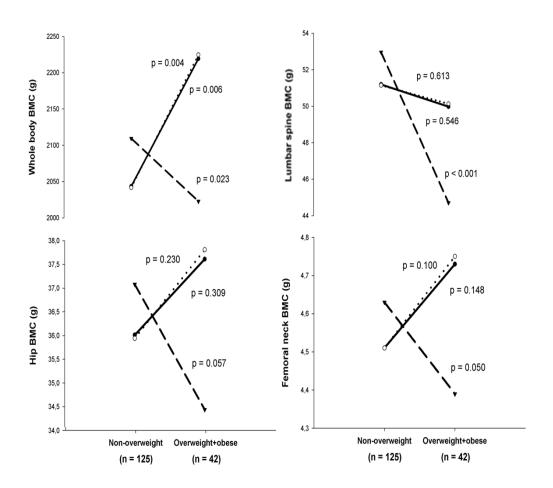


Figure 2.9. BMC and BMD in relation to weight status in boys. Whole body, total hip, lumbar spine and femoral neck. Solid lines (Model 1) show adjustments by confounders (height, calcium intake, and sexual maturation). Semi-solid (Model 2) lines show adjustments by (Model

1 + average physical activity). Dashed lines (Model 3) show adjustments by confounders (Model 2 + lean mass). With permission from (Gracia-Marco et al., 2012).

Prospective studies have shown that lean mass accretion peaks before the peak bone mass accretion, suggesting a causal association between these measures. Rauch et al. (Rauch et al., 2004) showed that peak in lean mass accretion occurred, on average, 0.51 years before peak BMC accretion in girls and by 0.36 years in boys. Further, peak lean mass accretion was the primary determinant of total body peak BMC accretion, explaining 50% of the variability in peak BMC accretion. Using peripheral quantitative computed tomography of the tibia, Xu et al. (Xu et al., 2009) examined multiple indicators of bone density and strength in relation to changes in lean mass of the lower leg in a prospective longitudinal study. They showed that the peak gains in muscle cross-sectional area, a measure of lean mass, occurred 1 year after the peak gains in tibia length and total cross-sectional area, and earlier than cortical CSA, total BMC and cortical volumetric BMD. Thus, there are dynamic changes in the distribution and density of bone that occur during puberty, not all of which are directly responding to changes in lean mass.

2.5 Osteoporosis and its consequences

Osteoporosis ("porous bones", from Greek: οστέον/osteon meaning "bone" and πόρος/poros meaning "pore") is defined by the National Institute of Health (US) as "A skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture" (NIH Consensus Development Panel on Osteoporosis Prevention and Therapy, 2001). The World Health Organization (WHO) further classifies osteoporosis as BMD 2.5 standard deviations below the mean value of healthy individuals of the same

sex and ethnicity at the age of 30 years (Kanis et al., 2000a). Approximately 200 million people are affected by osteoporosis worldwide and the prevalence is expected to increase due to an aging population (Cooper, 1999, Reginster and Burlet, 2006). The economic burden of osteoporosis in Europe is higher than most types of cancer (except lung cancer), or chronic cardiorespiratory diseases (Kanis et al., 2008, Johnell and Kanis, 2006) and represents a direct annual cost of ~ €31.7 billion to health care and social services (Kanis and Johnell, 2005b).

2.5.1 Fracture prevalence

Approximately 30 % of all postmenopausal women have osteoporosis in the United States and in Europe. At least 40 % of these women (Melton et al., 1992) and 15-30 % of men (Randell et al., 1995) will sustain one or more fragility fractures in their remaining lifetime. By 2050, the worldwide incidence of hip fractures is projected to increase by 310 % and 240 % in men and women respectively (Gullberg et al., 1997). The estimated number of hip fractures worldwide are expected to rise from 1.66 million in 1990 to 6.26 million in 2050 (Figure 2.10), even if age-adjusted incidence rates remain stable (Cooper et al., 1992).

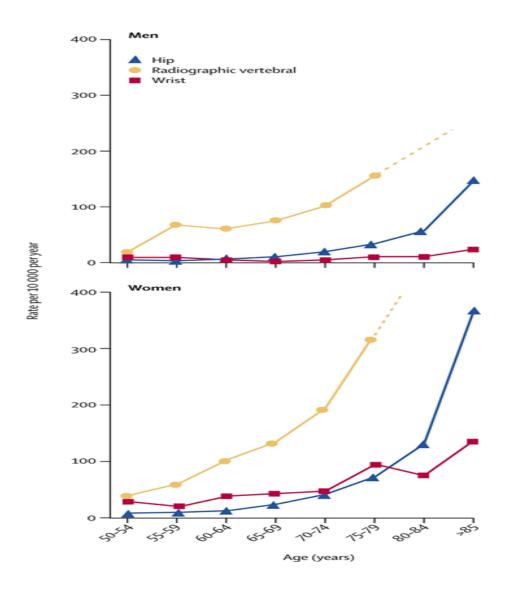


Figure 2.10. Age-specific and sex-specific incidence of radiographic vertebral, hip, and distal forearm fractures. With permission from (Cooper et al., 1992).

Patients with fractures (especially hip fractures but also vertebral fractures) suffer loss of quality of life and mobility, long-term disability and loss of independence (Cummings and Melton, 2002). Only half of those ambulatory before a hip fracture are able to walk independently afterwards (Kanis et al., 2000b). Fractures also increase the mortality and impose a financial burden on the health care system (Zethraeus et al., 2007). In men, osteoporotic fractures occur approximately 5-10 years later in life than in women, and after a hip fracture morbidity and mortality rates are higher among men (Kaufman et al.,

2013). Low BMD is a major risk factor for osteoporotic fracture (Johnell et al., 2005). Every SD decrease in BMD is associated with approximately a two-fold increase in the age-adjusted hip fracture risk in postmenopausal women, and with a three-fold risk increase in elderly men (Cummings et al., 1993).

Other important risk factors for osteoporotic fracture are increased age, female sex, previous fracture history, a family history of fracture and systemic glucocorticoid treatment (Kanis et al., 2004a). Additional risk factors are smoking, excessive alcohol intake and low body mass index (BMI), as well as increased risk of falling related to visual impairment, treatment with sedatives or reduced mobility (De Laet et al., 2005). A fracture risk assessment tool (FRAX®) developed by the (WHO) was introduced as a method to identify patients with high fracture risk. It is a country-specific computer-based algorithm available online which calculates fracture probability based on risk factors and patient characteristics, with or without available measurements of femoral neck BMD (Kanis et al., 2009).

2.5.2 Fractures during childhood and adolescence

The annual fracture incidence increases during adolescence and it has been shown that a prior fracture is associated with an 86 % increased risk of any fracture (Kanis et al., 2004b). In addition, a decrease of 1 SD in BMC or BMD is associated with an increase in fracture risk (Kalkwarf et al., 2011). The lifetime fracture risk is 40 % for girls and 64 % for boys, highlighting the importance of this period of life (Cooper et al., 2004a, Hedstrom et al., 2010, Jones et al., 2002). The incidence of non-osteoporotic fractures in 5 to 14-year-old girls

increases by 4 % per year and peaks later in life at the age of 85 years (Figure 2.11). In males the fracture incidence reaches 7 % per year in late adolescence, and peaks at the age of 15 to 24 years, which is greater compared to females (Donaldson et al., 2008).

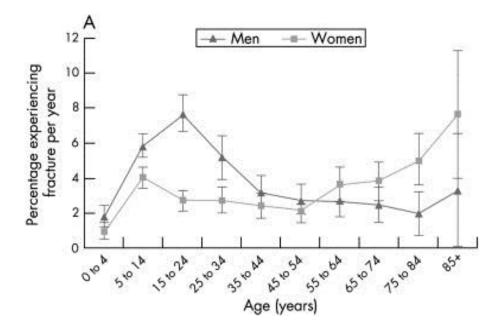


Figure 2.11. Annual fracture incidence and lifetime fracture prevalence per 100 people, by age and sex in the English general population 2002 – 2004. Note the rise in fracture incidence in the adolescent years. With permission from (Donaldson et al., 2008).

2.5.3 Causality and skeletal sites of paediatric fractures

The leading cause of paediatric fractures is falls (27 %), followed by accidents occurring during leisure activities (25 %), at home (14 %), on playgrounds (11 %), and traffic (11 %) and school accidents (8 %). The majority of the fractures in children occur at the upper extremities (~75 %), with the lower extremities representing 20 %, and less than 5 % are located in the axial skeleton and trunk (Brudvik and Hove, 2003). The most commonly fractured site in children is the forearm (lower arm, radius and ulna), comprising at least one third of all fractures (Cooper et al., 2004b). Forearm injuries occur most often at

the distal end and they are as common in girls as in boys, and the incidence increases with the age (Hedstrom et al., 2010). Upper arm fractures comprise 10 % of all paediatric fractures and distal humerus fractures are most common in children from 4 to 9 years, whereas the proximal humerus is more commonly injured in older children. Lower extremity fractures in paediatric patients usually occur at the lower leg (tibia and fibula) and foot sites (Rennie et al., 2007). Biological and behavioural differences are thought to explain the male predominance in fracture incidence and is mainly attributed to the greater participation to leisure activities (Joeris et al., 2014). In order to improve bone health and reducing the prevalence of osteoporosis and the fracture incidence in life, primary prevention remains the most important policy action in public health. It is important to acknowledge a common misconception that osteoporosis and osteoporotic fractures are always the result of bone loss. Bone loss commonly occurs in both men and women with age, however an individual who does not reach the optimal PBM during childhood and adolescence may develop osteoporosis without the occurrence of accelerated bone loss (NIH Consensus Development Panel on Osteoporosis Prevention and Therapy, 2001). Hence, it is important to highlight in the next chapters that osteoporosis has its origins in childhood and adolescence and that suboptimal bone growth during this period is as important as bone loss later in life for the development of osteoporosis (Gordon et al., 2016).

2.6 Determinants of bone health

Bone health during adulthood is determined by the maximum amount of bone mass attained during childhood and adolescence. Therefore, it is critical to understand the role of the determinants affecting bone acquisition during growth.

Osteoporosis has a strong genetic component and epidemiological studies show that heritable factors account 60-80 % of the variability in BMD (Mitchell et al., 2015, Stewart and Ralston, 2000, Bachrach, 2001), while environmental non-modifiable (e.g. hormones) (Vanderschueren et al., 2005, Bonjour and Chevalley, 2007) and modifiable (e.g. calcium, vitamin D and exercise) (Courteix et al., 2005, Ward et al., 2007, Lappe et al., 2014, Specker B, 2007, Mouratidou et al., 2013, Valtuena et al., 2012, Vlachopoulos et al., 2016) factors account for the remaining peak bone mass explained variance (Figure 2.11).

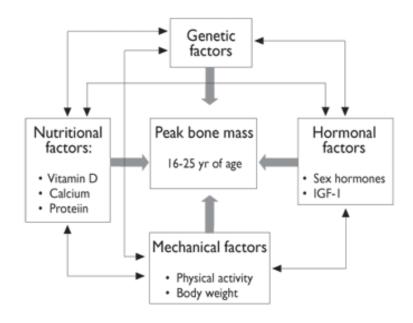


Figure 2.12. Physiological determinants of peak bone mass. The black arrows illustrate the interdependency of the four types of factors. With permission from (Bonjour et al., 2009).

2.6.1 Genetic predisposition

Osteoporosis is a polygenetic disease and no single gene explains the disease. In twin studies several genes have been identified to be associated with variations in BMD and many of these genes were identified as candidate genes in the pathways of either bone formation or bone resorption (Duncan and Brown,

2010). A meta-analysis including 17 genome-wide association studies and 32,961 individuals of European and Asian ancestry shed unique insight on the genetic architecture and pathophysiological mechanisms underlying BMD variation and fracture susceptibility (Estrada et al., 2012). The study identified that the genes explaining the variance of bone mass are involved in biological pathways, such as mesenchymal stem-cell differentiation (osteoblast/osteocyte), WNT signalling (osteoblast genesis) and RANK-RANKL-OPG pathway (osteoclast differentiation and activation). In addition, the study has identified 56 loci associated with BMD, and 14 loci associated with osteoporotic fracture (Estrada et al., 2012).

Previous studies identified the collagen 1 alpha 1 (Grant et al., 1996), the vitamin D receptor 16 (Morrison et al., 1994), and low-density lipoprotein receptor-related protein 5 (Boyden et al., 2002) as determinants of BMD. Many loci are also associated with BMD in childhood, some with sex (Chesi et al., 2015) and puberty (Mitchell et al., 2015) specific effects. The rare variant EN1 and a common variant SOX6 were associated with high BMD in both children and adults (Zheng et al., 2015), suggesting that the risk of osteoporosis may be established during childhood (Mitchell et al., 2016). Recent studies that used a "genetic risk score" based on loci identified in adult bone studies have shown that genetic risk for low BMD in adulthood was associated with decreased bone accretion from age 9 to 17 years (Warrington et al., 2015).

2.6.2 Hormones

Among endocrine factors, sex hormones (Riggs et al., 2002, Seeman, 2004), parathyroid hormone (PTH) (Pettway et al., 2008) and the growth hormone and insulin-like growth factor 1 (IGF-1) (Niu and Rosen, 2005) exert a specific impact on bone development, particularly during the phase of pubertal maturation. Androgen receptors have been localized in growth plate in humans during pubertal maturation (Abu et al., 1997, Nilsson et al., 2003). However, there is no evidence that androgens stimulate longitudinal bone growth by a direct action on the skeleton. In contrast, it is well-documented that estrogens play an essential role in longitudinal bone growth (Riggs et al., 2002). Estrogens can accelerate bone growth at the beginning of puberty and play a key role in the closing of growth plates in both genders (Vanderschueren et al., 2005). From birth to the end of adolescence, the IGF-1 system is essential for the harmonious development of the skeleton (Yakar and Rosen, 2003). During puberty, the plasma level of IGF-1 transiently rises according to a pattern similar to the curve of the gain in bone mass and size (Bonjour et al., 1994). IGF-1 positively influences the growth of the skeletal pieces in both length and width.

Thyroid hormones play pivotal role in linear development of skeleton. They are necessary to achieve peak bone mass, but the excess of thyroid hormones in childhood can lead to premature accretion of growth plates and short stature (Williams et al., 1998). PTH is an important regulator of 1-alpha-hydroxylase renal enzyme that helps to maintain the 25-hydroxyvitamin D (25(OH)D) in the normal range to allow an optimal calcium absorption. Regarding the PTH relationship with bone development, it has been shown that an increase of PTH levels can lead to a stimulation of osteoclast activity and bone turnover, which would help to maintain calcium homeostasis at the expense of bone mineral mass. Low levels of vitamin D, can reduce the absorption of calcium and

phosphorus by reducing the levels of ionized calcium and stimulating the secretion and action of PTH (Stagi et al., 2015).

2.6.3 Smoking and alcohol

Two lifestyle factors negatively influencing PBM are smoking and alcohol consumption (Emaus et al., 2014, Kanis et al., 2005). Smoking has been associated with reduced bone mass and increased fracture risk in adult men and women (Law and Hackshaw, 1997, Ward and Klesges, 2001). Less is known about the effect of smoking at the time of PBM accrual, but studies have demonstrated a negative relationship between smoking and BMD in both male and female adolescents (Hernandez et al., 2003, Neville et al., 2002). In the GOOD cohort, cross-sectional data showed that smoking was associated with lower BMD of especially the femoral neck, and reduced cortical thickness of the radius and tibia at age 18-20 years after adjusting for age, height, weight, physical activity, milk consumption and drinking habit (Tamaki et al., 2011). Longitudinal data demonstrated that smoking was associated with impaired bone mass development in young adulthood (Valimaki et al., 1994). Alcohol intake is considered a risk factor for osteoporotic fractures, even though most studies indicate that it takes a quite large amount equalling three or more units per day (1 unit=285 ml of beer, 120ml of wine, 30ml of spirits) to consider it a contributor to fracture risk (Kanis et al., 2005, Berg et al., 2008). However, there are no studies regarding the specific effects of alcohol consumption and bone acquisition during growth.

2.6.4 Nutrients: calcium and vitamin D

To achieve the full genetic potential for PBM accretion, sufficient modifiable environmental factors are required including nutrients and optimal skeletal loading (Davies et al., 2005, Bonjour et al., 2009). Nutrition is an important modifiable factor that can affect bone health. Over 99 % of calcium and 85 % of phosphorus are found in the skeleton serving as a reservoir in the case of deficiency, and play an essential role in calcium homeostasis (Abrams and Stuff, 1994). Vitamin D optimizes calcium absorption and affects bone mineralization (Kitchin and Morgan, 2007). Traditionally, calcium and vitamin D nutrients have been the main focus of osteoporosis prevention strategies. The dietary requirements for calcium and vitamin D have been summarized by the Institute of Medicine in a useful article for clinicians, professionals and the public (Table 2.1) (Ross et al., 2011).

Table 2.1. Calcium and vitamin D dietary reference intakes by life stage. Adapted with permission from (Ross et al., 2011).

Life stage groups (age and gender)	Calcium			Vitamin D	
	Recommended Dietary Allowances (mg/d)	Upper limit (mg/d) ^a	Recommended Daily allowances (IU/day)	Serum 25OHD level (ng/ml) ^b	Upper limit (IU/d) ^a
0–6 months (M+F)	200°	1000	400 ^c	20	1000
6–12 months (M+F)	260°	1500	400°	20	1500
1–3 years (M+F)	700	2500	600	20	2500
4–8 years (M+F)	1000	2500	600	20	3000
9–13 years (M+F)	1300	3000	600	20	4000
14–18 years (M+F)	1300	3000	600	20	4000
19–30 years (M+F)	1000	2500	600	20	4000
31–50 years (M+F)	1000	2500	600	20	4000
51-70 years (M)	1000	2000	600	20	4000
51–70 years (F)	1200	2000	600	20	4000
71+ years (M+F)	1200	2000	800	20	4000
Pregnant or lactating (F)					
14–18 years (F)	1300	3000	600	20	4000
19–50 years (F)	1000	2500	600	20	4000

M: Males, F: Females. ^a UL indicates level above which there is risk of adverse events. The UL is not intended as a target intake (no consistent evidence of greater benefit at intake levels above the RDA). ^b Measures of serum 250HD levels corresponding to the RDA and covering the requirements of at least 97.5% of the population. ^c Reflects AI reference value rather than RDA. RDAs have not been established for infants.

Previous studies investigating the associations between calcium intake and bone outcomes in children and adolescents, reported positive associations, but not always (Wang et al., 2003, Rizzoli et al., 2010). Calcium intake has been shown to increase bone mass accrual in an randomised controlled trial (RCT) in both prepubertal girls and boys (Bonjour et al., 1997). A 6-year longitudinal assessment of calcium intake on bone outcomes in a large diverse study cohort of 1743 children found that dietary calcium had a positive effect, after adjustment for age, height velocity, and physical activity, on bone accrual at the lumbar spine in nonblack females with no effect on other ethnic groups or in males (Lappe et al., 2014). Also, calcium supplementation RCTs indicate positive effects on BMD (Helen L Lambert, 2008, Matkovic V, 2005). A recent review of nine RCTs found that small (1-5%) bone accrual gains were associated with calcium supplementation (Weaver et al., 2016). It should be noted that only four of the RCTs adjusted for body size (height and weight) (Dibba et al., 2000, Cameron et al., 2004, Prentice et al., 2005, Cheng S, 2005). This is noteworthy as longitudinal growth complicates interpretation of bone mass changes.

A systematic review on the effects of dairy products and dietary calcium on bone mass revealed that only 9 out of 27 RCTs reported modest benefits in children's bone mass ranging from 1 % to 6 %, suggesting that there is no conclusive evidence of the positive effects of dairy products and dietary calcium on bone mass in children and adolescents (Lanou et al., 2005). A different meta-analysis included 21 RCTs (study duration 1-2 years) with 3,821 participants (83 % girls) aged between 4 and 17 years. The findings revealed that increasing the consumption of milk products and calcium intake non-

significantly improved total body BMC by 2 g. In addition, children with calcium intakes below 800 mg/day had a greater increase in BMC (49 g), and vitamin D added to the milk supplement could potentially improve by 35 g the BMC in the lumbar spine. Calcium/milk supplement groups alone showed a non-significant improvement of 2 g in total body BMC compared to control groups (Huncharek et al., 2008). It should be noted that dairy products, such as milk and yogurt, include other nutrients, such as protein, that might enhance the effect of calcium on bone outcomes. It has been found that the effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake (Vatanparast et al., 2007). A different meta-analysis of 19 studies, concluded that calcium supplementation in healthy children had no effect on BMD at the hip or spine, but a small positive effect was seen on BMD of the upper limb (Winzenberg et al., 2006). Few of the studies included in the review were performed in children with low baseline calcium intake.

Vitamin D is essential to bone mineralization because of its effects on intestinal calcium absorption and on bone mineral accrual (Sopher et al., 2015, Rizzoli et al., 2010). Vitamin D levels in the body are influenced by genetic factors, dietary intake and mainly by endogenous synthesis in the skin following sunlight exposure, and more specifically ultraviolet B radiation (Wang et al., 2010, Spiro and Buttriss, 2014). Clinical vitamin D (in)sufficiency in young people includes the following categories: severe deficiency of vitamin D when (25(OH)D levels are below 27.5 nmol/L, deficiency between 27.5 nmol/L and 50 nmol/L, insufficiency between 50 nmo/L and 75 nmol/L and sufficiency above 75 nmol/L (Gonzalez-Gross et al., 2012b). It should be noted that the definition of sufficient 25(OH)D levels in adolescents is controversial between countries and

continents and therefore predefined cut-offs are used to define optimal levels ≥75 mmol/L and insufficiency <75 mmol/L (Valtuena et al., 2012). Depending on the magnitude and the skeletal site, deficiency in vitamin D may lead to rickets and increase fragility by reducing the absorption of calcium in the skeleton (Pettifor, 2005).

Insufficient vitamin D status has been observed in European adolescents from 9 different countries, aged 12.5 to 17.5 years participating in the Healthy Lifestyle in the Europe by Nutrition in Adolescence (HELENA) study. After adjusting by age, sex and weight status, it was revealed that 15 % of adolescents were severely deficient, 27 % were deficient and 39 % insufficient for their 25(OH)D levels (Gonzalez-Gross et al., 2012b). The daily dose of vitamin D in children and adolescents is a matter of debate indicating that optimal vitamin D levels should be maintained throughout the year. On this note, in the northern hemisphere at latitudes greater than 40°N (north of Madrid), the sunlight is not strong enough to trigger vitamin D synthesis in the skin from October to March (Spiro and Buttriss, 2014). In a review and meta-analysis of 6 studies, the authors concluded that it is unlikely that vitamin D supplements are beneficial for bone health in children and adolescents with normal vitamin D levels, but supplementation in deficient children and adolescents could result in clinically important improvements in bone density (Winzenberg et al., 2011). In addition, subgroup analyses showed that the vitamin D effect was more pronounced in prepubertal or early pubertal vs post-pubertal girls, as well as in those with a lower compared with higher baseline 25(OH)D. Therefore, there may be a "critical window" in pre or early pubertal children with deficient vitamin D levels during which the skeleton is most receptive to the effects of vitamin D.

2.6.5 Interactions between nutrition and exercise

Calcium, vitamin D and exercise have been shown to independently influence bone mineral accrual in young people. However, there is evidence suggesting that exercise interacts with calcium intake (Ward et al., 2007, Lappe et al., 2014, Specker B, 2007) and vitamin D levels (Valtuena et al., 2012, Bonjour et al., 2013, Mouratidou et al., 2013) to improve bone status and that the combined effects of exercise and nutrition are greater than the isolated effects to improve bone development (Courteix et al., 2005, Vlachopoulos et al., 2016).

The effects of calcium and exercise on bone health were investigated in 113 pre-pubertal females for 12 months (Figure 2.13). Girls were separated into four groups: two exercise groups (7 ± 4 hours of sport participation per week) that received 800 mg of calcium phosphate (exercise/calcium) or placebo (exercise/placebo) and two sedentary groups (1± 1 hours of sport participation per week) that received 800 mg of calcium phosphate (sedentary/calcium) or placebo (sedentary/placebo) (Courteix et al., 2005). The findings showed that in the exercise/calcium group there was a 6.3 % greater BMD at the total body, 11 % at the lumbar spine, 8.2 % at femoral neck and 9.3 % at Ward's triangle compared to other groups. It should be mentioned that exercise might increase the calcium intake requirements by 422 mg due to calcium excretion through sweat following exercise at intensities. This was observed in adults but there is no evidence in young people (Bullen et al., 1999, Klesges et al., 1996).

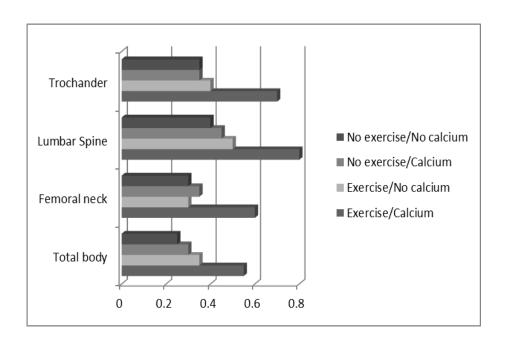


Figure 2.13. Bone mineral density gains (Δ) in four groups at total body, femoral neck, lumbar spine and trochanter sites after calcium supplementation and exercise intervention period of 12 months. Adapted with permission from (Courteix et al., 2005).

The positive combined associations between calcium, vitamin D and bone status have been shown in adolescents at different sites of the skeleton and at different maturation stages (Neville et al., 2002). The relationship between physical activity and 25(OH)D concentration was investigated in 100 adolescents aged 12.5-17.5 years after controlling for calcium and vitamin D intakes, maturation, age, sex, season, physical activity and fitness level. Findings revealed that the interaction between 25(OH)D levels and physical activity was significantly related to BMC at TB and legs. Furthermore, it was found that the active group of adolescents had increased total BMC when the vitamin D levels were sufficient (>75 nmol/L) (Valtuena et al., 2012). The beneficial effects of physical activity have been also demonstrated in participants with vitamin D deficiency. It has been reported that adjusted BMD for age, BMI, parathyroid hormone and bone turnover markers was positively

related to physical activity at total body, femoral neck and lumbar spine sites in 166 adolescent girls with vitamin D deficiency (Constantini et al., 2010).

Previous evidence indicates that the combined effects of calcium and vitamin D may not have a beneficial effect on BMC and BMD in adolescents. A study of 101 adolescents aged 12.5-17.5 years examined the relationship between calcium, vitamin D, milk intake, 25(OH)D and bone status after adjusting the BMC and BMD for a number of cofounding variables such as height, family affluence scale, maturation status, season and physical activity (Mouratidou et al., 2013). There was no association between calcium and vitamin D intake and BMC and BMD at any skeletal site. However, a positive relationship was found between milk intake and BMD and BMC in boys and BMD was associated with calcium intakes. In girls there was a significant positive relationship of 25(OH)D and BMC and BMD at total body, subtotal, left and right arm. These results are in agreement with the findings of a meta-analysis indicating that increases in bone mass of children following calcium, milk and vitamin D supplementation, were not positive in some studies due to genetic or environmental factors, such as physical activity (Huncharek et al., 2008).

The contribution of physical fitness to the relationship of bone status and dietary nutrients has been verified by a study that investigated the association of physical fitness and body composition with 25(OH)D levels in 1006 adolescents (Valtuena et al., 2013). Physical fitness was positively related to 25(OH)D levels in boys and upper limp muscular strength was associated with 25(OH)D levels in girls. In addition boys with higher fitness levels and lower BMI had

significantly higher 25(OH)D levels compared to those with lower fitness levels (Valtuena et al., 2013). A study in 755 males aged 18.7 years highlighted a significant interaction between milk consumption and exercise duration with DXA and pQCT bone outcomes (Ruffing et al., 2006). Specifically, there was a significant interaction between milk intake and prior exercise in relation to cortical thickness. In addition, only males with high prior exercise duration showed a skeletal benefit of the exercise if milk intake was greater than one glass per day (Figure 2.14).

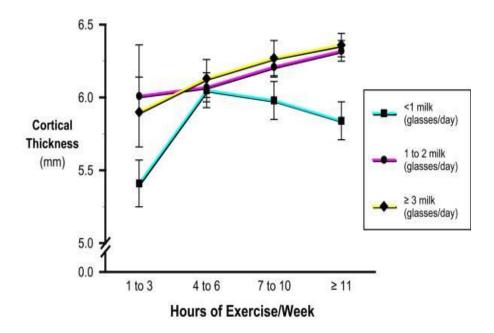


Figure 2.14. Interaction between milk and exercise during the prior year on cortical thickness of the tibia in 755 males aged 18.7 years. With permission from (Ruffing et al., 2006).

2.7 Bone mechanoadaptation ("May the force be with you")

Physical activity effects on bone can be mechanistically explained by mechanoadaptation (Tobias et al., 2014). According to Wolf's law, when the loading on a particular bone increases, the bone remodels itself over time to become stronger to resist that loading (Wolff, 1986). This principle provided a

basic understanding of skeletal modification and guided the future research focusing on the factors that influence bone physiology. The mechanostat theory, proposed by Frost, describes the relationship between loading of bones and subsequent adaptations of bone mass and geometry (Frost, 1987). The theory is based on the combination of ideas that mechanical forces and non-mechanical forces influence bone integrity. The mechanostat suggests that a group of mechanisms monitor how the bone is stressed, integrate this information, and stimulate bone cells to either increase or decrease bone strength in response to specific levels of mechanical usage (Frost, 1987, Frost, 2003).

Turner suggest that short-duration and high intensity loading movements of a sufficient magnitude stimulate bone cell responsiveness and induce bone adaptations by increasing bone strength. Thus, the muscle forces acting on bone must be rapid, powerful, and changing in magnitude and direction in order to stimulate bone adaptation (Turner, 1998). The relationship between muscle and bone led to the idea of a functional "bone-muscle unit," suggesting that muscle strength (either increased or decreased) should affect bone strength in the same direction (Schoenau and Frost, 2002). Furthermore, it was suggested that activities involving maximal-force muscle contractions and/or activities involving rapid accelerations of the body place substantial loads on bones and stimulate an increase in bone strength (Schoenau and Frost, 2002).

Table 2.2 Four regions of elastic bone deformation which result in different consequences on the control loop.

States	Strain Thresholds	Modelling Remodelling	Result
Disuse	Strain < circa 800 µStrain	Remodelling Bone adaptation and bone repair	Bone mass and bone strength is reduced.
Adapted State	Circa1500 µStrain < Strain > circa 800 µStrain	Remodelling Bone repair	Bone mass and bone strength stays constant
Overload	Strain > circa 1500 µStrain	Modelling Bone growth	Bone mass and bone strength is increased
Fracture	Strain > circa 15000 µStrain	Maximum elastically deformation exceeded	Bone fracture

According to this a typical bone, the tibia has a security margin of about 5 to 7 between typical load (2000 to 3000 μ Strain) and fracture load (about 15000 μ Strain).

Harold Frost combined the bone and muscle physiology with the mechanostat theory to develop the Utah paradigm of skeletal physiology (Frost, 2000). The Utah paradigm is based on the mechanostat theory and provides an explanation for the relationship between strong muscles and strong bones. The latter was explained by describing how the muscle (mechanical) forces transmitted to bone can play a dominant role in controlling the biological mechanisms that influence bone mass and geometry (Frost and Schonau, 2000). The Utah Paradigm has two basic propositions: Proposition 1 states that "Bones have the main purpose of providing only enough strength to keep voluntary physical loads, whether subnormal, normal, or supranormal, from causing spontaneous fractures" (Frost, 2001). This proposition includes the mechanostat theory, describes how bones adapt to previous and current mechanical usage, and indicates that the mechanical forces caused by muscle contractions primarily are responsible for meeting the thresholds required for stimulating bone adaptation (Frost, 2001, Frost, 2000). Proposition 2 states that

"All osteoporosis and osteopenias accompany reduced whole-bone strength" (Frost, 2000). Proposition 2 focuses into the relationship between tissue-level biological mechanisms, such as osteoclast and osteoblast activity, and mechanical factors, such as the loads placed on the bones, and how these mechanisms work together in determining whole bone strength in response to mechanical use or disuse (Frost, 2001, Frost, 2000). The Utah paradigm and the relationship between these mechanisms state that the biological mechanisms determining bone strength need bone cells and non-mechanical factors in order to influence bone strength (Figure 2.15). In addition, it states that mechanical factors guide the biological mechanisms in influencing bone strength, that neural physiology and anatomy are responsible for the control of the biological mechanisms, and that the non-mechanical factors involved can enhance or decrease but never replace the impact of mechanical forces on determining bone strength (Jee, 2000). Despite the bone loss due to disuse according to the Utah paradigm, it will be of interest to see whether inadequate loading or non-weight bearing loading, such as swimming and cycling, will result in bone deterioration.

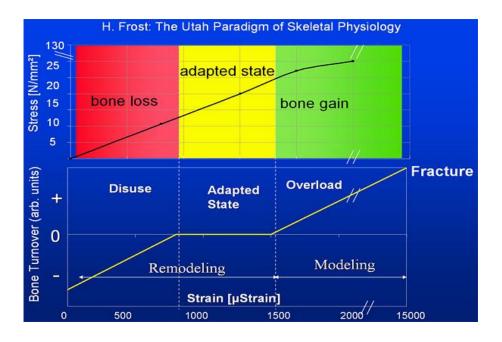


Figure 2.15. Mechanostat: Modeling and Remodeling Thresholds. Available for free use (Frost, 2000).

Taking into account the mechanostat theory and the Utah paradigm, in real life the bone adapts to the forces experienced by increasing mass and remodelling in a way to increase strength relative to the loading condition. The manner in which this occurs is twofold, affecting either the endosteal or periosteal cortical bone surfaces, or both surfaces concurrently. The greatest immediate contribution to fracture resistance is gained when new bone is added to the periosteal surface (Seeman and Delmas, 2006). Because bone is lost from the endosteal surface during adulthood, exercise-induced increases to the periosteum are likely to help maintaining the bone's resistance to fracture with age. Periosteal apposition is the predominant effect in response to increased physical activity during growth, particularly in early puberty. Results from animal studies suggest that early exercise-induced alterations to bone structure persist through senescence, significantly reducing fracture risk in older age (Warden et al., 2007). In addition to bone mass adaptations due to physical activity during childhood and adolescence it is important to measure the structural and strength adaptations of the skeleton when possible, as animal studies indicated that external loading induced only small changes in bone quantity (<10 %) in comparison to structural and strength adaptations (>60 %) (Warden and Fuchs, 2009). Figure 2.16 presents the theoretical representation of the additive effect of exercise-induced periosteal expansion on growth-related changes to the skeleton and the potential protective effect this may have on risk for fracture in later life (Warden and Fuchs, 2009).

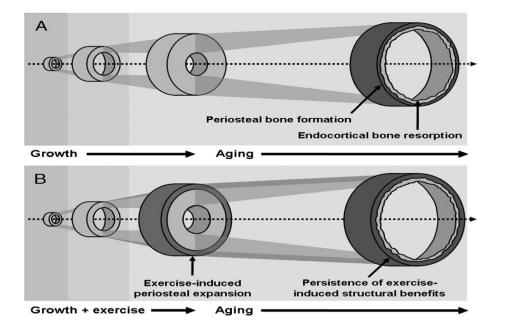


Figure 2.16. Bone structural changes attributed to growth and aging (A) contrasted with the additive effects of exercise during growth and the subsequent benefits to life-long skeletal health if effects persist into older adulthood (B). With permission from (Warden and Fuchs, 2009).

2.8 Physical activity and bone health

Physical activity is defined as any bodily movement produced by skeletal muscles that require energy expenditure above resting level (World Health Organization, 2010). Current recommendations for health and wellbeing indicate that children and adolescents (5-18 years) should engage in 60 or more minutes per day of moderate to vigorous-intensity physical activity (MVPA), including: 1) vigorous-intensity physical activity (VPA) at least 3 days per week; 2) muscle-strengthening physical activity at least 3 days per week; and 3) bone-strengthening physical activity at least 3 days per week (World Health Organization, 2010, U.S. Department of Health, 2008). Recent findings in UK suggest that less than 1 % of girls and only 7 % of boys are meeting the current physical activity recommendations for health (Department of Health, 2013) and

physical activity levels are known to decline by approximately 65% during adolescence (Dumith et al., 2011). In addition, a meta-analysis on the effectiveness of interventions to increase physical activity in children including 30 studies and 6,153 boys and girls with a median intervention duration of 26 weeks, found minimal increases in MVPA (approximately 4 minutes per day) (Metcalf et al., 2012). The later highlights the need for alternative physical activity interventions to improve bone health in children and adolescents.

The role of physical activity to improve bone and especially of weight-bearing nature is considered an important determinant of the accrual and maintenance of bone mass due to the ability of the skeleton to adapt to the loads under which it is placed. The engagement in physical activity is, therefore, particularly important for the skeleton during childhood and adolescence due to the ability of the skeleton to adapt to mechanical loading after exercise (Bielemann et al., 2013). A review of 19 cohort studies from childhood to adulthood highlighted the importance of physical activity during growth and its link with bone mass later in life. The positive association was higher in males than in females at the femoral neck and lumbar spine sites (Bielemann et al., 2013). The results of the six-year Saskatchewan Paediatric Bone Mineral Accrual study showed that girls and boys classified in the highest physical activity quartile, measured by questionnaires, acquired 17 % and 9 % higher adjusted total body BMC compared with their inactive peers one year after peak BMC velocity. At the lumbar spine site, active children displayed 18 % higher BMC (adjusted for weight and height) one year after peak BMC velocity. In addition, there was a difference of 7 and 11 % in active and inactive boys and girls respectively at the femoral neck (Bailey et al., 1999). In a continuation of this study, the authors

examined whether the positive effects of physical activity on BMC acquired in adolescence preserved into the second and third decade of life (Figure 2.17). Furthermore, active boys had 8-10 % greater adjusted BMC for weight, height, calcium intake, physical activity during adulthood and maturation status at total body, femoral neck and total hip in adulthood. In addition, active girls had 9-10 % higher adjusted BMC at total hip and femoral neck in young adulthood (Baxter-Jones et al., 2008). These results highlight the importance of physical activity not only for augmenting bone health status during childhood and adolescence but also in later adult life.

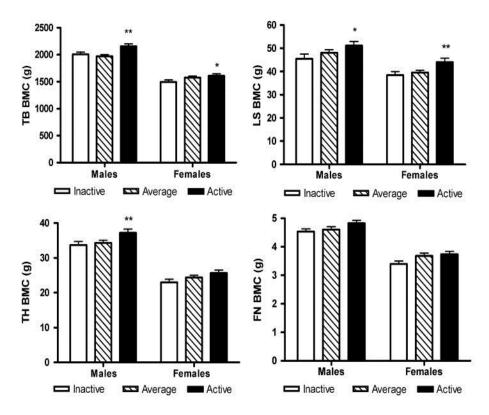


Figure 2.17. Adjusted total body, lumbar spine, total hip and femoral neck BMC by inactive, average and active adolescent activity groups at 1 year after peak height velocity. Adjusted means (SE) (height and weight covariates); **significantly greater (p<0.05) than inactive and average groups, *significantly greater (p<0.05) than inactive group. With permission from (Baxter-Jones et al., 2008).

2.9 Intensity of physical activity and bone health

The optimal thresholds of physical activity that induce positive changes on bone outcomes depend on the method used to assess physical activity, the cut-off points used to classify physical activity intensities and the time sampling interval (epoch) used, and the control of potential covariates. Findings from the crosssectional HELENA study in 380 adolescents found that the recommended amount of 60 minutes of MVPA per day was insufficient to classify adolescents in a group with increased bone mass. In addition, less than 41 and 45 minutes of MVPA per day are associated with reduced bone mass at the trochanter and femoral neck. At least 78 minutes of MVPA were needed to find an association with increased BMD at the femoral neck. Regarding vigorous physical activity (VPA), more than 28 minutes/day for the hip and intertrochanter and more than 32 minutes per day for the femoral neck were associated with increased BMD (Gracia-Marco et al., 2011a). In a different cross-sectional study in 4,457 11year-old boys and girls from the Avon Longitudinal Study of Parents and Children, it has been shown that MVPA were positively associated with lower limb BMC after controlling for height, lean and fat mass (Figure 2.18). More specifically, lower limb BMC was 4.9 % higher in children with MVPA levels in the top versus bottom quartile (Tobias et al., 2007).

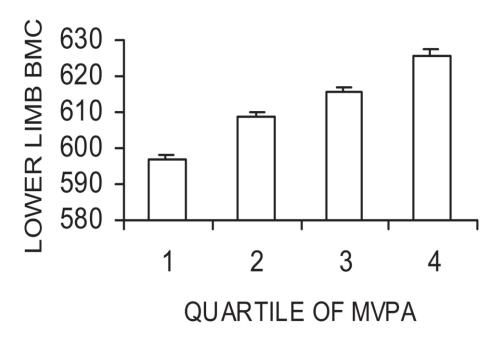


Figure 2.18. Effect of total MVPA on lower limb BMC. Figure shows mean BMC (g) \pm SE according to quartile of total MVPA. Results were adjusted for age of DXA scan, sex, socioeconomic factors, and height + lean mass + fat mass (n=4457). p < 0.001 (F test for difference between quartiles) with permission form (Tobias et al., 2007).

The sustained effect of childhood physical activity into adolescence and even young adulthood has been demonstrated in the Iowa Bone Development Study that observed 333 children at ages 5, 8, and 11 years by measuring physical activity using accelerometers and bone outcomes using DXA (Janz et al., 2010). Children were grouped by their activity level at age 5 years to investigate the effect of the age-5 physical activity on BMC at ages 8 and 11 years. Children in the most active quartile at age 5 years had adjusted BMC values at age 8 years ranging between 6 % and 14 % greater than those in the lowest activity quartile at age 5 years. By age 11 years, those who were most active at age 5 years still had 4-7 % greater BMC after adjusting for age, height, weight, and current physical activity (Figure 2.19). Although the magnitude of the benefits reduced

over time, the benefits of being highly active, even at age 5 years, positively affected bone mass into early adolescence.

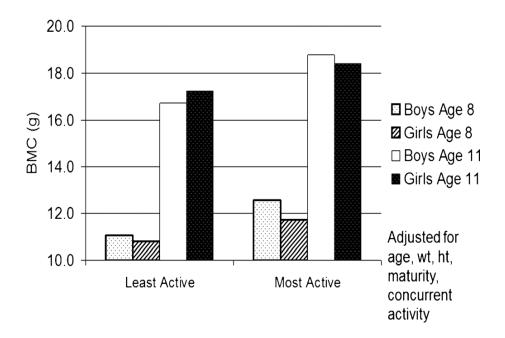


Figure 2.19. Mixed model means of Iowa Bone Development Study cohort contrasting the hip bone mineral content (BMC) (g) at ages 8 and 11 years in the most active and least physically active quartiles of boys and girls at age 5 years with adjustment for age, weight (wt), height (ht), maturity, and current physical activity (at age 8 and 11 years). N = 333 children. With permission from (Janz et al., 2010).

Despite the existing evidence on the effects of physical activity on bone development, not all types of physical activity have equivalent effects on bone development. Physical activity is a categorical term including light leisure activities but also others more vigorous such as organized sport or intentional, organised exercise. The osteogenic potential of a particular physical activity is conditional upon the magnitude of the applied load, the rate at which the load is applied, the duration of the loading bout, and the novel nature of the load (Bauer, 2003). Physical activities shown to have the greatest osteogenic effects on the growing skeleton are those characterized by a considerable loading

magnitude applied at a rapid rate. Greater forces, delivered quickly through activities, such as jumping, seem to convey the greatest benefits to bone mineralization and structure in children and adolescents. These activities typically are weight-bearing activities because body weight increases the magnitude of loading. Therefore, whilst participation in MVPA is considered beneficial for bone development in youth, this may not be the case for all forms of exercise as bone adaptations depend on the loading applied on the skeleton. Accordingly, it is important to understand how bone health outcomes are altered in response to different types of exercise or sports participation in childhood and adolescence.

2.10 Sport participation and bone health

Sports participation is an organised activity, which aims to maintain and improve physical ability and skills. Participation in sport is part of most children's and adolescent's lives and has numerous health benefits and is crucial for healthy skeletal development, however not all sports have a positive influence on bone mass. Most of the research conducted to date focusses on females, with limited evidence comparing bone mass in male athletes. Bone development is dependent on the mechanical load produced due to the specific sport practised and the forces applied on the skeleton that trigger bone modelling and remodelling (Wolff et al., 1999). Prolonged participation in weight bearing activities, may elicit greater improvements in BMC and BMD compared to non-weight bearing activities, which may decrease or not affect bone development in children and adolescents (Hind and Burrows, 2007). According to their potential to augment bone mass and geometry during growth, sports can be described as

osteogenic (weight-bearing and intense exercise) or non-osteogenic (non weight-bearing and light exercise) (Courteix et al., 1998, Bass et al., 2002, Duncan et al., 2002a, Faulkner et al., 2003, Ward et al., 2005, Tournis et al., 2010, Dowthwaite et al., 2012, Ferry et al., 2013, Maimoun et al., 2013a). However, previous evidence exists only female athletes as it was summarised in a recent systematic review on the influence of physical activity on bone strength in children and adolescents (Tan et al., 2014).

Football is the most popular sport worldwide and considered as an osteogenic sport and is related to augmented bone mass both in childhood and adolescence (Ara et al., 2006, Vicente-Rodriguez et al., 2004a, Krustrup et al., 2010, Calbet et al., 2001). A previous study showed a 13-24 % higher BMC in 22.3 year old footballers, who had been playing football for the last 12 years, than non-active controls (Calbet et al., 2001). A different study compared BMC in 11.7 year old male footballers, who trained for 2 to 5 hour per week, with controls at baseline and after 1 year of training. The findings showed that there were no differences between the groups at baseline, but after 1 year footballers gained significantly higher BMC at most skeletal sites compared to controls. Also, it was noted that the bone gains were greater at total body and weightbearing (the lumbar spine, total hip, and supporting leg) than non-weightbearing bones (dominant arm and non-dominant arm) in boys who became pubescent during the 1 year studied period than in boys who remained prepubescent (Zouch et al., 2014). Evidence in prepubescent male footballers shows that football participation of at least 3 hours per week exhibit greater BMC at the lumbar spine femoral neck and trochanter skeletal sites compared to non-athletic controls. In addition, the same study indicated that footballers

had significantly better performance in physical fitness tests, such as 20 m shuttle run test (20mSRT) and vertical jump (Vicente-Rodriguez et al., 2003). The authors also performed a multiple regression analysis showing that height, body composition and fitness tests were significant predictors of total body BMC (r=0.92) and BMD (r=0.69), highlighting their potential to detect bone status in adolescent athletes and children (Vicente-Rodriguez et al., 2003). A different study followed male footballers for 3 years and a comparison of bone status with controls showed that footballers gained twice as much femoral neck and intertrochanteric BMC than the control group (Figure 2.20) and their mean hip BMD by a third more than the control group (Vicente-Rodriguez et al., 2004a).

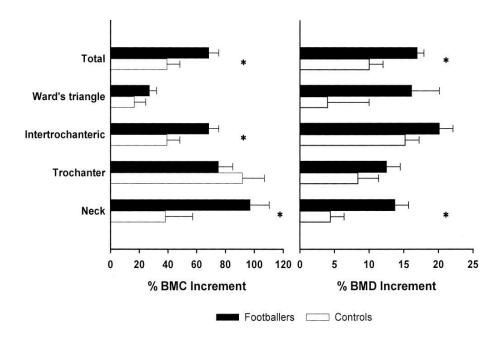


Figure 2.20. Increments in BMC and BMD at different skeletal sites after 3 years in footballers and controls. With permission from (Vicente-Rodriguez et al., 2004a).

In contrast, sports such as swimming and cycling could be considered "non-osteogenic" although the supporting evidence is unclear (Andreoli et al., 2012, Ferry et al., 2013, Greenway et al., 2012, Ferry et al., 2011, Dias Quiterio et al., 2011, Tenforde and Fredericson, 2011). A meta-analysis of 64 studies

summarised the effects of swimming on bone mass and reported that swimmers had lower BMD that athletes of high impact sports and similar values than controls. However, swimmers had higher bone turnover than controls and this may result in better bone structure (Gomez-Bruton et al., 2013). In addition, the pure swimming training was compared with swimming training with participation in other weight-bearing and with controls in adolescents using DXA and QUS bone outcomes. The findings showed that male pure swimmers had lower BMD and BMC at several skeletal sites than their control peers. However, those engaged in other sports other than swimming, had lower BMC only at lumbar spine. Also, male pure swimmers had lower BMD and BMC than swimmers who were involved in other sports. Female pure swimmers had higher arm BMD and lower leg BMC than female controls, while female swimmers who were involved in other sports had only lower leg BMC than female controls. No differences observed in QUS parameters between swimmers and controls and all the results were controlled for height, calcium intake, lean mass and pubertal status (Gomez-Bruton et al., 2015).

Evidence regarding the effects of cycling on bone health is still controversial. A recent systematic review on the effects of road cycling on bone health that included 31 studies concluded that cycling does not appear to confer any significant osteogenic benefit (Olmedillas et al., 2012). A study comparing bone mass in adolescent cyclists who trained for 10 hours a week for over two years with sedentary controls found no differences at total body BMC but leg BMC was lower in cyclists compared to controls, but the differences disappeared after controlling for body weight (Rico et al., 1993a). Some studies also report no difference on bone outcomes between cyclists and controls (Duncan et al.,

2002b, Warner et al., 2002, Barry and Kohrt, 2008, Rector et al., 2008). However, a study in adolescent cyclists (< 17 years of age) compared bone status with age matched controls who were involved in recreational sports activities. The results showed that cyclists had 10 % lower BMC in legs, adjusted for total lean mass and height, despite the 8 % higher BMC in the hip area compared with the control group (Olmedillas et al., 2011). In addition, the BMC of cyclists over 17 years was 26.5 %, 15.8 % and 14.4 % lower at the pelvis, femoral neck and legs skeletal sites while the BMD was 8.9 % to 24.5 % lower at the whole body, pelvis, total hip, trochanter, intertrochanter, femoral neck and legs (Olmedillas et al., 2011). These findings in swimmers and cyclists could indicate that participation in these sports might be a barrier for obtaining a high PBM which may compromise future bone health (Tenforde and Fredericson, 2011, Scofield and Hecht, 2012, Andreoli et al., 2012).

The importance of sport participation for bone development and the effects of different loading sports on BMD has been also highlighted by longitudinal studies. A 27-year follow up study in 154 Belgian boys aged 13 years at baseline showed long-term positive effects of osteogenic sport participation on BMD. Specifically engagement in high impact sports (ground reaction forces> 4 times body weight) during adolescence and adulthood resulted in significantly higher BMD (1.12 g/cm²) at lumbar spine compared to participants that stopped high impact sports in adulthood (BMD= 1.01 g/cm²) or did not engage in high impact sports during adolescence and in adulthood (BMD= 0.99 g/cm²) (Van Langendonck et al., 2003). A 14-year follow up of elite female gymnast has shown that premenarcheal gymnasts had significantly greater size-adjusted total body, lumbar spine, and femoral neck BMC (15 %, 17 %, and 12 %,

respectively) than non-gymnasts. Ten years after retirement, gymnasts had maintained similar size-adjusted total body, lumbar spine, and femoral neck BMC differences (p<0.05) (13 %, 19 %, and 13 %, respectively) when compared with non-gymnasts (Erlandson et al., 2012). A different 12-year longitudinal study investigating the effects of different loading sports (Figure 2.21), such as badminton and ice hockey, on BMD has shown that participation in osteogenic sports during adolescence can maximize peak BMD in males compared to non-osteogenic sports and controls (Tervo et al., 2010).

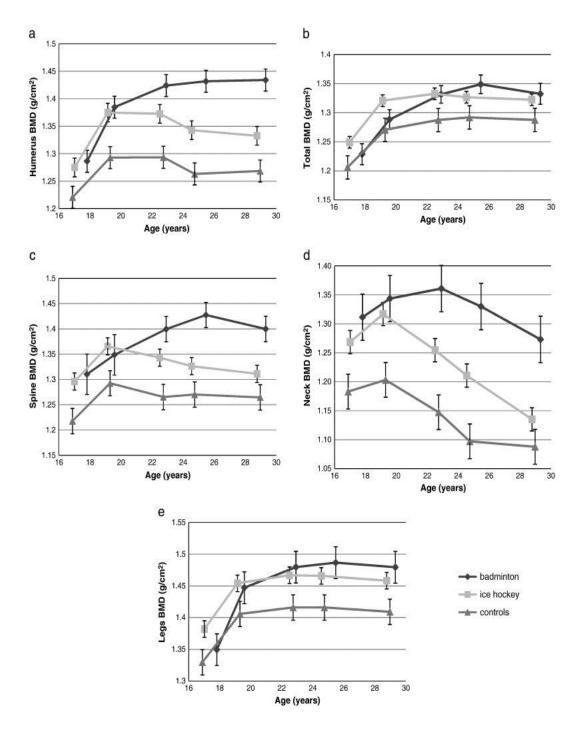


Figure 2.21. Changes in BMD during the 12-year study period. The mean BMD values presented are not adjusted for (a) humerous, (b) total body, (c) lumbar spine, (d) femoral neck and (e) legs skeletal sites. With permission from (Tervo et al., 2010).

A comparison between adolescent female swimmers and footballers found that swimmers had significantly lower BMD at all sites compared to footballers, and swimmers had lower values compared with the controls (Ferry et al., 2011). In

spite of the controversial surrounding sports such as swimming and cycling in terms of bone health in females, there is no evidence comparing the effects of these sports on bone outcomes of males athletes involved in these popular sports. In addition to BMD and BMC gains, exercise can also influence the structural bone outcomes (Hind et al., 2012b). A combination of bone quantity, quality and microarchitecture outcomes can provide important information regarding bone adaptations during growth. In addition, bone turnover markers can provide further cellular bone responses (Jurimae et al., 2010). A crosssectional study comparing bone geometry estimates using HSA in young (22 years) female football players and sedentary controls found that total hip BMD, femoral neck BMD and HSA parameters (7-17%) were significantly higher in football players compared to controls after adjusting for body weight (El Hage. 2013). Another cross-sectional study in female artistic gymnasts, rhythmic gymnasts, swimmers and controls showed that artistic gymnasts had higher BMD than swimmers and control at all bone sites and higher values than rhythmic gymnasts in the lumbar spine and radius (Maimoun et al., 2013b). The previous study assessed HSA parameters and reported that CSA, cortical thickness and the buckling ratio were significantly higher in both gymnast groups compared with swimmers and controls. Also, reduced bone remodelling was observed in rhythmic gymnasts compared with artistic gymnasts only when groups were subdivided according to menarcheal status (Maimoun et al., 2013b). In addition, after 1 year of sport specific training, female rhythmic gymnasts gained significantly higher BMD compared to swimmers and controls at the femoral neck region (Maimoun et al., 2013a). In a study of adolescent female athletes (Figure 2.22), it was reported a greater increase in subperiosteal width in footballers compared to swimmers, and the endocortical diameter significantly reduced in swimmers after 8 mouths of sports training (Ferry et al., 2013). The differences observed in bone mass between osteogenic and non-osteogenic sports are likely to be explained by the mechanical loading of the skeleton according to the impact of produced by the movement specific patterns (Greene and Naughton, 2006a). To date, studies evaluating bone-related outcomes in female athletic groups have mainly focused in BMD and BMC outcomes provided by DXA (Ubago-Guisado et al., 2015a, Falk et al., 2010, Maimoun et al., 2013b, Michalopoulou et al., 2013). To the author's knowledge, there are no studies comparing bone status of male adolescents across different sports using a combination of DXA, HSA, TBS, QUS and bone markers alongside the adjustment of outcomes for key confounding variables, such as age, maturation, height, calcium intake, regions specific lean mass and physical activity.

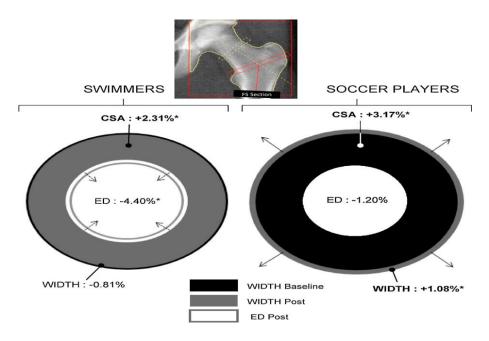


Figure 2.22. Schematic representation of the percentage geometric changes at the femoral shaft after an 8-month training season: subperiosteal width had significantly increased in football players, not in swimmers whereas endocortical diameter (ED) had contracted in swimmers, not in football players. With permission from (Ferry et al., 2013).

A recent systematic review and meta-analysis on the effect of swimming during childhood and adolescence on BMD concluded that swimming does not appear to be an effective sport for improving BMD, and swimmers might be in need of additional osteogenic exercises for increasing BMD values (Gomez-Bruton et al., 2016b). In addition, a different study indicated that swimmers not engaged in other sports had lower BMD at many skeleton sites than swimmers engaged in other sports (Gomez-Bruton et al., 2014). The latter indicates that if athletes of non-osteogenic sports participate in other weight-bearing exercises, they may have important benefits on their bone health. Therefore it would be of interest to test whether adding some weight-bearing exercises such as jumps, can enhance bone mass due to the high ground reaction forces applied in the growing skeleton (McKay et al., 2005a).

2.11 Jumping interventions and bone heath

Physical activities characterized by a high intensity and considerable loading magnitude shown to have a great osteogenic effect on the growing skeleton. Ground reaction forces, delivered through activities such as jumping, seem to induce the greatest benefits to bone mineralization and structure in children and adolescents (Figure 2.23). These activities typically are weight-bearing activities because body weight increases the magnitude of loading. Evidence supports the activities with the most osteogenic potential have ground reaction forces greater than 3.5 times body weight (per leg), with peak force occurring in less than 0.1 s. (McKay et al., 2005a, Hind and Burrows, 2007, Gunter et al., 2012). The peak force during walking (a low-impact activity) is lower in comparison to

running and even lower compared to jumping (a high-impact activity). In addition, it takes more the time to reach peak force during walking or running, but the time is much lower during jumping. This is important because the combination of force magnitude and the rate at which the force is applied, determines the impact of the activity.

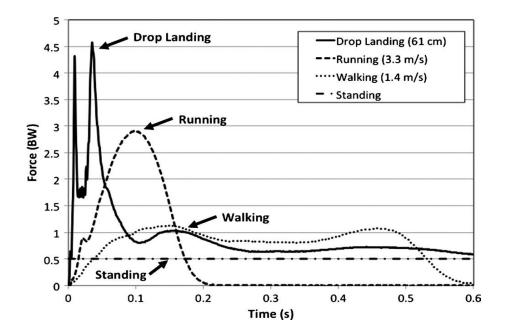


Figure 2.23. Ground reaction forces (measured in portions of bodyweight) and loading rates experienced by a prepubertal female child during quiet standing, walking, running, and a drop landing from a 61-cm height. All recorded values are from a single leg. Values for the drop jump were derived from a two-foot drop landing on dual force plates. Values for standing reflect 0.5 body weight per leg while standing on two feet, one foot on each plate. With permission from (Gunter et al., 2012).

The majority of jumping intervention studies conducted in school-based environment but no evidence exists for athletics groups. A systematic review of RCTs showed that weight-bearing activities (i.e. games, dance resistance training and jumping exercise) eliciting ground reaction forces of 2-9 times body weight can effectively improve bone mass by 0.9-4.9% in pre-pubertal children,

1.1-5.5 % in early pubertal and 0.3-1.9 % in pubertal children and adolescents (Hind and Burrows, 2007). These findings are in agreement with a recent meta-analysis of 27 studies addressing the impact of weight bearing activities (i.e. body weight, resistance training machines or both) on BMC and BMD of 2,985 boys and girls aged 10.3 ± 2.7 years. The results revealed small effect sizes of 0.17 (0.05-0.29) for BMC and 0.26 (0.02-0.49) for BMD. Research thus far indicates that at least 7 months of impact exercise are needed to induce a measurable change in bone mass in children (Gunter et al., 2008a, Gunter et al., 2008b, Hind and Burrows, 2007).

To date, the most of the exercise intervention studies used jumping intervention programmes due to the increased ground reaction forces applied on the skeleton and the easier implementation. Fuchs et al. found that 7 months of jumping intervention (drop landings from a 2-ft height) three times per week during the school days induced significantly greater BMC at the femoral neck (4.5 %) and lumbar spine (3.1 %) in children compared with controls after controlling for initial age and bone values, and changes in height and weight. Children started by performing 40-50 jumps per session, and increased to 100 jumps, and ground reaction forces were measured between 3.5 and 4.5 times body weight per leg (Fuchs et al., 2001). The researchers followed the participants over 14 months and found that the intervention group maintained 4 % greater femoral neck BMC and bone are compared to the control group (Fuchs and Snow, 2002). In a different study, MacKelvie et al conducted a 7-month intervention that included 10 minutes of varied jumping activities three times per week. They found that early pubertal girls gained between 1.5 % and 3.1 % more BMC at the femoral neck and lumbar spine, respectively, whereas

prepubertal boys gained approximately 1 % at the femoral neck and 1.6 % at the total body compared to controls. Measured ground reaction forces in this intervention ranged from 3.5 to 5 times body weight (Mackelvie et al., 2001, MacKelvie et al., 2002b). After 2 years of this jumping protocol, girls in the intervention group had accrued between 3.7 % and 4.6 % greater BMC at the lumbar spine and femoral neck region of the hip compared with controls, whereas boys gained 4.3 % more BMC at the femoral neck compared with controls (MacKelvie et al., 2004). These findings suggest an additive effect from repeated exposure over multiple school years. In contrast, Wiebe et al. exposed girls (6-10 years) to single-leg drop landings (three sessions per week, 50 landings per session) from heights of 14 and 28 cm and found no effect on bone mineral accrual despite the similar ground reaction forces (2.2 and 4.4 times body weight per leg) applied to the skeleton. Findings from a four-year longitudinal study of 205 pre-pubertal children aged 10 ± 1 years suggested that a seven-month jumping intervention significantly increased the adjusted BMC for age, sexual maturation and lean mass by 7.9 % at LS, 8.4 % at total hip, 7.7 % at femoral neck and 7.3 % at total body. Three years after the end of the intervention the effects decreased but remained significant at the above sites (Figure 2.24), accounting for 2.3 %, 3.2 %, 4.4 % and 2.9 % higher BMC respectively (Gunter et al., 2008b). A different intervention study conducted a 9month school based jumping intervention in pre and early pubertal (10.6 years) females. The intervention included 10 min bouts of jumping three times per week plus capoeira, along with usual physical education activities that represented the control group. The intervention groups gained 3.1 % greater BUA derived from QUS, and improved vertical jump (12.2 %) and estimated maximal oxygen consumption (9.6 %) compared to the control group (Nogueira

et al., 2014). The discrepancies in results may be explained by differing exercise doses between Wiebe et al. (Wiebe et al., 2008), Fuchs et al. (Fuchs et al., 2001), and Gunter et al. (Gunter et al., 2008b). In the study by Wiebe et al., the number of jumps per week was fewer (50 vs 100), and the height of the drops considerably was lower (14 and 28 cm vs 61 cm).

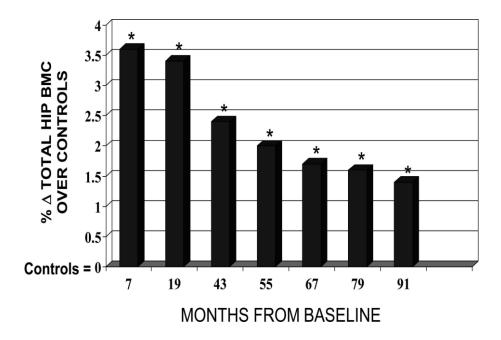


Figure 2.24. Jumping intervention effect on total hip BMC (Δ) in prepubertal children. Percentages of change in BMC at total hip after a 7 month jumping intervention programme compared to controls. The intervention participants had 3.6 % greater bone mass than controls immediately after the intervention and 1.4 % greater bone mass at the total hip than controls after 8 years. With permission from (Gunter et al., 2008b).

The skeleton of prepubertal and early pubertal children favourably responds to targeted weight-bearing interventions (MacKelvie et al., 2002b, Fuchs et al., 2001, Gunter et al., 2008b, MacKelvie et al., 2004), but the evidence is not as consistent for post pubertal adolescents. Two studies in late pubertal and post-pubertal female subjects indicate that resistance training (three times per week for 6.5 months) (Blimkie et al., 1996), plyometric training (three times per week

for 9 months) (Witzke and Snow, 2000), and step aerobics (two times per week for 9 months) (Heinonen et al., 2000) are insufficient to improve bone mass at loaded skeletal sites compared with age- and maturity matched controls. On the other hand, a study found positive effects of exercise after the pubertal growth spurt. Weeks et al. used an 8-month school-based jumping intervention, three times per week in 99 male and female subjects (13.8 years). The intervention included approximately 10 minutes of varied jumping activity in place of the usual physical education class warm-up, which was performed by controls. Ground reaction forces were not reported, but participants performed approximately 300 jumps per session and the average jump heights ranged from 20 to 40 cm. Results showed that female subjects had greater increases in femoral neck (4.9 %) and lumbar spine (1.5 %) BMC compared with controls. Male participants gained 4.3 % more total body BMC and 5.0 % greater calcaneal broadband ultrasound attenuation, derived from QUS, compared to controls (Weeks et al., 2008). The participants were followed there years after the intervention and femoral neck BMC (3.6 %) was significantly higher in the intervention group compared to controls, but the benefits of the intervention did not maintain in other bone outcomes (Weeks and Beck, 2012). This study supports to the notion that jumping may be a unique stimulus for healthy skeletal development throughout the growing years. However, the load should be sufficiently high and maintained for a sufficient period.

In addition, meta-analyses on the impact of exercise on bone adaptations highlighted the importance of measuring bone structure in addition to bone mass. In addition, it was highlighted the importance of controlling for confounding factors where possible, such as maturation stage, exercise mode,

intervention strategy, duration and frequency of exercise, design and duration of the study (Ishikawa et al., 2013, Hind and Burrows, 2007, Behringer et al., 2014). The different effects of exercise loading relative to biological age on bone mass and geometry were investigated by a 7-month jumping intervention study (consisting of 10-minute, 3 times per week) in prepubertal and early pubertal girls that randomized to a classroom-based jumping program (Petit et al., 2002). HSA was used to assess subperiosteal width, CSA, and CSMI of the femoral neck, intertrochanteric and femoral shaft sites. Among the prepubertal girls, there were no differences for change in any bone structural outcome. Among the early pubertal girls, the observed intervention responses were significantly greater gains in femoral neck (2.6 %) and intertrochanteric (1.7 %) BMD. Also, an increase was observed in bone cross-sectional area and cortical thickness attributable to less endosteal expansion at the femoral neck compared with controls (Petit et al., 2002). Mackelvie et al. (MacKelvie et al., 2004) used HSA to examine bone structural outcomes among boys, all of whom were prepubertal, exposed to a 2-yr dose of the same exercise program used by Petit et al. (Petit et al., 2002). Boys in the intervention group had close to 3% greater bone expansion on the periosteal and endosteal surfaces compared with controls. These findings are functionally important in that small gains to the periosteal surface significantly influence bone strength compared with gains on the endocortical surface and emphasize the importance of exercise before puberty to maximize periosteal apposition. These data have important implications because structural changes allow for increases in bone strength without significant increases in bone mass. Given bone is lost predominately from the endosteal surface during adulthood and not from the periosteal surface, physical activity during growth may offset fracture risk through structural

alterations. Therefore, it is of great importance to measure bone geometry, texture and strength where possible because the skeletal benefits of exercise would not be detected when only bone mass is measured.

Despite the evidence suggesting that jumping training has positive effects on bone mass in non-athletic paediatric populations, there are no studies investigating its effects in athletic groups that represent a large part of the population. This is critical to understand bone development due to sport participation and also to find out whether there is room for improvement for adolescents who may have sub-optimal bone development.

2.12 Thesis objectives

Considering the previous literature, the objective of the present thesis is to assess for first time the cross-sectional and longitudinal effect of sport participation in adolescent male athletes engaged in osteogenic (football) and non-osteogenic (swimming and cycling) sports in comparison with an active control group. Another objective is to examine the effect of a jumping intervention programme on bone outcomes in the adolescent male athletes. The specific aims of each chapter are provided below:

1. Chapter 4: to investigate the differences on bone mass and geometry in adolescent males involved in osteogenic (football) and non-osteogenic (swimming and cycling) sports in comparison with an active control group at baseline.

- 2. Chapter 5: to identify the determinants affecting bone mass and geometry in adolescent athletes at baseline.
- 3. Chapter 6: to assess the differences on bone mass and stiffness after 12-months of sport specific training in osteogenic and non-osteogenic sports in adolescent male athletes.
- 4. Chapter 7: to examine the 12-months of longitudinal adaptations of sport participation on bone mass at clinically relevant sites, hip geometry estimates at the femoral neck and lumbar spine texture in adolescent male athletes.
- 5. Chapter 8: to investigate the effect of a 9-month jumping intervention programme on total body bone mass change and physical fitness parameters in adolescent male athletes.
- 6. Chapter 9: to examine the ability of a 9-month jumping intervention to improve bone mass, geometry, texture at the femoral neck and lumbar spine, and to assess bone metabolism and nutritional markers before and after the intervention in adolescent male athletes.

3. Methodology

3.1 PRO-BONE study design

The PRO-BONE study has a longitudinal design and involves four cohorts (football, swimming, cycling and control) of males aged 12-14 years at the beginning of the study followed for 21 months. The timeline of the present PhD can be seen in Figure 3.1 below and the protocol of the study previously published (Vlachopoulos et al., 2015).

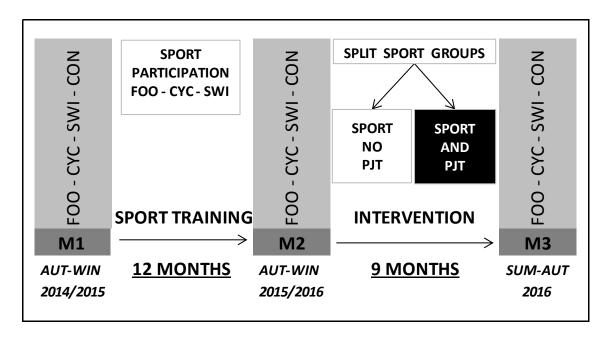


Figure 3.1. PhD timeline containing baseline measurements (M1), 1 year of sports training measurements – pre-intervention (M2) and post intervention measurements (M3). FOO: Football players; CYC: Cyclists; SWI: Swimmers; PJT: Plyometric Jump Training. Adapted from (Vlachopoulos et al., 2015).

3.2 Recruitment of the participants

Participants and parents/guardians were contacted via advert flyers, posters and social media to participate in this study and by contacting sport clubs and

schools from the South West of England. Where possible, a meeting was held to explain the project as well as to answer any questions. At the end of this meeting, consent/assent forms and information sheets were given out and participants and parents/guardians had 15 days to return the consent/assent forms. After these 15 days, a reminder (phone call or email) was provided to those not returning the consent/assent forms to check if they wish to participate. Seven more days were given to those that agreed to participate and in the 2nd reminder, they were asked to send the interest and consent/assent forms signed.

Participants were screened for eligibility, based on the inclusion/exclusion criteria outlined below, by a member of the research team depending on the information provided in the interest form. If eligible, the baseline assessment was scheduled for the participant. All participants and parents involved in this project were carefully informed about the risks and benefits of the study and required to sign the approved assent and consent forms before their visit to the laboratory at the Children's Health and Exercise Research Centre (CHERC, University of Exeter).

The total number of participants that were recruited and measured at baseline were N=121 (41 swimmers, 37 footballers, 29 cyclists and 14 controls). After 1 year of sport specific training, N=118 participants (39 swimmers, 37 footballers, 28 cyclists and 14 controls) remained in the study. Following the intervention, N=105 participants (37 swimmers, 30 footballers, 26 cyclists and 12 controls) completed the 3rd visit of the study.

3.3 Inclusion and exclusion criteria

The inclusion criteria were: 1) males 12-14 years old, engaged (≥ 3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports in the last 3 years or more; 2) male adolescents 12 -14 years old not engaged in any of these sports (≥ 3 h/week) in the last 3 or more years (control group). The criteria are based on the findings showing that 3 hours per week of participation can induce improvement in bone mass in children and adolescents (Ara et al., 2006).

The exclusion criteria were: 1) participation in another clinical trial; 2) any acute infection lasting until < 1 week before inclusion; 3) medical history of diseases or medications affecting bone metabolism or the presence of an injury (before inclusion) that may affect participation in their respective sports and/or any variable considered in the present study (i.e. doing the plyometric jump training); 4) non-Caucasian participants. The latter is included since there are differences in body composition (bone, fat and fat-free mass) and biochemical markers (i.e. osteocalcin) between ethnic groups (Bachrach et al., 1999).

3.4 Ethics approval

The methods and procedures of the PRO-BONE study were checked and approved by: the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496), the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060). All personal information obtained

confidentially and access to database was restricted to the researchers of the study. All measurements were carried out by qualified and experienced researchers who undertook a Disclosure and Barring Service check to work with young people.

3.5 Anthropometry and maturity status

The following measurements were undertaken at the 3 visits. Height (cm) and body mass (kg) were measured using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK; precision 0.1 cm; range 60-210 cm) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK; precision 100 g; range 2-200 kg) respectively. BMI calculated as body mass (kg) divided by the height (m) squared. All anthropometrical measurements were performed three times and the mean was calculated. Maturity status was assessed using two methods. Firstly self-reported by the participants during each visit using adapted drawings of the five stages (Tanner) of pubic hair development (Tanner and Whitehouse, 1976). Secondly using predicted age from PHV (R² = 0.90; standard error=0.50 vears) providing an indicator of somatic maturity (Moore et al., 2015).

3.6 Bone status measurements

3.6.1 Dual-energy x-ray absorptiometry

A Lunar Prodigy DXA scanner (GE Healthcare Inc, Wisconsin, USA) was used to scan participants at four sites due to the evidence of site specific impact of sports participation (Ginty et al., 2005b, Magkos et al., 2007, Ishikawa et al., 2013): 1) Lumbar spine (mean of L1-L4), 2) right hip, 3) left hip, 4) total body

(Figure 3.2). The DXA equipment was calibrated at the start of each testing day by using a lumbar spine phantom as recommended by the manufacturer. The body was segmented in accordance to standard procedures to evaluate regional bone mass and fat distribution. Participants were asked to remain still and they were scanned in the supine position. The position of the hip was controlled using a wedge to ensure the same joint position was used among all participants. The scan modes were automatically selected by the scanner software (standard or thick). All DXA scans and analyses were performed using the enCORE software version 14.10.022 (GE Healthcare Inc, Wisconsin, USA). The BMC (g), BMD (g·cm²) and bone area (cm²) were obtained for the scanned skeletal sites. From total body scans, fat mass (g), lean mass (g) and body fat (g and %) were obtained. All scans were undertaken by the same researcher and following the guidelines of the International Society of Clinical Densitometry (Crabtree et al., 2014) and after obtaining theoretical and practical bone densitometry training. The COVs were not determined in the present study due to ethical considerations of additional radiation exposure, but previous paediatric studies have shown that the COVs of DXA were between 0.64 % and 1.16 % depending on the region (Johnson and Dawson-Hughes, 1991, Shepherd et al., 2011).



Figure 3.2. Measurement of bone mineral density at the femoral neck using DXA as part of the PRO-BONE study. The photo was taken with permission from participant and parent.

3.6.2 Hip structural analysis

Bone geometry estimates were obtained using the HSA software from the narrow neck region across the narrowest point of the femoral neck. The HSA programme is based on previously described principles (Martin and Burr, 1984) and uses the distribution of bone mineral mass in line of pixels across the bone axis to measure the structural dimensions of bone cross sections (Beck et al., 1990). The geometric properties of the bone were obtained and the following variables used: 1) the cortical width neck (mm), which is the narrowest width of the femoral neck; 2) the diameter of the femoral neck (mm); 3) the CSA (mm³), which is the total bone surface area excluding the soft tissue area and the trabecular; 4) the CSMI (mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; 5) section modulus (mm³), which is an indicator of maximum bending strength in a cross

section. The COVs previously found to be between 7.9 % and 11.7 % (Khoo et al., 2005).

3.6.3 Trabecular bone score

TBS is a DXA based technological tool that provides an indirect textural index of trabecular microarchitecture in the lumbar spine and has been shown to significantly predict fracture risk independently of BMC (Hans et al., 2011a). TBS assesses DXA images of the lumbar spine scans using a grey-level analysis as the slope at the origin of the log-log representation of the experimental variogram (Pothuaud et al., 2009). All TBS analyses were performed by the same trained researcher using the TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The calculation was performed at the lumbar spine region of interest as in the BMC measurement. The COVs of TBS in relation to BMC has been reported to be between 1.1 % to 1.9 % (Silva et al., 2014).

3.6.4 Qualitative ultrasound

QUS measurements were performed with a Lunar Achilles Insight GE Healthcare Inc., Wisconsin, USA) and the results were obtained from the OsteoReport PC software version 5.x+ (TM Insight GE Healthcare, Milwaukee, Wisconsin, USA). The same device was used throughout the study and the calibration was completed prior to each visit. A standard procedure was followed according to instructions of the manufacturer. Participants placed on a stable chair in a comfortable position directly in front of the Achilles device. The

position of the leg placed against the calf support so the foot, calf and thigh were aligned with the centre of the calf support and the positioner. The QUS device provided three outcome variables, the BUS, the SOS and the bone stiffness. The BUA indicated the absorption of sound waves measured in decibels per megahertz. The SOS indicated the stiffness of a material by the ratio of the traversed distance to the transit time, expressed in meters per second. The bone stiffness was calculated by a linear combination of BUA and SOS: bone stiffness = (0.67 x BUA) + (0.28 x SOS) – 420. The real-time image of the calcaneus and the region of interest ensured that the measurement is precise (Jaworski et al., 1995). Dominant and non-dominant feet were measured twice and the mean of the two measurements calculated and used for statistical analyses. Previously the correlation between QUS and BMD measurements ranged between r = 0.44-0.70 in 11-16-year-old participants (Sundberg et al., 1998). The COVs of QUS in children range from 0.5 % to 1.2 % for SOS (Halaba et al., 2005) and from 2 % to 5 % for BUA (Dib et al., 2005).

3.7 Biochemical markers

Capillary blood samples (0.5 mL) were collected at a non-training weekend day in the morning in heparin fluoride coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) and centrifuged at 3000 rpm for 15 minutes at 4°C. Serum samples were stored at -80°C until analysis in a single session. Total serum levels of PINP, CTX-I, 25(OH)D and total calcium were analysed following guidelines (Vasikaran et al., 2011). ELISA kits (Abbexa Ltd., Cambridge, UK) for PINP (test range: 6-400 pg·mL⁻¹, sensitivity: 1.2 pg·mL⁻¹, inter and intra-assay COVs: 3.1 % and 8.2 % respectively), CTX-I (test range: 0.1-7.0 ng·mL⁻¹,

sensitivity: 0.03 ng·mL⁻¹, inter and intra-assay CVs: 4.9 % and 6.8 % respectively), and 25(OH)D (test range: 3-80 ng·mL⁻¹, sensitivity: 1.2 ng·mL⁻¹, inter and intra-assay CVs: 6.1 % and 8.6 % respectively) were used. Total calcium serum was measured using direct colorimetric assay (Cayman Chemical Company, MI, U.S.A.) and had a sensitivity of 0.25 mg·dL⁻¹ and the absorbance was read at 570-590 nm (inter and intra-assay COVs: 5.1 % and 7.3 % respectively).

3.8 Physical activity, training characteristics and diet intakes

Physical activity was measured for seven consecutive days at pre- and post-intervention using wrist accelerometers (GENEActiv, Cambridgeshire, UK). Participants were instructed to place the accelerometer at their non-dominant wrist for 7 days and the time sampling interval (epoch) was set at 100 Hz to obtain the data in 1 s epochs. For the present study, the time spent in moderate physical activity and vigorous physical activity was calculated using a cut-off point of 1140-3599 counts per minute and ≥ 3600 counts per minute, respectively (Phillips et al., 2013). Weekly training hours were obtained using face to face questionnaire during each visit to the research centre. Total energy, protein, calcium and vitamin D intake were assessed using a 24-h food recall. Total energy, calcium and protein intake were estimated using the CompEat Pro software (Nutrition systems, VIS Visual Information Systems Ltd., UK). Previous research in children indicated that Pearson correlations for energy and nutrient intake ranged from 0.52 to 0.86 and the agreement between recalled and observed food items to be 75 % (Weber et al., 2004).

3.9 Cardiorespiratory and muscular fitness

A battery of tests was used to assess attributes of physical fitness that may play an important role in the development of skeletal mass and strength during growth and maturation (Baptista et al., 2016, Kemper HC, 2000). Cardiorespiratory fitness was estimated using the 20 m shuttle run test (20mSRT) (Leger et al., 1988) and was completed in the same sports hall and under similar encouragement at all visits. The participants were tested at the end of the day following a standardized warm up and were equally encouraged to continue the test until they reached maximal effort. They were asked to run between two lines set 20 m apart by following the pace of the audio signals produced from a CD player. The starting speed was 8.5 km/h and increased by 0.5 km/h each minute. The test was terminated when the participant failed to reach the line two consecutive times. The last completed shuttle determined the score of the test and the number of shuttles completed was taken as an indicator of cardiorespiratory fitness. The agreement between 20mSRT and maximal oxygen consumption (VO_{2max}) has been shown to range from R²=0.68 to R²=0.92 in adolescents (Castro-Pinero et al., 2010, Ruiz et al., 2009) and that the criterion-related validity of Leger's protocol was r = 0.78 in children and adolescents (Mayorga-Vega et al., 2015).

Muscular fitness was assessed using the standing long jump (SLJ) test and the vertical jump using was assessed using the counter movement jump (CMJ) test at least 30 minutes before performing the 20mSRT and following a standardized warm up. The starting position of the standing long jump test is exactly behind a line and with feet at shoulder's width apart. Participants are allowed to swing

their arms during the eccentric contraction phase and they are advised to jump as far as possible in order to land with both feet in a non- slippery hard surface. The distance (cm) measured between the starting line and the participant's heels. The CMJ test was assessed on a jump mat (Probotics Inc, Alabama, USA) which calculates jump height based on flight time. The participants performed the CMJ test with their feet shoulder width apart after having received instructions as to how much can they bend their knees and the position of their arms, they are asked to jump as high as possible. Then, they were placed in a standing position with their feet shoulder width apart at the jump mat. For both jump tests three maximal jumps were performed and the best score was used. The max height and distance (in cm) of the maximal efforts is used as criterion of measure. Previous reports indicate that SLJ and CMJ accounted for 44.4 % and for 40.8 % of the variation in 1RM leg press in adolescents (Ortega et al., 2008, Milliken et al., 2008).

3.10 Jumping intervention

Following 12 months of sport specific training (measurement point M2 in Figure 3.1), the sport group participants were simply randomized into two sub-groups to perform a 9-month jumping intervention programme as follows: 1) intervention-sport: intervention-swimming (INT- SWI), intervention-football (INT-FOO), intervention-cycling (INT-CYC) and 2) control-sport: control-swimming (CON-SWI), control-football (CON-FOO), control-cycling (CON-CYC). It has been shown that 7 to 9 months of plyometric jump training can effectively improve BMC and/or BMD at different skeletal sites in children and adolescents and the benefits were maintained for 3 years after the intervention (Gunter et al., 2008b, Witzke and Snow, 2000). Therefore, a progressive plyometric jump

training (approximately 10 min/day) consisted from CMJ was performed by intervention groups 3 to 4 times per week depending on the level of progression. Both the intensity and number of jumps increased progressively in 3 levels of 12 weeks each. Intensity was modified using an adjustable weight vest. With this, a 2 to 5 kg increase in body weight was achieved at the end of the intervention (see Table 3.1). The reliability and validity of the CMJ has been previously reported (Acero et al., 2011, Markovic et al., 2004). At the second visit trained staff ensured that participants fully understood and correctly executed the CMJs, and a research assistant observed, demonstrated and reviewed the jumps. Participants were instructed to perform a number of CMJ on a hard surface. The CMJ activates the stretch-shortening cycle in the muscles, resulting in greater power production in the legs with previous evidence in children showing that 12 CMJ produce ground reaction forces 5.3 times body weight and rates of force of 493 times body weight/s, as shown in an independent sample of boys and girls (McKay et al., 2005a). The participants were instructed to start from a standing position, then to bend the knees up to 90° and immediately perform the vertical jump as high as possible each time, landing back on the ground on both feet simultaneously. A jump diary was used to record the number of jumps performed and was checked every three months to check the compliance of the jumping intervention programme.

Table 3.1. PRO BONE study jumping intervention training progression

Level	Exercise	Vest weights (kg)	Repetitions	² Sets/ day (³ Rest)	⁴ Trainings/ week	Jumps/ week
1	¹ CMJ	-	20	3	3	180
Total level 1 (12 weeks)				180 x 12 =	2160	
2	¹ CMJ	2.5	20	4	3	240
Total level 2 (12 weeks)					240 x 12 =	2880
3	¹ CMJ	5	20	4	4	320
Total level 3 (12 weeks) 320 x 12 = 3840					3840	
Total levels (36 weeks) 8880						

¹Countermovement jump. ²Sets = 20 CMJ. ³Rest between sets = 30 seconds. ⁴When 3 sets/day, jumps suggested to be performed in the morning before going to school (1 set), after school (1 set) and before going to bed (1 set). When 4 sets/day, jumps performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1 set). ⁵No significant differences between the intervention groups at any level of the intervention.

3.11 Sample size

The sample size calculated according to the primary interest variable, total body BMD of cyclists (Olmedillas et al., 2011) in order to achieve 90 % of statistical power to detect differences between the groups. Taking into account a significance level of 5 % and assuming that the mean of the reference group was 1.133 units (SD = 0.127) and the mean of the experimental group was 1.002 units (SD = 0.093), 9 participants required for the reference group and 9 participants for the experimental group (total = 18 participants). Given the longitudinal nature of the current project, the sample size adjusted assuming an attrition rate of 40 %, yielding a sample size of 15 participants for each group (e.g. 15 intervention cyclists + 15 control cyclists = 30 cyclists). Following the recruitment period, a total of 121 participants were found eligible to take part in the study, comprising of: cyclists (n=29), footballers (n=37), swimmers (n=41) and controls (n=14).

3.12 Statistical analyses

Descriptive data of mean and SD are calculated unless otherwise stated. Statistical analyses performed using the SPSS IBM statistics (version 21.0 and 22.0 for Windows, Chicago, IL, USA) and statistical significance level was set at P < 0.05. The distribution of the variables was checked and verified by using Shapiro-Wilk tests, skewness and kurtosis values, visual check of histograms, Q-Q and box plots. Unadjusted data are shown as mean and standard deviation (SD) while adjusted data presented as mean and standard error (SE). A description of the chapter-specific statistical analysis is shown below:

Chapter 4: One-way analysis of variance (ANOVA) was performed to detect between-group differences on bone-related outcomes. One-way analysis of covariance (ANCOVA) with Bonferroni post hoc was used to detect between-group differences on bone-related outcomes after accounting for age (Webber et al., 2009), height (Nevill et al., 2002), lean mass (Gracia-Marco et al., 2012) calcium intake (Boot et al., 1997), and MVPA (Gracia-Marco et al., 2011a). In addition, differences (in %) between groups were calculated for all variables to quantify the magnitude of the differences.

Chapter 5: To identify the determinants of bone related outcomes a correlation matrix using Pearson correlation coefficients was performed. Multiple linear regression analyses were used to examine the contribution of the determinants (sport participation, height, lean mass, fat mass, total calcium, 25(OH)D, MVPA, vertical jump and 20mSRT) to bone outcomes. The selection of the predictors was based on their relationship with bone outcomes (Petit et al., 2005, Macdonald et al., 2006, Sioen et al., 2012). All predictors were entered into the regression models simultaneously. For the multiple linear regressions, the

standardised regression coefficients (β) are reported and significance was set at alpha level of 0.05. The squared semi-partial correlation coefficients (sr^2) were used to determine the contribution of each predictor in the overall variance of the model after removing shared contributions with other predictors.

Chapter 6 and 7: To identify the effect of 1 year of sport specific training on bone status between the groups, ANCOVA with Bonferroni post hoc was used after accounting for age, height, lean mass, MVPA and the bone outcomes at T0 as covariates.

Chapters 8 and 9: To detect the differences in characteristics and fitness parameters pre- and post-intervention, paired t-tests were used. To detect differences in bone outcomes between the intervention and the non-intervention groups, the 9-month bone gains (Δ) were calculated and ANCOVA was used after controlling for baseline bone status, change in lean mass and post maturity status (years from PHV) (Moore et al., 2015). Percentages of difference between the intervention and non-intervention groups were used to quantify the magnitude of the differences in adjusted bone outcome gains.

4. The impact of sport participation on bone mass and geometry in adolescent males

4.1 Abstract

Purpose: Exercise is an effective approach for developing bone mass and adolescence is a key period to optimize bone health. However, sports specific training may have different effects on bone outcomes. This study examined the differences on bone outcomes between osteogenic (football) and nonosteogenic (swimming and cycling) sports and a control group in adolescent males. Methods: One hundred and twenty one males (13.1±0.1 years) were measured: 41 swimmers, 37 footballers, 29 cyclists and 14 controls. Dual energy X-ray absorptiometry measured bone mineral density (BMD) and content (BMC) at lumbar spine, right and left hip and total body. Hip structural analysis evaluated bone geometry at the femoral neck. Quantitative ultrasound evaluated bone stiffness at both feet. Results: Footballers had significantly higher BMD at total body less head (7-9 %), total hip (12-21 %) and legs (7-11 %) compared to all groups and significantly higher BMD at the femoral neck than controls (14 %). Cyclists had higher BMD at the trochanter (10 %) and BMC at the arms (10 %) compared to controls. Geometrical analysis showed that footballers had significantly higher cross-sectional area (8-19 %) compared to all groups, cross-sectional moment of inertia (17 %) compared to controls and section modulus compared to cyclists (11 %) and controls (21 %). Footballers had significantly higher bone stiffness compared to all groups (10-20 %) at the dominant foot and (12-13 %) at the non-dominant foot compared to swimmers and controls. Conclusions: Adolescent male footballers exhibited higher bone density, geometry and stiffness compared to swimmers, cyclists and controls. Although swimmers and cyclists had higher bone outcomes compared to controls, these differences were not significant.

Keywords: adolescence, bone mass, bone geometry, bone stiffness, exercise.

4.2. Introduction

Osteoporosis is a disease characterized by reduced bone mass and deterioration of bone microarchitecture, resulting in increased risk of fragility fractures. Bone mass acquisition during adolescence is not only an important determinant of skeletal growth but also for reducing the risk of osteoporosis later in life (Nikander et al., 2010). In this regard, a 10 % increase in peak bone mass during adolescence might reduce the risk of fracture later in life by 50 % and delay the onset of osteoporosis by 13 years (Rizzoli et al., 2010). Therefore, early prevention remains one of the most prudent approaches to improve bone health status in later adult life.

It is known that 20 % of the variation in peak bone mass can be explained by lifestyle factors, including physical activity (PA) and diet (i.e. calcium and vitamin D intakes) (Vlachopoulos et al., 2016, Michalopoulou et al., 2013). In terms of PA, a favourable osteogenic response can be obtained when high-impact, intensive and weight-bearing exercise is performed, due to the mechanical load imposed on the bone tissue (Greene and Naughton, 2006b). For example, football is considered an "osteogenic" sport and augments bone mineral density (BMD) and content (BMC) at the weight-bearing sites in early and late pubertal males (Nebigh et al., 2009). In contrast, sports such as swimming and cycling have been considered "non-osteogenic" (Vicente-

Rodriguez et al., 2004a), although the supporting evidence is unclear. Previous evidence found that adolescent male swimmers to have lower adjusted BMC and BMD compared to controls (Gomez-Bruton et al., 2015). A recent systematic review concluded that swimmers have similar bone mass with sedentary controls (Gomez-Bruton et al., 2016b). Similarly, although there are reports of cycling showing no effect on bone-related outcomes in adolescents, some studies suggest cycling during adolescence may negatively impact bone health and compromise the acquisition of a high peak bone mass (Olmedillas et al., 2011). There is limited evidence evaluating the effects of osteogenic and non-osteogenic sports on bone outcomes in adolescent males and further research is needed to investigate this discrepancy in the literature.

With football, swimming, and cycling among the most popular sports during childhood and adolescence in the United Kingdom, understanding the contribution of these sports to bone health is important. To date, studies evaluating bone-related outcomes in athletic groups have mainly focused in BMD and BMC outcomes provided by Dual energy X-ray Absorptiometry (DXA). But a more comprehensive evaluation of bone structure, as well researched can be obtained using the Hip Structural Analysis (HSA) software from DXA (Beck et al., 1990). The parameters obtained from HSA software reflect bone strength at the narrow neck site of the clinical important site of the hip. A previous study showed that adolescent female footballers had greater hip strength compared to swimmers and controls, while swimmers had lower bone mass at the narrow neck than footballers and controls (Ferry et al., 2011). Another method to assess bone properties is Quantitative Ultrasound (QUS), which is a non-radiation technique and provides measurements of the bone stiffness changes at the calcaneus site. Currently, there are no studies evaluating bone outcomes

in male adolescent athletes using a combination of DXA, HSA and QUS outcomes. Furthermore, there is a lack of consistency when controlling for the use of confounding variables in the assessment of bone outcomes in youth sports. This is important as uncritical use of confounders can lead to size related artefacts (Prentice et al., 1994). Previous studies typically use confounders such as age, height, weight, calcium intake, fat mass, fat-free mass and lean mass (Maimoun et al., 2013b, Zemel et al., 2011). However, the most common inconsistencies observed in many studies are the lack of consideration for size adjustments in adolescent participants and the lack of site specific adjustment of the skeletal outcomes. Therefore, more studies are needed to assess the bone outcomes by taking into account the relevant confounders according to participant characteristics.

The PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) study was designed to investigate whether the bone properties, assessed by DXA, HSA and QUS, differ between 12-14 year old males who perform osteogenic (football) and non-osteogenic (swimming, cycling) sports in comparison to a control group after controlling for a comprehensive set of confounders. We hypothesised that adolescent males engaged in football will have higher bone outcomes compared to those engaged in cycling and swimming and compared to a control group, and that adolescent males engaged in cycling and swimming will have similar bone outcomes.

4.3 Methods

4.3.1 Study design and participants

The study represents a cross-sectional analysis of the baseline data derived from the PRO-BONE study, which is a 33 month longitudinal design including a 9-month jump intervention programme. The purpose, methodology and sample size of the PRO-BONE study have been justified elsewhere (Vlachopoulos et al., 2015). Data were collected between autumn and winter 2014/15 in 121 adolescent males: 41 swimmers, 37 footballers, 29 cyclists and 14 controls. The inclusion and exclusion criteria were: 1) males 12–14 years old, engaged (≥3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or more; 2) males 12–14 years old not engaged in any of these sports (≥3 h/week) in the last 3 or more years (control group); 3) participants not taking part in another clinical trial; 4) participants not having any acute infection lasting until < 1 week before inclusion; 5) participants had to be free of any medical history of diseases or medications affecting bone metabolism or the presence of an injury; 6) white Caucasian ethnicity.

Participants were recruited from athletic clubs and schools across the South West of England. Written informed consent and assent forms were signed from parents and participants accordingly and all participants completed the first visit at the research centre as part of the study. The methods and procedures of the study have been checked and approved by: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

4.3.2 Anthropometry and sexual maturity

Height (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK; precision 0.1 cm; range 60–210 cm) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK; precision 0.1 kg; range 2–200 kg) respectively. Body mass index was calculated as body mass (kg) divided by the height (m) squared. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair (Tanner and Whitehouse, 1976).

4.3.3 Dual energy x-ray absorptiometry

A DXA scanner (GE Healthcare Inc., Wisconsin, USA) was used to measure BMD (g/cm²), BMC (g), bone area (BA, cm²), fat mass (g) and lean mass (g). Four scans were performed to obtain data for the lumbar spine (LS, L1-L4), right and left hip (including femoral neck, Ward's triangle, trochanter and shaft sub-regions; the mean of right and left hip scans was used), and the total body scan. The total body scan was then used to obtain data for specific regions such as: arms, legs, pelvis and total body excluding head. All DXA scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the GE encore software (2006, version 14.10.022). The positioning of the participants and the analyses of the results were undertaken according to International Society of Clinical Densitometry (Crabtree et al., 2014).

4.3.4 Hip structural analysis

Using the HSA software, analyses were performed at the narrow neck region across the narrowest point of the femoral neck. The HSA programme uses the distribution of bone mineral mass in line of pixels across the bone axis to measure the structural dimensions of bone cross sections (Beck et al., 1990).

The geometric properties of the bone were obtained and the following variables used: 1) the cortical width neck (mm), which is the narrowest width of the femoral neck; 2) the diameter of the femoral neck (mm); 3) the cross sectional area (CSA, mm³), which is the total bone surface area excluding the soft tissue area and the trabecular; 4) the cross-sectional moment of inertia (CSMI, mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; 5) section modulus (mm³), which is an indicator of maximum bending strength in a cross section; and 6) the hip strength index, which is an advanced feature that has been added to the more recent versions of GE enCore software and indicates the risk of fracture forces generated during a fall on the greater trochanter and the CSA short term precision percentage coefficient of variation has been reported to be between 2.4 % and 7.9 % (Khoo et al., 2005).

4.3.5 Quantitative ultrasound

QUS measurements were performed with a Lunar Achilles Insight ((GE Healthcare Inc., Wisconsin, USA) and the OsteoReport PC (software version 5.x+). The stiffness index is then calculated by a linear combination of broadband ultrasound attenuation (BUA) and speed of sound (SOS) as follows: Stiffness index = (0.67 x BUA) + (0.28 x SOS) – 420. Both feet were measured twice and the mean of the two measures was used for statistical analyses of the dominant and non-dominant foot. For the purpose of this study only stiffness index values were used. QUS is considered a valid and radiation-free method compared to DXA to assess bone health in children (Baroncelli, 2008).

4.3.6 Physical activity and diet

PA was measured for seven consecutive days by using wrist accelerometers (GENEActiv, GENEA, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (Phillips et al., 2013). Participants were instructed to place the accelerometer on their non-dominant wrist and data was collected at 100 Hz. Data were analysed at 1 s epoch intervals to establish time spent in different intensities. Time spent in moderate PA and vigorous PA (VPA) was calculated using a cut-off point of 1140-3599 counts per minute and ≥ 3600 counts per minute, respectively (Phillips et al., 2013). Moderate-to-vigorous PA (MVPA) was calculated using a cut-off point of ≥ 1140 counts per minute. Weekly training hours were obtained by face to face questions during the visit of the participants at the research centre.

Dietary calcium, vitamin D and energy intake were assessed using a 24 hour food recall. The validity and reliability of self-reported dietary intake has been previously reported in children (Weber et al., 2004). Total energy, calcium and vitamin D intake were estimated using the CompEat Pro software (Nutrition systems, VIS Visual Information Systems Ltd., UK).

4.3.7 Statistical analysis

Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA) and descriptive data are reported as mean and SD. The distribution of the variables was checked and verified using Shapiro-Wilk's test, skewness and kurtosis values, visual check of histograms, Q-Q and box plots. The analysis of the data was completed in two stages: 1) raw (unadjusted) data using one-way analysis of variance (ANOVA) with Bonferroni post hoc to detect between-group differences on bone-related outcomes (DXA, HSA and QUS), and 2) adjusted data using one-way analysis of covariance

(ANCOVA) with Bonferroni post hoc taking into account the following relevant confounders: age, height, region-specific lean mass (trunk, total body, arms and legs), calcium intake and MVPA (Gracia-Marco et al., 2011a, Weber et al., 2004, Gracia-Marco et al., 2012, Ubago-Guisado et al., 2015a). A preliminary analysis showed maturation to have no effect on bone outcomes after accounting for age and thus was not included in the model. Percentages of difference between groups for all variables were used to quantify the magnitude of the differences. Statistical significance level was set at P < 0.05 and differences of P <0.001 were also indicated.

4.4 Results

4.4.1 Descriptive characteristics of the study sample

Table 4.1 presents the descriptive characteristics of the participants. Swimmers were older, taller, heavier and had more lean mass than the footballers. Footballers spent more time doing MVPA and VPA than swimmers and controls. Cyclists were older and spent more time doing VPA than controls and they also spent more time doing MVPA and VPA than the swimmers. In addition, swimmers and footballers trained more hours on average than the cyclists. Finally, controls had more fat mass than all the other groups.

Table 4.1. Descriptive characteristics of the participants

Parameters	Swimmers	Footballers	Cyclists	Controls
	(n=41)	(n=37)	(n=29)	(n=14)
Age (yrs)	13.4 (1.0) ^{b,dd}	12.8 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
Height (cm)	165.5 (9.7) ^{bb,d}	155.2 (9.3)	160.8 (9.9)	154.5 (9.9)
Body mass (kg)	52.4 (9.0) ^{bb}	44.3 (7.9)	49.5 (12.3)	48.3 (13.0)
BMI (kg/m²)	19.0 (1.7)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
Lean mass (kg)	41.6 (9.1) ^{b,dd}	35.4 (7.2)	37.7 (7.5)	31.7 (5.5)
Fat mass (kg)	8.3 (3.2)	6.6 (2.4)	8.6 (7.2)	14.1 (8.5) ^{a,bb,c}
Percentage of body	17.1 (7.1)	15.8 (5.6)	17.8 (8.9)	29.0
fat (%)				(10.5) ^{aa,bb,cc}
Weekly training	9.5 (5.1) ^{cc}	10.0 (2.3) ^{cc}	5.1 (2.1)	-
hours (h)				
Pubertal maturation	(15/25/13/45/2)	(24/35/24/16/0)	(14/28/28/27/3)	(29/21/21/29/0)
(I/II/III/IV/V) (%)				
MVPA (min/day)	85.9 (30.4)	119.8 (29.7) ^{aa,d}	107.2 (33.3) ^a	83.2 (26.8)
VPA (min/day)	11.9 (7.3)	22.5 (9.0) ^{aa,dd}	18.5 (12.8) ^{á,d}	8.9 (4.0)
Energy intake	2084.5 (560.6)	2093.7 (755.4)	2219.8 (843.4)	1748.9 (434.6)
(kcal/day)				
Calcium intake	988.8 (429.7)	1017.9 (504.5)	1014.9 (601.9)	881.5 (380.7)
(mg/day)	,	,	,	,
Vitamin D intake	1.84 (1.5)	1.82 (1.89)	1.95 (1.69)	1.52 (1.39)
(µg/day)				

Values presented as mean \pm SD. BMI: Body mass index, MVPA: Moderate to vigorous physical activity, VPA: Vigorous physical activity. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls). ^{a,b,c,d} p<0.05, ^{aa,bb,cc,dd} p<0.001.

4.4.2 DXA region-specific BMD, BMC and BA

Table 4.2 shows the raw differences for the four groups at different sites. Controls had significantly lower BMD and BMC compared with footballers (BMD: ranged from 6.7 % to 30.1 %, BMC: ranged from 18.1 % to 52.4 %), swimmers (BMD: 10.9 % to 17.9 %, BMC: 26.7 % to 57.1 %) and cyclists (BMD: 8.3 % to 17.9 %, BMC: 21.0 % to 40.9 %) for all sites except for the lumbar spine and arms. In addition, controls had significantly lower BA compared to swimmers (BA: 11.6 % to 37.8 %). Footballers had 7.5 %, 10.4 % and 10.1 % significantly higher BMD at total hip, trochanter and Ward's triangle sites than the swimmers. In addition, they had 7.8 %, 10.4 % and 10.4 % significantly higher BMD at total hip, trochanter and Ward's triangle sites than the cyclists. Finally, swimmers had 6.1 % significantly higher BMD and 23.1 %BMC than footballers at the arms as well as 8.9 %, 9.9 % and 17.7 % greater BA at the shaft, lumbar spine and arms, respectively.

Table 4.2. Raw data for DXA region-specific bone mineral content (BMC, g), density (BMD, g/cm²) and area (BA, cm²) of all participants

area (BA, cm²) of all participants						
Parameter		Swimmers	Footballers	Cyclists	Controls	
S		(n=41)	(n=37)	(n=29)	(n=14)	
TBLH	BMD BMC BA	0.918 (0.067) ^d 1630.66 (333.56) ^d 1762.63 (250.99) ^{b,d}	0.931 (0.071) ^{dd} 1473.49 (338.60) 1564.89 (248.35)	0.905 (0.086) ^d 1498.27 (362.08) 1636.10 (261.69)	0.828 (0.071) 1234.38 (347.86) 1469.43 (300.17)	
Total hip	BMD BMC BA	0.962 (0.107) ^{dd} 28.87 (5.52) ^{dd} 29.86 (3.83) ^d	1.034 (0.085) ^{a,c,dd} 28.78 (6.18) ^{dd} 27.60 (4.21)	0.959 (0.114) ^{dd} 27.59 (5.97) ^d 28.54 (3.86)	0.830 (0.116) 21.12 (5.55) 25.14 (3.79)	
Femoral Neck	BMD BMC BA	0.948 (0.098) ^d 4.46 (0.65) ^{dd} 4.70 (0.43) ^d	1.001 (0.081) ^{dd} 4.53 (0.74) ^{dd} 4.51 (0.46)	0.975 (0.192) ^d 4.40 (0.79) ^d 4.61 (0.43) ^d	0.832 (0.118) 3.52 (0.73) 4.21 (0.45)	
Ward's triangle	BMD BMC BA	0.928 (0.111) ^d 2.31 (0.49) ^d 2.48 (0.43) ^d	1.022 (0.096) a,c,dd 2.40 (0.59) ^{dd} 2.34 (0.44)	0.926 (0.127) ^d 2.23 (0.55) ^d 2.40 (0.42)	0.799 (0.120) 1.64 (0.45) 2.04 (0.36)	
Trochante r	BMD BMC BA	0.799 (0.089) ^{dd} 9.08 (2.41) ^d 11.26 (2.30) ^d	0.882 (0.078) ^{aa,cc,dd} 9.31 (2.67) ^{dd} 10.41 (2.34)	0.799 (0.108) ^{dd} 8.61 (2.39) ^d 10.66 (2.15)	0.678 (0.098) 6.11 (2.17) 8.82 (2.12)	
Shaft	BMD BMC BA	1.100 (0.140) ^d 15.33 (2.62) ^{dd} 13.91 (1.36) ^{b,d}	1.170 (0.109) ^{dd} 14.94 (2.97) ^{dd} 12.67 (1.65)	1.090 (0.132) ^d 14.58 (2.98) ^d 13.27 (1.51)	0.941 (0.150) 11.49 (2.74) 12.11 (1.49)	
Lumbar Spine	BMD BMC BA	0.892 (0.114) ^d 43.26 (11.21) ^d 47.94 (7.37) ^{b,d}	0.883 (0.095) 38.54 (8.93) 43.17 (8.82)	0.867 (0.122) 39.50 (11.04) 44.88 (6.99)	0.791 (0.101) 32.64 (8.67) 40.79 (6.99)	
Arms	BMD BMC BA	0.784 (0.071) ^{b,dd} 244.93 (64.87) ^{bb,dd} 308.22 (58.14) ^{bb,dd}	0.736 (0.047) 188.34 (48.05) 253.62 (51.89)	0.747 (0.069) ^d 212.89 (59.27) ^d 281.00 (58.00) ^d	0.690 (0.049) 155.89 (40.58) 223.71 (45.67)	
Legs	BMD BMC BA	1.091 (0.010) ^d 779.05 (141.65) ^d 709.83 (80.64) ^d	1.124 (0.106) ^{dd} 747.84 (175.02) 657.46 (96.32)	1.077 (0.116) ^d 745.39 (179.21) 684.24 (108.91)	0.975 (0.103) 612.28 (179.74) 617.50 (102.23)	
Pelvis	BMD BMC BA	0.994 (0.087) ^d 246.55 (57.43) ^d 245.85 (41.36) ^{dd}	1.025 (0.103) ^{dd} 238.35 (63.80) ^d 229.19 (40.89) ^d	0.989 (0.130) ^d 227.93 (63.75) ^d 226.69 (37.38)	0.888 (0.087) 174.81 (45.97) 194.07 (34.96)	

TBLH: Total body less head. Values are presented as mean ± SD.Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.001.

Figures 4.1 and 4.2 (also supplementary table 4.1) present adjusted differences for the sports groups at different sites compared to the control group. Once the confounders were controlled for, differences remained significant and higher mainly in the football group compared to the other groups. More specifically, footballers had significantly higher BMD (8.8 % to 25.1 %) and BMC (7.9 % to 29.5 %) than controls at all sites except for the lumbar spine and arms. In addition, footballers had significantly higher BMD and BMC at all sites except for the lumbar spine and arms than swimmers (BMD: 6.9 % to 13.9 %, BMC: 8.4 % to 20.5 %) and cyclists (BMD: 5.2 % to 12.7 %, BMC: 6.7 % to 18.9 %). BA of footballers was significantly higher at pelvis site compared to the other groups (7.1 % to 8.9 %). Cyclists had 10.3 % significantly higher BMD only at the trochanter, 9.8 % higher BMC and 7.3 % higher BA only at the arms compared to controls. There was no significant difference in the other skeletal sites between cyclists and controls. However, cyclists had non-significant higher bone outcomes (BMD: 3.4 % to 11.0 %, BMC: 1.1 % to 11.8 %) in the most sites of the skeleton. At lumbar spine cyclist had non-significant lower BMC (-1.9 %) compared to controls. No significant difference were found between swimmers and controls at any skeletal sites. However, swimmers had non-significant higher bone outcomes in most skeletal sites (BMD: 0.3 % to 9.7 %, BMC: 0.8 % to 10.8 %). At the lumbar spine swimmers had nonsignificant lower bone outcomes (BMD: -0.8 %, BMC: -4.6 %) compared to controls. Cyclists and swimmers had similar BMD, BMC and BA (-0.9 % to 5.0 %) with no significant differences at any skeletal site.

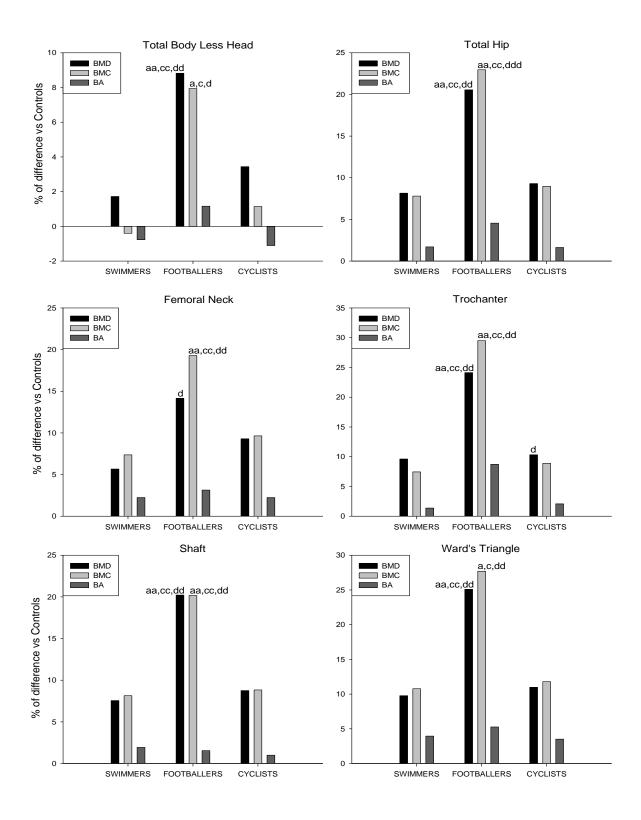


Figure 4.1. Difference (%) in adjusted bone mineral density (BMD), content (BMC) and bone area (BA) between the sports groups and controls at the total body less head and hip sites. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.001.

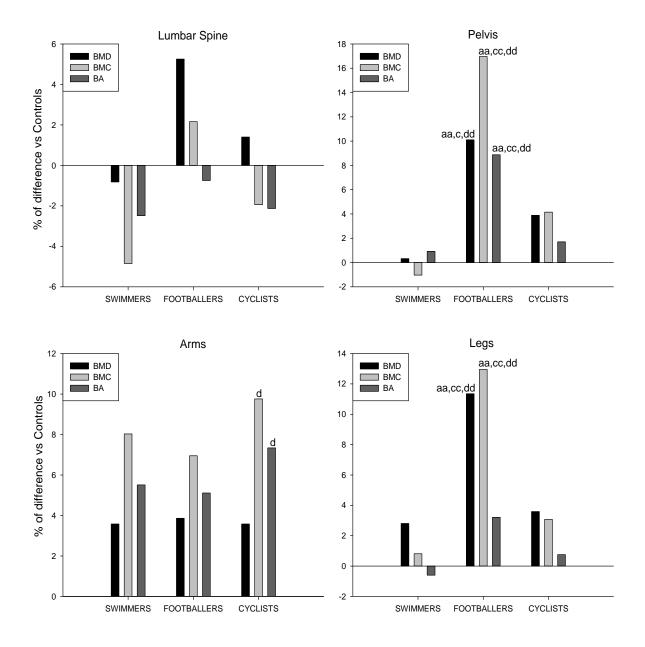


Figure 4.2. Difference (%) in adjusted bone mineral density (BMD), content (BMC) and bone area (BA) between the sports groups and controls at the lumbar spine, pelvis, arms, and legs. Letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.001.

Supplementary table 4.1. Adjusted data for DXA region-specific bone mineral content (BMC, g), density

(BMD, g/cm²) and area (BA, cm²) of all participants

Parameter Swimmers Footballers Cyclists Controls s (n=41) (n=37) (n=29) (n=14) TBLH BMD 0.888 (0.008) 0.950 (0.008) ^{aa,cc,dd} 0.903 (0.008) 0.873 (0.013)
TBLH BMD 0.888 (0.008) 0.950 (0.008) aa,cc,dd 0.903 (0.008) 0.873 (0.013)
TBLH BMD 0.888 (0.008) 0.950 (0.008) ^{aa,cc,dd} 0.903 (0.008) 0.873 (0.013)
===== · · · · · · · · · · · · · · · · ·
BMC 1462.44 (21.27) 1584.97 (22.09) ^{a,c,d} 1485.07 1468.36
BA 1628.11 (13.78) 1659.72 (14.31) (23.13) (35.64)
1622.53 1640.69
(14.98) (23.08)
Total hip BMD 0.942 (0.015) 1.050 (0.016) ^{aa,cc,dd} 0.952 (0.016) 0.871 (0.025)
BMC 26.67 (0.49) 30.42 (0.50) 4a,cc,dd 26.96 (0.52) 24.74 (0.79)
BA 28.12 (0.25) 28.88 (0.26) 28.10 (0.27) 27.65 (0.41)
Femoral BMD 0.934 (0.019) 1.009 (0.020) ^d 0.966 (0.020) 0.884 (0.031)
Neck BMC 4.23 (0.08) 4.70 (0.08) ^{aa,c,dd} 4.32 (0.08) 3.94 (0.12)
BA 4.56 (0.05) 4.60 (0.05) 4.56 (0.05) 4.46 (0.08)
Ward's BMD 0.910 (0.018) 1.037 (0.019) ^{aa,cc,dd} 0.920 (0.019) 0.829 (0.030)
triangle BMC 2.16 (0.06) 2.49 (0.06) ^{a,c,dd} 2.18 (0.06) 1.95 (0.10)
BA 2.37 (0.05) 2.40 (0.05) 2.36 (0.05) 2.28 (0.08)
Trochanter BMD 0.786 (0.014) 0.890 (0.014) ^{aa,cc,dd} 0.791 (0.014) ^d 0.717 (0.022)
BMC 8.23 (0.22) 9.92 (0.22) ^{aa,cc,dd} 8.34 (0.23) 7.66 (0.36)
BA 10.35 (0.19) 11.10 (0.20) 10.42 (0.20) 10.21 (0.31)
Shaft BMD 1.069 (0.019) 1.195 (0.020) aa,cc,dd 1.081 (0.020) 0.994 (0.031)
BMC 14.21 (0.25) 15.79 (0.26) aa,cc,dd 14.30 (0.27) 13.14 (0.42)
Lumbar BMD 0.850 (0.014) 0.902 (0.015) 0.869 (0.015) 0.857 (0.024)
Spine BMC 38.29 (0.89) 41.11 (0.93) 39.46 (0.98) 40.24 (1.51)
BA 44.49 (0.53) 45.28 (0.55) 44.65 (0.58) 45.62 (0.90)
Arms BMD 0.752 (0.006) 0.754 (0.007) 0.752 (0.007) 0.726 (0.011)
BMC 211.40 (3.15) 209.28 (3.19) 214.77 (3.41) ^d 195.68 (5.22)
BA 276.16 (3.12) 275.13 (3.16) 280.95 (3.38) ^d 261.75 (5.17)
Legs BMD 1.060 (0.011) 1.148 (0.011) ^{aa,cc,dd} 1.068 (0.012) 1.031 (0.018)
BMC 713.53 (10.85) 799.47 729.54 (11.51) 707.81 (17.72)
BA 666.45 (6.82) (11.13) ^{aa,cc,dd} 675.52 (7.23) 670.46 (11.13)
691.97 (6.99)
Pelvis BMD 0.954 (0.012) 1.047 (0.013) ^{aa,c,dd} 0.988 (0.014) 0.951 (0.021)
BMC 216.33 (4.61) 255.68 (4.78) aa,cc,dd 227.69 (5.03) 218.61 (7.76)
BA 224.41 (2.76) 242.12 (2.86) ^{aa,cc,dd} 226.18 (3.01) 222.39 (4.65)

TBLH: Total body less head. Values are presented as mean ± SE. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and p<0.001. Adjusted for age, height, calcium intake, MVPA and region-specific lean mass.

4.4.3 Bone geometry - Hip Structural Analysis

The adjusted geometrical differences in narrow neck site between the groups are presented in Figure 4.3 and the raw differences are presented in supplementary table 4.2. Footballers had a significantly higher CSMI than controls (17.4 %), greater section modulus than cyclists (10.7 %) and controls (21.0 %), significantly higher CSA than swimmers (10.8 %), cyclists (8.7 %) and controls (19.3 %) and a significantly greater hip strength index than swimmers (20.7 %) and controls (38.9 %). Cyclists had only a significantly higher hip strength index compared to controls (28.6 %). Cyclists had non-significant higher geometrical outcomes compared to controls (CSMI: 6.4 %, Section modulus: 9.3 %, CSA: 9.8 %). Swimmers had non-significant higher geometrical outcomes compared to controls (CSMI: 7.8 %, Section modulus: 10.9 %, CSA: 7.6 %, hip strength index: 15.1 %). Cyclists compared to swimmers had similar geometrical outcomes with minimal differences.

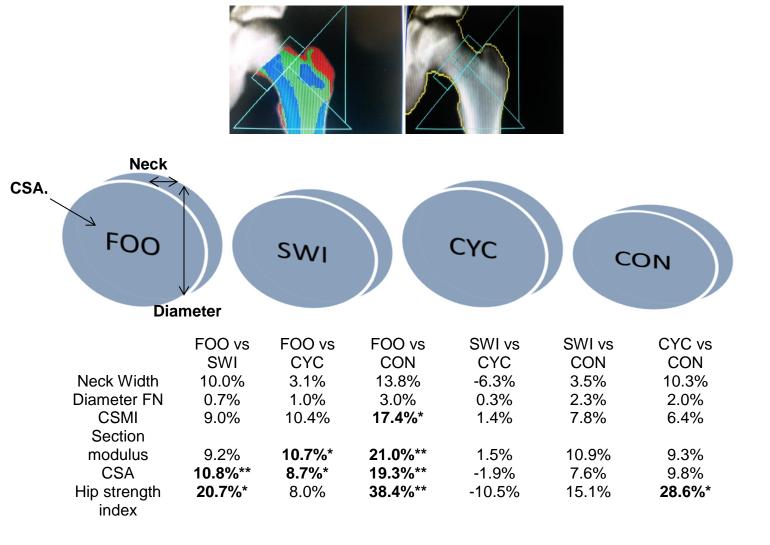


Figure 4.3. Percentage of difference in adjusted geometrical parameters of the narrow neck site between groups. Neck Width (mm), CSA: Cross sectional area, CSMI: Cross sectional moment of inertia, FN: femoral neck.* p<0.05 and ** p<0.001.

Supplementary table 4.2. Raw and adjusted data for Hip Structural Analysis (HSA) and

Quantitative Ultrasound (QUS) parameters of all participants

Parameters	Swimmers	Footballers	Cyclists	Controls
	(n=41)	(n=37)	(n=29)	(n=14)
Neck Width (mm)	5.9 (1.9)	6.7 (1.8)	6.5 (2.0)	5.3 (1.3)
Adjusted Neck Width (mm)	6.0 (0.3)	6.6 (0.3)	6.4 (0.3)	5.8 (0.5)
Diameter of FN (mm)	31.4 (2.7) ^d	30.5 (2.8)	30.9 (2.6) ^d	28.4 (2.5)
Adjusted Diameter of FN (mm)	30.7 (0.3)	30.9 (0.3)	30.6 (0.3)	30.0 (0.5)
CSMI (mm ⁴)	8944 (2574) ^d	8471 (2607) ^d	8403 (2552) ^d	6021 (2673)
Adjusted CSMI (mm ⁴)	8212 (244)	8947 (250) ^d	8102 (259)	7618 (398)
Section Modulus (mm ³)	558.3 (121.4) ^{dd}	548.1 (116.7) ^{dd}	530.8 (123.3) ^{dd}	395.0 (123.4)
Adjusted Section Modulus (mm ³)	523.6 (12.0)	571.5 (12.3) ^{c,dd}	516.1 (12.7)	472.2 (19.6)
CSA (mm²)	137.2 (20.2) ^{dd}	140.9 (20.4) ^{dd}	135.9 (22.7) ^d	109.8 (21.0)
Adjusted CSA (mm ²)	131.1 (2.3)	145.3 (2.4) ^{aa,c,dd}	133.7 (2.4)	121.8 (3.8)
Hip Strength Index	1.45 (0.35)	1.75 (0.37) ^{a,dd}	1.62 (0.34) ^d	1.26 (0.37)
NA OLDanain and	04 C (40 0)d	400.4	00 7 (44 F)d	04.0 (44.0)
SI Dominant	91.6 (13.2) ^d	100.4 (12.4) ^{a,dd}	92.7 (11.5) ^d	81.3 (11.2)
Adjusted SI Dominant Foot	89.6 (2.0)	101.6 (2.1) ^{aa,c,dd}	92.1 (2.2)	84.3 (3.3)
SI Non-Dominant Foot	89.6 (11.9)	97.8 (10.5) ^{a,dd}	92.7 (13.8)	83.1 (12.2)
Adjusted SI Non-Dominant Foot	87.9 (1.9)	98.6 (1.9) ^{a,d}	91.9 (2.0)	86.0 (2.9)

Values presented as mean ± SD. CSA: Cross sectional area, CSMI: Cross sectional moment of inertia, FN: femoral neck. NA: Not Applicable adjustment for Hip Strength Index. SI: Stiffness Index. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls). a,b,c,d p<0.05, aa,bb,cc,dd p<0.001. Adjusted for age, height, calcium intake, MVPA and region-specific lean mass.

4.4.4 Quantitative ultrasound

The adjusted bone stiffness values of the dominant and non-dominant foot are presented in Figure 4 and the raw differences are presented at supplementary table 2. Footballers had significantly higher bone stiffness in the dominant foot than swimmers (13.4 %), cyclists (10.3 %) and controls (20.1 %). In addition, footballers had significantly greater bone stiffness than swimmers (12.2 %) and controls (12.9 %) at the non-dominant foot. No significant differences were found between dominant vs. non-dominant foot within each group of participants. Cyclists had higher (non-significant) stiffness index compared to controls in both dominant (8.9 %) and non-dominant foot (5.3 %). Swimmers had higher (non-significant) bone stiffness at the dominant (5.9 %) and the non-dominant (0.7 %) foot. Cyclists compared to swimmers had higher (non-significant) bone stiffness at the dominant (2.7 %) and the non-dominant (4.4 %) foot.

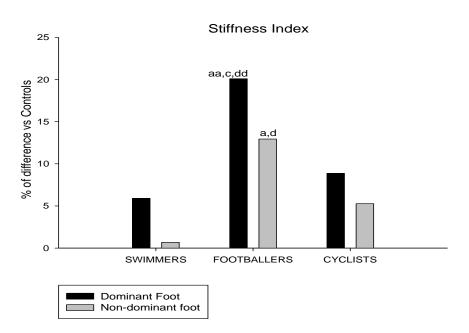


Figure 4.4. Difference (%) in adjusted stiffness index (SI) (mean±SE) between the sports groups and the controls at the dominant and non-dominant foot. Letters denote a higher significant difference with: a (swimmers), c (cyclists) and d (controls). a,b,c,d p<0.05 and

aa,bb,cc,dd p<0.001. No differences observed between dominant and non-dominant foot at the groups.

4.5 Discussion

The key findings from this study are: 1) footballers presented greater adjusted BMD and BMC including clinical relevant sites, an enhanced hip structural geometry at the narrow neck and a greater bone stiffness index compared to swimmers, cyclists and controls, and 2) swimmers and cyclists had similar bone mass, geometry and bone stiffness and both groups had higher but not significant bone outcomes compared to controls. The impact of osteogenic (football) and non-osteogenic sports (swimming and cycling) on bone-related outcomes has not previously been compared in adolescent male athletes and there is equivocal evidence on the effects of these sports on bone outcomes (Vicente-Rodriguez et al., 2004b, Olmedillas et al., 2011, Gomez-Bruton et al., 2015). To date, there are no studies published using a combination of methods such as DXA, HSA and QUS to assess bone outcomes in this population and there are a lack of studies taking into consideration the relevant confounders based on the characteristics of the groups studied. The findings of the present study therefore provide a more comprehensive assessment into the effect of sports participation on bone outcomes in male adolescents.

4.5.1 Bone outcomes in footballers vs controls

Participation in osteogenic sports during adolescence can induce greater adjusted BMD compared to leisure active controls at many sites of the skeleton due to the mechanical loading applied (Maimoun et al., 2013b). A previous study reported a 10.7 % and 10.5 % higher adjusted BMC at the total hip and

lumbar spine respectively, in prepubescent male football players (n= 39) compared to active controls (n= 13) (Zouch et al., 2008). The magnitude of the differences might differ among studies due to the use of different confounders and the characteristics of the participants.

In parallel with the findings for BMD and BMC, the geometrical adaptations examined by HSA at the narrow neck of the femoral neck also supported the higher bone geometry in footballers (Figure 3). One study in oligoamenorrheic female athletes showed that engagement in weight-bearing sports for 4 hours per week resulted in significantly higher CSMI and section modulus compared to non-athletes (Ackerman et al., 2013), which is consistent with the improved structural rigidity we found in footballers.

Previous studies using QUS technique observed positive associations between PA and calcaneal bone stiffness index in a sample of Flemish children and adolescents (De Smet et al., 2015). Our results are in agreement with a study reporting that child and adolescent football players have significantly higher QUS parameters at lower extremities compared with age matched controls (Falk et al., 2010).

4.5.2 Bone outcomes in swimmers vs controls

A recent meta-analysis of fourteen studies summarized that swimming does not induce improvements in BMD during childhood and adolescence and that swimmers present similar BMD compared to sedentary controls (Gomez-Bruton et al., 2016b). We found similar BMD and BMC between swimmers and active controls concurs with this meta-analysis, which presents neutral effects of swimming on BMD and BMC at most skeletal sites of the skeleton compared to active controls. The latter could be due to the fact that swimmers and controls

have similar bone profile as muscle contraction is not enough to produce bone adaptations (Maimoun et al., 2013b).

The HSA at the narrow neck site showed that adolescent male swimmers have similar bone geometry parameters compared to active controls. To our knowledge there is no previous evidence using the HSA technique in adolescent male swimmers. Only one study have used HSA in elite adolescent female swimmers and showed that they had similar bone geometry compared with controls (Maimoun et al., 2013b). The latter study highlighted the importance of lean body mass as it was highly correlated with CSA and hip strength index.

Regarding QUS, we found similar bone stiffness index in both dominant and non-dominant foot of swimmers compared to controls. A previous study in adolescents reported similar QUS parameters between swimmers and controls and indicated that bone adaptations due to swimming might be counterbalanced by other weight-bearing activities (up to 3 hours per week) (Gomez-Bruton et al., 2015), however this cannot be the case in our study because the QUS parameters were controlled for MVPA.

4.5.3 Bone outcomes in cyclists vs controls

A systematic review revealed that road cyclists did not have any osteogenic benefits due to the non-mechanical loading character of the sport (Olmedillas et al., 2012). A previous study conducted in adolescent female cyclists showed they had similar BMD compared to non-athletic controls after adjusting for years since menarche, lean mass and sport specific training (Duncan et al., 2002b). According to our study, the skeletal differences between cyclists and active controls are site dependent and more specifically we found significantly greater

BMC at the arms after controlling for region-specific lean mass and MVPA among other confounding factors.

There is no previous evidence using HSA technique in adolescent cyclists and only a few studies used volumetric bone parameters. One study conducted in adolescent female cyclists found no significant differences in CSA and CSMI in cyclists compared to controls (Duncan et al., 2002b) which is in accordance with our study. However, it should be noted that in our study the HSA revealed cyclists had significantly higher hip strength index than controls (28.6 %), something that was not observed with BMD and BMC at any of the hip variables analysed with DXA. This might be explained by the fact that geometrical bone outcomes may differ from BMD and BMC when using DXA.

The effect of cycling on bone properties using QUS has not been previously evaluated and to the best of our knowledge this study is first that provides evidence for this population. We did not find differences on stiffness index between cyclists and controls, but cyclist had non-significant higher stiffness index in both the dominant (8.9 %) and non-dominant (5.3 %) foot compared to controls. Our results support the findings of previous studies that the loading pattern of sports participation may influence the bone stiffness index in adolescents (Falk et al., 2003).

4.5.4 Comparison of bone outcomes between footballers, swimmers and cyclists

In the present study the comparison between osteogenic (football) and nonosteogenic sports (cycling and swimming) showed that adolescent male footballers had significantly greater adjusted BMD and BMC compared to swimmers and cyclists at all sites of the skeleton except for the lumbar spine and the arms. A previous study in athletic adolescent females reported that 3 hours per week of football participation induced greater improvements in height and lean mass adjusted BMD and BMC compared to swimmers at femoral neck and other sites of the skeleton (Ubago-Guisado et al., 2015a). Only one study investigated the effects on bone mass between adolescent male footballers and swimmers and reported greater BMD at the femoral neck site in the footballers (Silva et al., 2011). To our knowledge, no previous evidence exists on the assessment of bone mass between footballers and cyclists in adolescents. Only one study in children reported positive associations between BMD and football participation and negative associations found between BMD and cycling participation (Slemenda et al., 1991).

In our study there was no significant difference observed in BMD and BMC between adolescent male swimmers and cyclists at any site of the skeleton. A recent review summarised the impact of sport participation on peak bone mass and it reported that both swimming and cycling may not be associated with significant improvements in bone health (Tenforde et al., 2015). The comparison of BMD and BMC between swimmers and cyclists has been assessed only once in female adolescents, reporting similar values at all skeletal sites after taking into account potential confounders (Duncan et al., 2002b). The differences observed in the current study are likely to be explained by the non weight-bearing environment of both swimming and cycling and by the mechanical loading of the skeleton according to the impact of produced by the sport specific patterns. In addition, weight training and the plyometric exercises might induce higher bone mass in adolescent athletes (Gunter et al., 2008a). A study in adolescent swimmers has shown that participation of adolescent swimmers in other weight-bearing sports or activities involving

plyometric exercises can induce higher BMD and BMC (Gomez-Bruton et al., 2015). In our study, a subsample of our participants has been asked about weight training and we have shown that almost all footballers were involved in plyometric exercise training. A large number (70.7 %) of the swimmers reported participation in plyometric training, but only a few cyclists (37.9 %) were doing plyometric exercises. The participation in plyometric training or other weight-bearing activities might explain the difference on bone outcomes between adolescent athletes and needs further investigation to quantify the impact of weight training on bone outcomes.

In parallel with the BMD and BMC findings, the bone geometry evaluated by HSA at the narrow neck of the femoral neck was also higher in footballers compared to swimmers and cyclists. Previous research in adolescent female footballers and swimmers showed that the CSA area and section modulus were significantly higher in footballers compared to swimmers at the narrow neck site which is in agreement with our results (Ferry et al., 2011). There is no evidence comparing football and cycling in children and adolescents and the only evidence exists in young females (21 - 28 years) which found that footballers had approximately 10 % higher CSA compared to cyclists and after adjusting for age, weight and height, these results are in accordance with our findings (Nikander et al., 2005).

In relation to QUS parameters we found improved bone stiffness in footballers compared to cyclists and swimmers at the dominant foot and higher bone stiffness in footballers compared to swimmers at the non-dominant foot. As there is no evidence using QUS in similar age athletic groups, we identified a study in young adults (18-22 years) that reported higher bone stiffness in

footballers compared to swimmers at the dominant and non-dominant heels (Yung et al., 2005) which complies with the findings of the present study.

The strengths of the current study are 1) the investigation of bone outcomes across three male adolescent athletic groups that were not compared before: 2) the combination of DXA, HSA software and QUS outcomes which provides a thorough insight of the differences in BMD, BMC, bone geometry and bone stiffness; 3) the rigorous methodology and strong internal validity to control for specific confounders. It should be noted that the limitation of the cross-sectional study precludes any determination of causality in our findings. Nevertheless, our population had strict age inclusion criteria and sport participation characteristics. The limitations of the self-reported maturation assessment should be noted. Most of the participants of our control group met the physical activity guidelines due to inclusion criteria used, but we know that most adolescents of this age do not meet the guidelines (Riddoch et al., 2007). However, in this specific case, it seems reasonable to propose that sport participation could have different effects on bone-related outcomes depending on the characteristics of the sport practiced. Therefore, more studies are needed to focus on the determinants affecting bone health for athletic groups during youth.

4.6 Conclusions

This study is the first to investigate the impact of weight-bearing (football) and non weight-bearing sports on bone outcomes in adolescent males. The findings of this study indicate that participation in weight-bearing sports, such as football, can induce greater improvements in bone mass, bone geometry and stiffness index compared to non weight-bearing sports, such as swimming and cycling and compared to controls. Swimmers and cyclists had similar bone outcomes

and both groups had higher bone outcomes compared to controls, but these differences were not statistically significant. These findings add to the sport participation recommendations that specific musculoskeletal training may affect the bone development during adolescence. Further longitudinal analyses of the specific sports are needed for this population in order to identify if these effects will be different after a longer period of sports participation.

5. Determinants of bone outcomes in adolescent athletes at baseline

5.1 Abstract

Purpose: The determinants of areal bone mineral density (aBMD) and hip geometry estimates in adolescent athletes are poorly understood. This study aimed to identify the determinants of aBMD and hip geometry estimates in adolescent male athletes. Methods: One hundred and twenty-one males (13.1±0.1 years) were measured: 41 swimmers, 37 footballers, 29 cyclists and 14 controls. Dual energy X-ray absorptiometry (DXA) measured aBMD at lumbar spine, femoral neck (FN) and total body. Hip structural analysis evaluated hip geometry estimates at the FN. Multiple linear regression examined the contribution of the sports practised, height, lean and fat mass, serum calcium and vitamin D, moderate to vigorous physical activity (MVPA), vertical jump and cardiorespiratory fitness (CRF) with aBMD and hip geometry estimates. Results: Region specific lean mass was the strongest positive predictor of aBMD (β = 0.614 - 0.931) and football participation was the next strongest predictor ($\beta = 0.304 - 0.579$). Height ($\beta = 0.235 - 0.380$), fat mass ($\beta = 0.380$) 0.189), serum calcium (β = 0.103), serum vitamin D (β = 0.104 - 0.139) and vertical jump (β = 0.146 - 0.203) were associated with aBMD across various specific sites. All hip geometry estimates were associated with lean mass (β = 0.370 - 0.568) and height ($\beta = 0.338 - 0.430$). Football participation was associated with hip cross-sectional area (β = 0.322) and MVPA (β = 0.140 -0.142). CRF (β = 0.183 - 0.207) was associated with section modulus and cross-sectional moment of inertia. Conclusions: Region specific lean mass is the strongest determinant of aBMD and hip geometry estimates in adolescent male athletes. Football participation and height were important determinants for aBMD and hip geometry estimates while the contribution of the other predictors was site specific.

Keywords body composition; bone mass; exercise, lean mass, predictors, sport participation.

5.2 Introduction

During growth and maturation changes in bone density and geometry occur in order to withstand the forces applied through external loading of the skeleton (Frost, 2003). Peak bone mass is achieved by early adulthood and is largely determined by non-modifiable genetic factors (Duren et al., 2007). However, modifiable factors, such as nutrition (Mouratidou et al., 2013) and physical activity (Gracia-Marco et al., 2011a), are also known to alter peak bone mass. Exercise can significantly enhance areal bone mineral density (aBMD) and strength at loaded sites in children but not in adults (Nikander et al., 2010). Optimal bone development can be achieved with adequate status of key nutrients, such as calcium and vitamin D, and may attenuate exercise-induced adaptations of aBMD (Harvey et al., 2012). The type of sport practised can affect the skeletal development differently depending on training characteristics (Ubago-Guisado et al., 2015a). Participation in weight bearing sports, such as football, is associated with greater areal bone mineral density than non-weight bearing sports, such as swimming and cycling (Ferry et al., 2011, Vlachopoulos et al., 2017c). However, it is poorly understood why the differences exist between different athletic groups and there is limited understanding of the determinants of aBMD and hip geometry in adolescent male athletes.

Total body lean mass has a positive association with aBMD in the growing skeleton (Gracia-Marco, 2016) but controversy currently exists surrounding the association between fat mass and aBMD (El Hage et al., 2009). There is no evidence distinguishing the site specific effects of lean mass and fat mass on aBMD in adolescent athletes and there are inconsistencies in the use of confounders to adjust bone parameters in non-athletic groups. Data on nonathletic prepubescent females indicate that leg lean mass is the most important predictor of bone mineral content at the leg and femoral neck sites (Daly et al., 2008). Although a positive association between fat mass and aBMD has been reported in non-athletic adolescents males and females (Sayers and Tobias, 2010), this is explained by an increase in lean mass (Gracia-Marco et al., 2012). To date, there is no evidence explaining the effects of lean and fat mass on bone outcomes in adolescent athletes, which is of great interest due to the importance of body composition in athletic groups. Cardiorespiratory (CRF) and muscular fitness (vertical jump) have also been found to be positively associated with bone outcomes in non-athletic adolescents (Gracia-Marco et al., 2011c, Baptista et al., 2016), but their contribution on bone parameters in adolescent athletes is poorly understood.

Geometric properties of the hip, such as cross-sectional area (CSA), obtained by using hip structural analysis (HSA) software, can provide further insight into the determinants of bone hip geometry estimates (Khoo et al., 2005). During growth, bones adapt their geometry due to increases in height, lean and fat mass (Daly et al., 2008) and geometric parameters of the femur neck (FN) are closely adapted to lean mass (Petit et al., 2005). The primary predictor of bone hip geometry in non-athletic boys and girls is muscle CSA, accounting for 10 – 16 % of the variance (Macdonald et al., 2006), while other factors such as

moderate to vigorous physical activity (MVPA) can have a site specific influence on bone geometry (Michalopoulou et al., 2013).

As highlighted above, numerous factors have been shown to be related to bone outcomes in non-athletic adolescents, but the determinants of aBMD and hip geometry in adolescent male athletes have yet to be comprehensively investigated. Therefore, this study aims to provide novel insight into the contribution of the independent predictors of sports participation (football, swimming and cycling), height, region specific lean and fat mass, serum calcium and vitamin D, MVPA, muscular fitness and CRF (all adjusted by each other) on aBMD and hip geometry estimates in adolescent male athletes. It is hypothesized that football participation, lean mass and height would be the most important determinants of aBMD and hip geometry estimates in adolescent male athletes. It is proposed that other modifiable factors (e.g. nutrition, MVPA and fitness) would have a small but significant contribution on bone outcomes.

5.3 Methods

5.3.1 Study design and participants

Participants comprised 121 adolescent males (41 swimmers, 37 footballers, 29 cyclists and 14 controls) participating in the PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) longitudinal study (Vlachopoulos et al., 2015). The data in the current study are taken from the baseline data of the PRO-BONE study and was completed between autumn and winter 2014/15. The inclusion and exclusion criteria were: 1) males 12–14 years old, engaged (≥3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or more; 2) active males 12–14 years old who were not engaged in

football, cycling and swimming (≥3 h/week) in the last 3 or more years but who were physically active (control group); 3) not taking part in another clinical trial; 4) not having an acute infection lasting until < 1 week before inclusion; 5) to be free of any medical history of diseases or medications affecting bone metabolism; 6) to be white Caucasian.

Participants were recruited from athletic clubs and schools across the South West of England. Written informed consent and assent forms were signed from parents and participants accordingly and all participants completed the first visit at the research centre as part of the study. The methods and procedures of the study have been checked and approved by: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

5.3.2 Dual energy x-ray absorptiometry

A dual energy X-ray absorptiometry (DXA) scanner (GE Healthcare Inc., Wisconsin, USA) was used to measure aBMD (g/cm²), fat mass (g) and lean mass (g) at specific regions of the body. Four scans were performed to obtain data for the lumbar spine (LS, L1-L4), bilateral proximal femora (the mean of both was used for the current analysis) and the total body. The total body scan was then used to obtain data for specific regions such as: arms, legs and total body less head (TBLH). All DXA scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the GE encore software (2006, version 14.10.022).

5.3.3 Hip structural analysis

Hip geometry estimates at the FN were determined using HSA software which analyses the distribution of bone mineral mass in a line of pixels across the bone axis. The hip geometry estimates of the bone were obtained and the following variables used: 1) the cross sectional area (CSA, mm²), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia (CSMI, mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; and 3) section modulus (Z, mm³), which is an indicator of maximum bending strength in a cross section. The short term precision percentage coefficient of variation of these variables has been reported to be between 2.4 % and 10.1 % (Khoo et al., 2005).

5.3.4 Anthropometry, physical activity and nutritional markers

Height (cm) and body mass (kg) were measured using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Body mass index was calculated as body mass (kg) divided by the height (m) squared. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair (Tanner and Whitehouse, 1976).

Physical activity was measured for seven consecutive days using validated wrist accelerometers (GENEActiv, GENEA, UK) (Esliger et al., 2011). Participants were instructed to place the accelerometer on their non-dominant wrist and data was collected at 100 Hz. Data were analysed using 1 s epoch to establish time spent in MVPA using a cut-off point of ≥ 1140 counts per minute previously validated in youth (Phillips et al., 2013).

Total serum levels of calcium and 25 hydroxyvitamin D [25(OH)D] were analysed. Serum samples were analysed by using ELISA kits (Abbexa Ltd., Cambridge, UK) for 25(OH)D and had a test range of 3 - 80 μg·mL⁻¹ and a sensitivity of 1.2 μg·mL⁻¹ (inter and intra-assay CVs: 5.7 % and 9.5 % respectively). Total serum levels of calcium was measured using direct colorimetric assay (Cayman Chemical Company, MI, U.S.A.) and had linear assay range of 0.25-10 mg·mL⁻¹ (inter and intra-assay CVs: 8.1 % and 12.8 % respectively).

5.3.5 Physical fitness

The fitness tests used in the present investigation have been shown to be reliable and valid in youth (Ortega et al., 2008). A counter movement vertical jump test was used to provide an estimate of lower limb muscular power. The jumps were performed on a jump mat (Probotics Inc., Huntsville, USA) which calculates jump height based on flight time. Each participant performed three maximal vertical jumps and the highest jump was used for the analysis.

Cardiorespiratory fitness was evaluated using the 20 m shuttle run test (Leger et al., 1988). The test ended when the participants failed to reach the line on two consecutive occasions. The last completed shuttle determined the score of the test and the number of shuttles completed was taken as an indicator of CRF.

5.3.6 Statistical analyses

Data were analysed using SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA) and descriptive data are reported as mean and standard deviation (SD). The normal distribution of the raw variables and of the regression model residuals was checked and verified using Shapiro-Wilk's test, skewness and kurtosis values, visual check of histograms, Q-Q and box plots.

Collinearity was checked for the variables using the variance inflation factor (VIF) and tolerance levels. One way analysis of variance (ANOVA) with Bonferroni post hoc comparisons and Chi-Square tests were used to detect between-group mean differences for the descriptive variables (table 1).

Multiple linear regression analyses were used to examine the contribution of sport participation, height, lean mass, fat mass, total calcium, 25(OH)D, MVPA, vertical jump and 20 m shuttle run test to bone outcomes. The selection of the predictors was based on their relationship with bone outcomes (Petit et al., 2005, Macdonald et al., 2006, Sioen et al., 2012). To account for the differences between the sports groups a dummy variable was computed (footballers, swimmers, cyclists and controls) and controls were selected as the reference group. In a preliminary analysis we found that Tanner stage was not a significant predictor after adjusting for height and age and consequently was not included in the model. All remaining predictors were entered into the regression models simultaneously. For the multiple linear regressions, the standardised regression coefficients (β) are reported and significance was set at alpha level of 0.05. The squared semi-partial correlation coefficients (sr^2) were used to determine the contribution of each predictor in the overall variance of the model after removing shared contributions with other predictors.

5.4 Results

5.4.1 Characteristics of the participants

Table 5.1. Descriptive characteristics of the participants

Characteristics	All	Swimmers	Footballers	Cyclists	Controls
Ondi deteristics	(n=121)	(n=41)	(n=37)	(n=29)	(n=14)
Age (yrs) Height (cm) Pubertal maturation (I/II/III/IV/V) (%) Body composition	13.1 (1.0) 159.9 (10.6) (18/29/21/30/2)	13.4 (1.0) ^{b,dd} 165.5 (9.7) ^{bb,d} (13/25/13/46/3	12.8 (0.9) 155.2 (9.3) (24/35/25/16)	13.2 (1.0) ^d 160.8 (9.9) (13/28/28/28/3	12.3 (0.5) 154.5 (9.9) (29/21/21/29)
Body mass (kg) BMI (kg/m²) Lean mass (kg) Fat mass (kg)	48.7 (10.4) 18.9 (2.3) 37.6 (8.4) 8.5 (5.5)	52.4 (9.0) ^{bb} 19.0 (1.7) 41.6 (9.1) ^{b,dd} 8.3 (3.2)	44.3 (7.9) 18.3 (1.4) 35.4 (7.2) 6.6 (2.4)	49.5 (12.3) 18.9 (3.3) 37.7 (7.5) 8.6 (7.2)	48.3 (13.0) 20.0 (3.4) 31.7 (5.5) 14.1 (8.5) ^{a,bb,c}
Micronutrient status Total Calcium (mg/dl) 25 (OH)D (μg/l) Physical activity and fitness	9.98 (0.41) 14.13 (1.25)	10.01 (0.46) 13.75 (1.19)	9.97 (0.4) 14.44 (1.63)	9.94 (0.41) 14.38 (0.58)	10.0 (0.35) 13.92 (0.94)
MVPA (min/day) Vertical jump height (cm) CRF (No of shuttles) Weekly training hours (h) Bone mineral density (DXA)	101.3 (33.8) 41.0 (6.7) 69.3 (24.2) 7.5 (4.8)	85.9 (30.4) 42.3 (6.9) ^d 69.6 (20.3) ^{dd} 9.5 (5.1) ^{cc}	119.8 (29.7) ^{aa,d} 41.4 (6.0) ^d 82.9 (17.6) ^{a,c,dd} 10.0 (2.3) ^{cc}	107.2 (33.3) ^a 41.0 (6.8) 69.6 (21.2) ^{dd} 5.1 (2.1)	83.2 (26.8) 35.9 (5.8) 31.8 (16.1)
TBLH BMD (g/c m²) Lumbar Spine BMD (g/cm²)	0.908 (0.079) 0.872 (0.112)	0.918 (0.067) ^d 0.892 (0.114) ^d	0.931 (0.071) ^{dd} 0.883 (0.095)	0.905 (0.086) ^d 0.867 (0.122)	0.828 (0.071) 0.791 (0.101)
Femoral Neck BMD (g/c m ²)	0.9516 (0.110)	0.948 (0.098) ^d	1.001 (0.081) ^{dd}	0.975 (0.192) ^d	0.832 (0.118)
Total Hip BMD (g/c m²)	0.968 (0.119)	0.962 (0.107) ^{dd}	1.034 (0.085) ^{a,c,dd}	0.959 (0.116) ^{dd}	0.830 (0.116)
Legs BMD (g/c m ²) Arms BMD (g/c m ²)	1.084 (0.113) 0.750 (0.068)	1.091 (0.010) 0.784 (0.071) ^{b,dd}	1.124 (0.106) 0.736 (0.047)	1.077 (0.116) 0.747 (0.069) ^d	0.975 (0.103) 0.690 (0.049)
Bone geometry (HSA) CSA (mm²) Z (mm³) CSMI (mm⁴)	134.9 (22.7) 530.9 (126.5) 8331.5 (2644)	137.2 (20.2) ^{dd} 558.3 (121.4) ^{dd} 8943.5 (2574) ^d	140.9 (20.4) ^{dd} 548.1 (116.7) ^{dd} 8471.6 (2607) ^d	135.9 (22.7) ^d 530.8 (123.3) ^{dd} 8403.1 (2552) ^d	109.8 (21.0) 395.0 (123.4) 6020.7 (2673)

Values presented as mean ± SD. BMD: Bone mineral density, BMI: Body mass index, CRF: Cardiorespiratory fitness, CSMI: Cross sectional moment of inertia, CSA: Cross sectional area, DXA: Dual-energy X-ray absorptiometry, MVPA: Moderate to vigorous physical activity, Z: section modulus, 25(OH)D: 25-hydroxyvitamin D. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls), a,b,c,d p<0.05, aa,bb,cc,dd p<0.001.

The raw descriptive characteristics of the participants and the differences between sports groups are presented in Table 5.1. Swimmers were significantly older, taller, heavier and had more lean mass than the footballers and controls, and cyclists were significantly older than controls. All groups were similar for total serum calcium and 25(OH)D. Swimmers had significantly higher muscular and CRF than the controls. Footballers spent significantly more time in MVPA compared to swimmers and controls and had a significantly higher CRF compared to all the other groups. Cyclists had significantly higher MVPA than swimmers and significantly higher CRF than controls.

The unadjusted data showed that swimmers had significantly higher aBMD at the arms compared to footballers and higher aBMD at all sites except for the legs compared to controls. Footballers had significantly higher aBMD at TBLH, FN compared to controls and higher aBMD at TH compared to all groups. Cyclists had significantly higher aBMD at all sites expect LS and legs compared to controls. Swimmers, footballers and cyclists had significantly enhanced hip geometry estimates compared to controls.

Table 5.2. Multiple regression models for aBMD variables in adolescent male athletes

1 abie 5.2.	Predictors		eis for ai	P var	iables in ado	lescent male atl		sr ²	Р
	riedictors	β STD	sr- value	value		riedictors	β STD	sr- value	value
		טוט	value S	value S			310	value S	value S
TBLH	Footballers	.374	.031	<.001	Total Hip	Footballers	.549	.068	<.001
aBMD	Swimmers	.077	.002	.404	aBMD	Swimmers	.161	.007	.211
$(R^2=0.75)$	Cyclists	.139	.002	.114	$(R^2=0.53)$	Cyclists	.212	.014	.080
(14 -0.70)	Height	.056	.000	.662	(11 =0.00)	Height	.216	.007	.215
	Lean mass	.617	.045	<.001		Lean mass	.226	.006	.238
	Fat mass	.189	.015	.013		Fat mass	020	.000	.857
	Calcium	.082	.006	.125		Calcium	.109	.010	.137
	25(OH)D	.083	.006	.120		25(OH)D	.035	.001	.628
	MVPA	003	.000	.955		MVPA	.026	.000	.741
	Vertical	.146	.010	.043		Vertical jump	.133	.008	.192
	jump	.136	.006	.115		CRF	.102	.003	.382
	CRF					.			.00_
Lumbar	Footballers	.094	.002	.475	Legs	Footballers	.304	.034	<.001
Spine	Swimmers	061	.001	.603	aBMD	Swimmers	.024	.002	.689
aBMD	Cyclists	008	.000	.945	$(R^2=0.75)$	Cyclists	061	.003	.347
$(R^2=0.59)$	Height	090	.001	.582		Height	.068	.001	.596
	Lean mass	.703	.058	<.001		Lean mass	.614	.046	<.001
	Fat mass	014	.000	.885		Fat mass	.147	.008	.067
	Calcium	.084	.006	.222		Calcium	.055	.003	.305
	25(OH)D	.139	.016	.043		25(OH)D	.067	.004	.212
	MVPA	.007	.000	.927		MVPA	021	.000	.711
	Vertical	.203	.019	.028		Vertical jump	.107	.005	.153
	jump	.000	.000	1.000		CRF	.153	.008	.076
	CRF								
F	Coothallas:	400	050	004	A	Caathallau-	4.40	004	404
Femur	Footballers	.486	.053	.001	Arms aBMD	Footballers	.140	.004	.161
Neck aBMD	Swimmers	.131	.005	.326		Swimmers	.170	.008	.058
авии (R ² =0.49)	Cyclists	.208	.013	.099 .038	$(R^2=0.76)$	Cyclists	.158 .235	.008 . 013	.066 .016
(K =0.49)	Height	.380 .052	.021 .000	. 036 .792		Height	.235 .931	.013 .161	.016 <.001
	Lean mass Fat mass	.052 .074	.000	.792 .515		Lean mass Fat mass	.132	.007	.069
	Calcium		.002	.311		Calcium	.132 .103	.007 . 009	.069 . 049
	25(OH)D	.077 .015	.005	.311 .847		25(OH)D	.103	.009	.049 .045
	MVPA	.015	.000	.635		MVPA	037	.009	. 503
	Vertical jump	.039	.001	.083		Vertical jump	037 .058	.001	.303
	CRF	.164	.015	.063		CRF	.066	.002	.404 .406
	UNF	. 104	.000	.∠∪0		ONF	.000	.002	.400

β: standardised regression coefficient, aBMD: Areal bone mineral density, CRF: Cardiorespiratory fitness, MVPA: Moderate to vigorous physical activity, sr²: Squared semi-partial correlation coefficients, 25(OH)D: 25-hydroxyvitamin D.

5.4.2 Determinants of bone density and hip geometry estimates

Multivariate regression models significantly explained 49.0% - 76.4% (on average, 60.0%) of the variance in the aBMD outcomes (Table 5.2). Region specific lean mass and football participation were consistently the strongest significant predictors of aBMD at TBLH, LS, TH, legs and arms ($\beta = 0.614$ -0.931, sr² = 0.031 - 0.161, P < 0.01). Football participation (compared to the control group) was positively associated with aBMD at TBLH, FN, TH and legs $(\beta = 0.304 - 0.579, \text{ sr}^2 = 0.031 - 0.068, P < 0.01)$. Height was positively associated with aBMD at FN and arms ($\beta = 0.235 - 0.380$, sr² = 0.021, P < 0.05). Region specific fat mass was positively associated with aBMD at TBLH (β = 0.189, $sr^2 = 0.015$, P < 0.05). Serum calcium was positively associated with aBMD at the arms ($\beta = 0.103$, sr² = 0.009, P < 0.05). In addition, serum 25(OH)D was positively associated with aBMD at the arms and LS (β = 0.104 -0.139, sr² = 0.009, P < 0.05). Muscular fitness was positively associated with aBMD at TBLH and LS ($\beta = 0.146 - 0.203$, sr² = 0.010 - 0.019, P < 0.05). CRF was not associated with aBMD outcomes at any skeletal site after accounting for the other predictors.

Table 5.3. Multiple regression models for bone geometry estimates in adolescent male athletes

	Predictors	β	sr ²	P	-	Predictors	β	sr ²	Р
		STD	value	value			STD	values	values
			S	S					
CSA	Footballers	.322	.023	.004	Z	Footballers	.157	.005	.109
$(R^2=0.72)$	Swimmers	.068	.001	.495	$(R^2=0.78)$	Swimmers	.019	.000	.831
	Cyclists	.123	.005	.190		Cyclists	.005	.000	.951
	Height	.394	.023	.004		Height	.417	.025	.001
	Lean mass	.370	.017	.014		Lean mass	.430	.023	.001
	Fat mass	010	.000	.905		Fat mass	040	.001	.592
	Calcium	.025	.001	.657		Calcium	.016	.000	.755
	25(OH)D	038	.001	.498		25(OH)D	054	.003	.281
	MVPA	.103	.008	.093		MVPA	.142	.014	.010
	Vertical jump	.029	.000	.713		Vertical	024	.000	.734
	CRF	.178	.010	.051		jump	.207	.014	.012
						CRF			
CSMI	Footballers	.064	.001	.506					
$(R^2=0.78)$	Swimmers	030	.000	.732					
(Cyclists	038	.000	.645					
	Height	.338	.017	.005					
	Lean mass	.568	.039	<.001					
	Fat mass	087	.003	.245					
	Calcium	011	.000	.820					
	25(OH)D	048	.002	.334					
	MVPA	.140	.014	.011					
	Vertical jump	090	.003	.199					
	CRF	.183	.011	.024					

CRF .183 .011 .024

β: standardised regression coefficient, aBMD: Areal bone mineral density, CRF: Cardiorespiratory fitness, MVPA: Moderate to vigorous physical activity, sr²: Squared semi-partial correlation coefficients, 25(OH)D: 25-hydroxyvitamin D.

In the multivariate regression analysis of the hip geometry estimates (Table 5.3) the predictors explained 71.7% - 77.8% (on average, 75.7%) of the variance. Region specific lean mass was the strongest significant predictor and was positively associated with CSA, CSMI and Z (β = 0.370 - 0.568, sr² = 0.017 - 0.039, P < 0.05). Football participation (compared to the control group) was positively associated with CSA (β = 0.322, sr² = 0.023, P < 0.01). Height was positively associated CSA, CSMI and Z (β = 0.338 - 0.430, sr² = 0.017 - 0.025, P < 0.001). MVPA was positively associated with CSMI and Z (β = 0.140 - 0.142, sr² = 0.014, P < 0.05). CRF was positively associated with CSMI and Z (β = 0.183 - 0.207, sr² = 0.011 - 0.014, P < 0.05).

5.5 Discussion

The present study aimed to identify, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes involved in football, swimming and cycling. It has recently been shown that football has a beneficial impact on bone outcomes in comparison to cycling and swimming in adolescent males (Vlachopoulos et al., 2017c). However the determinants responsible for these differences are not known for this population. In support with our hypothesis, region specific lean mass was the primary explanatory variable on aBMD and hip geometry estimates at most sites of the skeleton. In addition, we found that only participation in football was a significant predictor of aBMD and hip geometry estimates when contrasted to the control group. Finally, it was observed that modifiable factors such as nutrition status (calcium and vitamin D), MVPA and physical fitness (vertical jump and CRF) had a small but significant contribution to bone density and hip geometry estimates across specific sites of the skeleton.

5.5.1 Determinants of bone mineral density

The determinants explained an important significant variance of aBMD at different skeletal sites (49.0 % - 76.4 %, average 64.5 %) with previous findings in non-athletic population reporting that a similar model of determinants explained 40 % - 83 % of the variance in BMC in prepubertal girls (Daly et al., 2008). Region specific lean mass was consistently the strongest determinant of aBMD at TBLH, LS, legs and arms. A previous study in non-athletic boys and girls reported that total lean mass was the best predictor of total body and lumber spine aBMD, but they did not report the relationship with other sites of the skeleton (El Hage et al., 2009). Another study in non-athletic children found that total lean mass was the strongest predictor of the aBMD at total body, and LS (Hrafnkelsson et al., 2010). However the study did not distinguished the site specific relationship of lean and bone mass which was considered in the present study. It is of great interest to understand the region specific relationship of lean mass and aBMD due to the site specific adaptation of the skeleton during external loading, specifically in athletic populations (Baxter-Jones et al., 2008). It is still not clear to what extent fat mass is associated with aBMD in adolescents and especially after adjusting for confounding factors. In our study we found that region specific fat mass only had a positive association with TBLH aBMD, suggesting that after accounting for other covariates its influence on bone development in athletic male adolescents is negligible, perhaps due to the strong effect of region specific lean mass. In addition, an increase in fat mass in adolescent athletes can have a negative effect on their performance (Ubago-Guisado et al., 2015b).

Football participation was positively associated with aBMD at TBLH, FN, TH and legs. There was no significant contribution of swimming and cycling in

contrast to the control group on aBMD at any sites of the skeleton. The contribution of football on aBMD was independent of lean mass and is likely to be explained by the intermittent and high-intensity characteristics of football that can produce high strains on the skeleton and stimulate bone mineral acquisition (Ubago-Guisado et al., 2015a). The concentric contractions during football generate greater forces compared to cycling and swimming and this might explain the increased skeletal loading in this group (Soderman et al., 2000). In addition, our findings show that height was positively significant associated with aBMD at the arms and FN. Similarly with our results, previously it was found that height had a weak and site specific relationship with BMC at different skeletal sites in non-athletic children (Hrafnkelsson et al., 2010). The movement characteristics of the sports practised seems to be important for bone acquisition and the present study found that football participation is one of the most important determinants, possibly because it includes high intensity concentric contractions that can enhance aBMD in adolescents.

Both MVPA and nutrition (calcium and vitamin D) are considered to be essential for optimal bone growth, but their contribution was diminished once other factors (e.g. height, lean mass, sports participation) were considered. In the present analysis we found that blood serum calcium and 25(OH)D had a small contribution on aBMD at the arms and only 25(OH)D contributed to aBMD at LS. Previous findings indicated that dietary calcium and 25(OH)D can have a weak, but significant contribution on specific sites of the skeleton in adolescents (Macdonald et al., 2006). The sites of the skeleton, such as arms, are less loaded through sport and nutritional factors may have a potential influence. The site specific relationship between nutrition and bone outcomes can be attributed to the interactions of nutrients in relation to bone health (Ilich and Kerstetter,

2000). In relation to the contribution of MVPA and fitness on aBMD, we found that vertical jump height was the only significant predictor of aBMD at TBLH and LS. These findings show that overall MVPA does not appear to be important once participation in a particular sport is considered in the regression model. This suggests the characteristics of the sport practised and the contribution of lean mass mediates the relationship between fitness and bone outcomes (Vicente-Rodriguez et al., 2008).

5.5.2 Determinants of hip geometry estimates

Using the HSA method we showed that the multiple regression model can explain a large proportion of the variance (71.7 % - 77.8 %, average 75.7 %) in geometrical parameters (CSA, CSMI, Z) at the narrow neck site of the hip. To the best of our knowledge, this is the first study examining the determinants of HSA outcomes in adolescent athletes. The strongest predictor for the geometrical parameters was the region specific lean mass followed by height. The contribution of region specific lean mass was consistent for all the geometrical parameters. The findings of the present study highlight the influence of region specific lean mass on hip geometry estimates during adolescence which is linked with bone outcomes in young adulthood (Baxter-Jones et al., 2008). Despite the lack of significant association between region specific lean mass and aBMD at the FN and TH, all the geometrical parameters of the narrow neck of the femur were significantly associated with region specific lean mass. This may reflect previous work in children and adolescents showing that HSA can provide more in depth geometrical evaluation at the hip site compared to BMD outcomes (Petit et al., 2005). In addition, studies using peripheral quantitative computed tomography (pQCT) found that muscle cross sectional area was the strongest predictor of bone strength parameters in early pubertal boys and girls (Macdonald et al., 2006). The latter study highlighted the importance of using site specific lean mass to understand its contribution to hip geometry estimates. On the other hand, region specific fat mass was not associated with any geometrical parameters and this is in agreement with findings in non-athletic adolescent females indicating that fat mass was not associated with CSA (Pollock et al., 2007).

Height was associated with all hip geometry estimates showing that the size of an adolescent athlete plays an important role in modifying hip geometry estimates. A previous study reported that femoral length is one of the most important predictors of CSA and Z in female adolescents (Daly et al., 2008) highlighting the importance of bone length at the hip. In addition, football participation was associated with CSA in hip geometry estimates of female footballers (El Hage, 2013). There was no contribution of swimming and cycling on geometrical parameters which is similar with the findings on aBMD outcomes. The different contribution of height and football in geometrical parameters compared to aBMD parameters might be due to fact that we used height and not femoral length to control for the size in geometrical parameters. Also, the estimated geometrical parameters might not be affected from the external loading that football applies at the narrow neck site and higher forces might be needed (Korhonen et al., 2012).

All groups of the present study had similar serum levels of calcium and vitamin D and there was no association found between serum levels of calcium and vitamin D and geometrical bone outcomes, which is consistent with no contribution of hip related aBMD outcomes in the present study. In contrast, MVPA was a significant predictor of CSMI and Z independent of the sport participation suggesting that MVPA might induce changes in geometrical

parameters and not aBMD due to mechanical stimuli applied at the hip site (Michalopoulou et al., 2013). The association between MVPA and bone outcomes was evident for the geometrical parameters but not for the aBMD parameters. This may be explained by previous findings showing that geometrical adaptations can occur before the adaptation of aBMD outcomes due to the initial respond inside the bone to the change in external strains (Wang et al., 2005, Haapasalo et al., 2000). CRF was a significant predictor of CSMI and Z after accounting for all the other predicting determinants, but there was no association with vertical jump. The different associations between fitness parameters and MVPA with aBMD and the geometrical parameters might be attributed to the sensitivity of the geometrical parameters of the hip to detect changes (Khoo et al., 2005). The bone structure at the hip and specifically CSA site might be associated with CRF due to the use lower leg muscle units during the sport specific movements. The training characteristics are dominant in the present study and our population was at the 75th percentile for CRF compared to same age and ethnicity matched population (Sandercock et al., 2012).

5.5.3 Limitations

To our knowledge, this is the first study conducted in adolescent male athletes to examine the determinants of aBMD and hip geometry estimates. A large list of predictors has been included and their effects have been adjusted by each other. In addition, the present study uses region specific lean mass as predictor of aBMD and hip geometry estimates due to the site specific adaptations of the skeleton during exercise and growth (Ireland et al., 2013). The cross-sectional analysis of the present study is a limitation and cannot prove cause and effect between the determinants and bone outcomes studies. In spite of using DXA as

a surrogate estimate of lean mass due to the 2 component model, DXA-derived lean mass has been found to be highly correlated (r = 0.82) with muscle cross sectional area measured by pQCT (Petit et al., 2005).

5.6 Conclusions

The present study has shown, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes. Region specific lean mass was consistently the most important determinant of aBMD and hip geometry estimates parameters in adolescent male athletes. Football participation and height were found to be important determinants for the aBMD and HSA parameters, respectively. Calcium and 25(OH)D had a small site specific contribution only on aBMD. MVPA and CRF positively influenced only the geometrical parameters and vertical jump was associated with aBMD parameters. Studies focusing on bone outcomes of young athletes should account for the region specific lean mass differences due to the site-specific adaptations of the skeleton to external loading. Future practical approaches of sports clubs should include weight-bearing and muscle strengthening exercises, such as jumps, which can optimise bone outcomes during the important period of adolescence.

6. The effect of 12-month participation in osteogenic and non-osteogenic sports on bone development in adolescent male athletes

6.1 Abstract

Objectives: Research investigating the longitudinal effects of the most popular sports on bone development in adolescent males is scarce. The aim is to investigate the effect of 12-month participation in osteogenic and non-osteogenic sports on bone development.

Design: A 12-month study was conducted in adolescent males involved in football, swimming and cycling and compared with an active control group.

Methods: 116 adolescent males (13.1±0.1 years at baseline): 37 footballers, 37 swimmers, 28 cyclists and 14 active controls were followed for 12 months. Bone mineral content (BMC) was measured by dual-energy x-ray absorptiometry, and bone stiffness was measured by quantitative ultrasound. Bone outcomes at 12 months were adjusted for baseline bone status, age, height, lean mass and moderate to vigorous physical activity.

Results: Footballers had higher improvement in adjusted BMC at the total body, total hip, shaft, Ward's triangle, legs and bone stiffness compared to cyclists (6.3 to 8.0 %). Footballers had significantly higher adjusted BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %). There was no significant difference between swimmers and cyclists for any bone outcomes. Swimming and cycling participation resulted in non-significant lower bone development at most sites of the skeleton compared to controls (-4.3 to -0.6 %).

Conclusions: Football participation induces significantly greater improvements in BMC and bone stiffness over 12 months compared to cycling and swimming.

Keywords: adolescence; bone mass; bone stiffness; cycling; football; swimming; weight-bearing exercise.

6.2 Introduction

Bone development occurs most rapidly during childhood and adolescence, with 80-90 % of peak bone mass (PBM) acquired by late adolescence depending on the site of the skeleton (Gordon et al., 2016). PBM is largely determined by genetics (Bonjour et al., 2007) and by modifiable factors, such as nutrition and physical activity (PA) (Gracia-Marco et al., 2011a, Mouratidou et al., 2013). Exercise during this period of life can enhance bone mineral content (BMC) and bone mineral density (BMD) (Behringer et al., 2014) and be maintained into adulthood (Baxter-Jones et al., 2008). Football, cycling and swimming are among the most popular sports performed by adolescents around the world (Sport England, 2016). However, participation in these sports may have different effects on bone development (Vlachopoulos et al., 2017c). Participation in "osteogenic" sports, such us football, can augment BMC at the loaded sites of the skeleton (Zouch et al., 2014, Ubago-Guisado et al., 2015a). However, participation in "non-osteogenic sports", such as swimming and cycling, may have a negative or no impact on bone outcomes (Olmedillas et al., 2011), which may compromise the achievement of a higher PBM and increase the risk of osteoporotic fractures in adulthood. From a public health perspective, understanding how the most popular sports worldwide among youth affect bone development is of great importance.

Cross-sectional studies have evaluated differences in BMC between adolescents engaged in different sports in comparison to a control group

(Olmedillas et al., 2011). Specifically, footballers were found to have higher adjusted-BMC and BMD at most sites of the skeleton compared with agematched controls (Zouch et al., 2014). In contrast, previous evidence found that adolescent male swimmers had lower adjusted-BMC and BMD at several sites compared to controls (Gomez-Bruton et al., 2015), but a recent systematic review concluded that swimmers have similar bone mass compared to sedentary controls (Gomez-Bruton et al., 2016b). Similarly, in a cross-sectional analysis we found that adolescent male swimmers and cyclists had lower bone outcomes compared to footballers (Vlachopoulos et al., 2017c). However, other studies showed that cycling during adolescence may negatively influence bone health (Olmedillas et al., 2011, Rico et al., 1993a). To date, there are only a few longitudinal studies on this topic and it was found that 3 years of football participation increased femoral neck BMD by 10 % and improved femoral neck and intertrochanteric BMC twice as much compared to age-matched controls in prepubertal males (Vicente-Rodriguez et al., 2004a). Previously, 8 months of football training significantly improved bone outcomes at total body, intertrochanteric site, lumbar spine and femoral neck in female adolescent footballers, whereas 8 months of swimming training had no effect on bone outcomes in female adolescent swimmers (Ferry et al., 2013). Research investigating the longitudinal effects of the most popular sports on bone development in adolescent males is scarce (Vlachopoulos et al., 2017a). It should be noted that a comprehensive analysis of potential confounders, such as lean mass and objectively measured moderate-to-vigorous PA (MVPA) should be used to control for important predictors of bone status in these sports (Vlachopoulos et al., 2017d).

In addition to Dual energy X-ray Absorptiometry (DXA), Quantitative Ultrasound (QUS) can indicate the risk of osteoporotic fractures at the calcaneus site that is particularly important for adolescent athletes due to their high prevalence of injuries (Krieg et al., 2008, Frush and Lindenfeld, 2009). In a cross-sectional study, it was shown that swimming had no effect on bone stiffness compared to age-matched controls in adolescent males and females (Gomez-Bruton et al., 2015). Also, in a cross-sectional analysis it was found that footballers had higher bone stiffness than controls but there were no differences in swimmers and cyclists compared to controls (Vlachopoulos et al., 2017c). However, there is lack of longitudinal studies comparing the effects of osteogenic and nonosteogenic sports on QUS bone outcomes in adolescent males athletes (Boreham and McKay, 2011). Therefore, the purpose of this study is to investigate the effect of 12-month participation on BMC and bone stiffness in osteogenic (football) and that non-osteogenic sports (swimming and cycling) compared to an active control group after controlling for baseline bone outcomes, age, height, lean mass and MVPA.

6.3 Methods

The present study represents a 12-month analysis of sport participation as part of the PRO-BONE study, whose purpose and methodology have been described elsewhere (Vlachopoulos et al., 2015). For the present study, data obtained at baseline (T0) during autumn/winter 2014/15 and at follow-up (T1) during autumn/winter 2015/2016 were used (mean difference of visits = 372 days). Five participants were excluded because they did not complete the second visit (n=3) or they had missing data (n=2). For the present study, 116

adolescent males (37 swimmers, 37 footballers, 28 cyclists and 14 active controls not engaged in these sports more than 3 hour per week) aged 13.1 years ± 1.0 at T0 and 14.1 years ± 1.0 at T1 were included. The inclusion criteria at T0 were: 1) males 12-14 years old, engaged (≥3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or more; 2) males 12-14 years old not engaged in any of these sports (≥3 h/week) in the last 3 or more years (control group). The exclusion criteria were at T0 were: 1) participants not taking part in another clinical trial; 2) participants not having any acute infection lasting until < 1 week before inclusion; 3) participants free of any medical history of diseases or medications affecting bone metabolism or injured; 4) white Caucasian ethnicity. Ethics approval received from the following committees: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West - Cornwall & Plymouth, ref. number 14/SW/0060).

A DXA scanner (GE Healthcare Inc., Wisconsin, USA) was used to measure BMC (g), fat mass (g) and lean mass (g, excluding bone and fat mass). The total body scan was used to obtain BMC at the arms, legs, and total body (excluding head). Dual hip scans were performed to obtain BMC for total hip, femoral neck, Ward's triangle, trochanter and shaft sub-regions and the mean of right and left hip scans was used. The coefficient of variation (CV) for measurement reliability was not determined in the present study. Previous paediatric studies have shown that the DXA between-day CV was between 1.0 % and 2.9 % depending on the region (Johnson and Dawson-Hughes, 1991). In

addition, QUS measurements were performed with a Lunar Achilles Insight (GE Healthcare Inc., Wisconsin, USA). This portable device measures bone stiffness using ultrasound waves. QUS is a non-ionising radiation technique and evaluates bone stiffness based on broadband ultrasound attenuation (dB/MHz) and speed of sound (m/s) (Baroncelli, 2008). The real-time image of the calcaneus and the region of interest ensures that the measurement is reliable and valid to assess bone health as demonstrated in paediatric population (Sioen et al., 2016). Daily calibration was completed at all visits and measurements were taken according to the standard procedure provided by the manufacturer. The positioning was standardised between visits by using an adapter for the children's feet in order to get the same position of the calcaneus. Both feet were measured twice and the mean of the two measures was used for statistical analyses.

Height (cm) and body mass (kg) were measured by using standard procedures and sexual maturity was self-reported using adapted drawings of the five stages of pubic hair development (Tanner and Whitehouse, 1976). Physical activity was measured for seven consecutive days at T0 and T1 using wrist accelerometers (GENEActiv, GENEA, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (Phillips et al., 2013). Data were collected at 100 Hz and analysed at 1 s epoch intervals to establish time spent in MVPA using a validated cut-point (Phillips et al., 2013). Weekly training hours were obtained by face to face interviews at T0 at T1. In addition, the coaches indicated participation in weight-training exercises for a subsample of participants.

Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA). Data were normally distributed and presented

as mean and standard deviation. Data were analysed in two stages: 1) raw (unadjusted) data using one-way analysis of variance (ANOVA) with Bonferroni post hoc or Chi-Square tests at T0 and T1 to detect the differences in BMC, and 2) adjusted data using one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect the differences between the groups at T1 after controlling for: bone status at T0, age, height, lean mass, MVPA and maturity status (Vlachopoulos et al., 2017d, Wilkinson et al., 2017, Ubago-Guisado et al., 2017). Paired t-tests were used to compare differences in values between T0 and T1. Preliminary analyses showed bone outcome results did not change when maturity was used as confounder instead of age. Thus, maturity was not included in the model. Percentages of difference between groups were used to quantify the magnitude of the differences. Significance was set at p < 0.05 and p < 0.01.

6.4 Results

Table 6.1 presents the descriptive characteristics of the participants at T0 and T1. From T0 to T1 all the descriptive characteristics significantly increased in all groups except MVPA in all groups and body fat percentage in sports groups that significantly decreased. Between-group differences at T1 showed that swimmers were older, taller, heavier and had more lean mass than the footballers and controls. Swimmers were more mature than footballers and controls. Swimmers trained more hours per week and had more years of training than cyclists. Footballers spent more time doing MVPA than swimmers and controls. In addition, footballers trained more hours per week and had more training years than cyclists and swimmers. Cyclists were older than controls and

spent more time doing MVPA compared to swimmers and controls. Controls had a higher body fat percentage than all sports groups.

Table 6.1. Descriptive characteristics of the participants at baseline (T0) and after 12 months (T1) of sport

participation

participation				
N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)
Age (yrs)	,	,	,	,
TO TO	13.5 (1.0) ^{b,dd}	12.9 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
T1	13.5 (1.0) ^{b,dd} 14.6 (1.0) ^{b,dd,*}	13.9 (0.9)*	13.2 (1.0) ^d 14.2 (1.0) ^{d,*}	13.2 (0.5)*
Height (cm)				
T0	165.1 (9.7) ^{bb,d}	155.2 (9.3)	160.7 (10)	154.5 (9.9)
T1	165.1 (9.7) ^{bb,d} 171.6 (8.9) ^{bb,dd,*}	162.7 (10.3) [^]	166.6 (10.7) [^]	160.7 (10.5) [^]
Body mass (kg)				
T0	51.9 (8.7) ^{bb}	44.3 (7.9)	49.3 (12.5)	48.3 (13.0)
T1	58.9 (8.2) ^{b,*}	50.8 (9.7)	54.7 (12.5)	55.2 (15.6) [*]
BMI (kg/m²)				
T0	18.9 (1.6) _*	18.3 (1.4) _*	18.9 (3.3) _*	20.0 (3.4) _*
T1	19.9 (2.0)	19.0 (1.8)	21.0 (3.1)	21.0 (3.7)
Lean mass (kg)	h dd		()	
T0	41.1 (9.0) ^{b,dd} 47.8 (8.7) ^{b,dd,*}	35.4 (7.2)	37.5 (7.5)	31.7 (5.5)
T1	47.8 (8.7) ^{5,00,}	41.2 (9.2)	42.9 (8.2)	36.8 (7.1)
Body fat (%)	()*			and a supplied that the supplied to the suppli
T0	17.3 (7.3) [*]	15.7 (5.6)*	18.0 (9.0)*	29.0 (10.5) ^{aa,bb,cc}
T1	14.4 (6.4)	14.5 (6.0)	16.1 (9.2)	27.9 (10.9) ^{aa,bb,cc}
Tanner stages (1-				
5; %) To	(40/05/40/40/0)	(0.4/05/0.4/4.0/0)	(4.4/00/05/00/4)	(00/04/04/00/0)
T0	(16/25/16/43/0) (5/11/11/51/22) ^{b,d,*}	(24/35/24/16/0)	(14/28/25/28/4)	(29/21/21/29/0)
T1	(5/11/11/51/22)	(6/16/35/43/0)	(7/11/14/57/11)	(0/21/43/36/0)
Training (h/week)	9.4 (5.1) ^{cc}	10.0 (2.2) ^{CC}	E 0 (0 4)	
T0 T1	8.9 (3.6) ^{cc}	10.0 (2.3) ^{cc} 9.4 (1.7) ^{cc}	5.2 (2.1)	-
Years of training	0.9 (3.0)	9.4 (1.7)	5.6 (2.0)	-
Tears or training	5.9 (2.5) ^{cc}	7.5 (2.3) ^{a,cc}	3.9 (1.3)	_
T1	6.9 (2.5) ^{cc,*}	8.5 (2.3) ^{a,cc,*}	4.9 (1.3)	_
MVPA (min/day)	0.3 (2.3)	0.0 (2.0)	T.3 (1.3)	=
TO	85.0 (30.9) [*]	119.8 (29.7) ^{aa,dd,*}	106.5 (33.7) ^{a,*}	83.2 (26.8)*
T1	62.9 (21.8)	92.4 (25.7) ^{aa,dd}	85.6 (21.8) ^{aa,d}	64.3 (18.1)
		dy mass index MV/DA	· Moderate to vigorou	s physical activity TO

Values presented as mean (SD). BMI: Body mass index, MVPA: Moderate to vigorous physical activity. To = baseline values, T1 = 1 year values. Superscript letters denote a higher significant difference between sports: a (swimmers), b (footballers), c (cyclists), d (controls), a,b,c,d p<0.05, aa,bb,cc,dd p<0.001 and within each sports group at T0 and T1: p<0.05.

Table 6.2 shows the adjusted BMC and bone stiffness at T0 and T1 between the groups and Figure 6.1 shows the adjusted BMC and bone stiffness differences (%) between the sports groups and controls at T1. At T1 footballers had significantly higher BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %). Also, at T1 footballers had significantly higher BMC at total body, total hip, Ward's triangle, shaft and legs compared to cyclists (6.3 to 8.0 %). At T1 footballers had non-significantly higher bone outcomes than controls (3.3 to 8.4 %). The adjusted bone stiffness was significantly higher in footballers compared to cyclists (7.8 %) at T1. Swimmers and cyclists had similar bone outcomes at T1 (-0.6 to 4.3 %) and both groups had no significant differences at any of the bone outcomes compared to controls (-4.5 to 4.7 %).

Table 6.2. Adjusted bone mineral content (BMC, g) and bone stiffness at baseline (T0) and after 12

months (T1) of sports participation in adolescent males

N = 116	Swimmers	Footballers	Cyclists	Controls
	(N = 37)	(N = 37)	(N = 28)	(N = 14)
TBLH (g)				
T0	1453.9 (21.1)	1574.5 (21.5) ^{a,c,d}	1459.9 (22.7)	1451.8 (34.4)
T1	1752.9 (20.9)	1846.7 (20.9) ^{a,cc}	1737.0 (21.9)	1787.1 (33.6)
Total hip (g)				
T0	26.50 (0.50)	30.24 (0.51) ^{aa,cc,d}	26.62 (0.53)	24.61 (0.79)
T1	32.04 (0.42)	33.53 (0.44) ^c	31.26 (0.45)	31.70 (0.70)
Ward's (g)				
T0	2.15 (0.06)	2.48 (0.06) ^{a,c,dd}	2.14 (0.06)	1.92 (0.1)
T1	2.66 (0.05)	2.74 (0.05) ^c	2.55 (0.05)	2.63 (0.08)
Trochanter (g)				
T0	8.11 (0.23)	9.85 (0.23) ^{aa,cc,d}	8.22 (0.24)	7.60 (0.36)
T1	10.99 (0.27)	11.38 (0.28)	10.59 (0.28)	10.50 (0.43)
Shaft(g)				
T0	14.16 (0.26)	15.71 (0.26) ^{a,cc,d}	14.12 (0.27)	13.09 (0.41)
T1	16.20 (0.16)	17.09 (0.17) ^{a,cc}	16.08 (0.17)	16.30 (0.27)
Arms (g)				
T0	209.27 (3.23)	207.24 (3.19)	211.98 (3.48) ^d	193.43 (5.22)
T1	252.85 (2.95)	258.71 (2.85)	254.39 (3.15)	249.52 (4.84)
Legs (g)				
T0	215.69 (4.51)	253.79 (4.59) ^{aa,cc,d}	223.08 (4.89)	216.31 (7.43)
T1	854.67 (9.67)	902.89 (9.82) ^{a,cc}	836.26 (9.85)	873.90 (15.24)
Stiffness index				
T0	89 (2)	100 (2) ^{a,c,d}	91 (2)	86 (3)
_T1	97 (1)	101 (1) ^c	93 (1)	98 (2)

Values are presented as mean (SE). TBLH: Total body less head. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.001. At T0 BMC values were adjusted for age, height, MVPA and lean mass. At T1 BMC values were adjusted for age, height, MVPA, lean mass and for baseline BMC (T0).

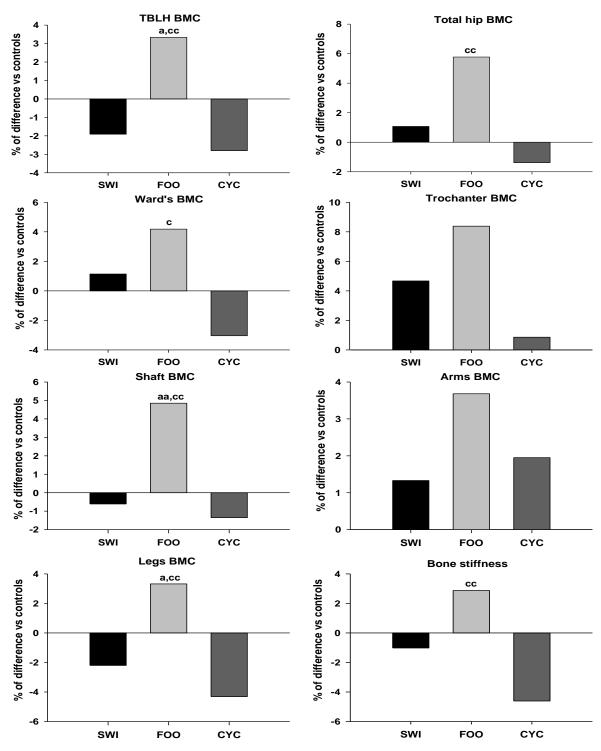


Figure 6.1. Differences (%) in adjusted bone mineral content (BMC) between the sports groups and controls after 1 year. The results adjusted for age, height, lean mass, moderate to vigorous physical activity and bone outcomes at baseline (T0). TBLH: Total body less head. Letters denote a significant difference with: a (Swimmers, SWI), b (Footballers, FOO), c (Cyclists, CYC) and d (Controls). ^{a,b,c,d} p<0.05 and ^{aa,bb,cc,dd} p<0.01.

Supplementary table 6.1 shows the unadjusted change in bone outcomes at T0 and T1. At T1 BMC significantly increased at all skeletal sites in swimmers (10.3 to 21.0 %), footballers (13.6 to 23 %), cyclists (9.9 to 19.0 %) and controls (14.8 to 21.0 %) compared to T0. In addition, bone stiffness significantly increased in swimmers (4.5 %), footballers (6.9 %) and controls (5.1 %) from T0 to T1, but the increase was not significantly different in cyclists (0.9 %).

Supplementary table 6.1. Unadjusted bone mineral content (BMC, g) and bone stiffness at

baseline (T0) and after 12 months (T1) of sports participation in adolescent males

N = 116	Swimmers	Footballers	Cyclists	Controls
	(N = 37)	(N = 37)	(N = 28)	(N = 14)
TBLH (g)				
T0	1622.8 (325.4) ^{dd}	1473.5 (338.6)	14789.0	1234.4 (347.9)
T1	1923.9 (327.8) ^{dd}	1791.3 (453.0)	(353.2) 1725.4 (396.2)	1504.8 (433.7)
Total hip (g)				
T0	28.79 (5.56) ^{dd}	28.78 (6.18) ^{dd}	27.31 (5.92) ^d	21.12 (5.55)
T1	33.52 (5.69) ^{dd}	34.56 (7.76) ^{dd}	31.17 (6.28) ^d	25.21 (6.49)
Ward's (g)				
T0	2.29 (0.50) ^{dd}	2.40 (0.59) ^{dd}	2.19 (0.51) ^d	1.64 (0.45)
T1	2.76 (0.63) ^d	2.92 (0.77) ^{dd}	2.51 (0.70)	1.98 (0.56)
Trochanter				
(g)	9.01 (2.35) ^d	9.31 (2.67) ^{dd}	8.49 (2.34) ^d	6.11 (2.17)
T0	11.41 (2.57) ^d	12.09 (3.84) ^{dd}	10.48 (2.76) ^d	7.73 (2.87)
T1				
Shaft (g)				
T0	15.31 (2.69) ^{dd}	14.94 (2.97) ^d	14.46 (2.96) ^d	11.49 (2.74)
T1	17.07 (2.59) ^d	17.3 (3.34) ^{dd}	16.05 (2.92)	13.49 (2.93)
Arms (g)				
T0	243.39	188.34 (48.05)	210.62 (59.05) ^d	155.89 (40.58)
T1	(64.01) ^{bb,dd} 297.38 (66.37) ^{b,dd}	235.65 (71.68)	254.04 (70.70) ^d	193.5 (57.54)
Legs (g)				
T0	775.78 (136.24) ^d	747.84 (175.02)	733.99 (171.45)	612.28 (179.47)
T1	906.04 (139.86) ^d	900.62 (224.16)	835.18 (177.26)	746.29 (217.99)
Bone				
stiffness	91 (12)	99 (11) ^{a,dd}	92 (23)	82 (11)
T0	95 (14)	106 (12) ^{a,cc,dd}	93 (14)	87 (14)
T1		(D) TDIII T-(-1 b	.h. l h	and the contract of

Values are presented as mean (SD). TBLH: Total body less head. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). ^{a,b,c,d} p<0.05 and ^{aa,bb,cc,dd} p<0.001.

6.5 Discussion

The main findings of the present study are: 1) after 12 months of sports participation, footballers had significantly higher BMC and bone stiffness gains compared to swimmers and cyclists, and higher but non-significant BMC and bone stiffness compared to active controls; 2) after 12 months swimmers and cyclists had similar BMC and bone stiffness, and both groups had no significant differences in BMC and bone stiffness compared to controls.

The present study shows that after 12 months footballers had higher adjusted BMC compared to cyclists and swimmers at most skeletal sites. The only study comparing these sports was conducted in female adolescent swimmers and footballers and showed that 8 months period of sport-specific training increased total body BMD by 2.9 % in footballers, whereas BMD remained constant in swimmers (Ferry et al., 2013). The present study found that footballers had 2.4 % higher adjusted BMC compared to swimmers after 12 months. Cross-sectional evidence in adolescent males found that footballers had greater BMD at the femoral neck compared to swimmers (Silva et al., 2011). The differences observed in BMC gains among the sports groups in the present study might be explained by the plyometric exercises included in the football training that can induce higher bone mass in adolescent athletes despite the reduced lean mass in footballers compared to swimmers (Gunter et al., 2008a). In this regard, Larsen et al. found that a 10-month programme that included small-sided ball games improved BMD at the legs and total body compared to controls, and BMD at the legs compared to a circuit strength training (Larsen et al., 2016).

In the present study, BMC development over 12 months was similar between adolescent male swimmers and cyclists at any skeletal sites. This is in line with

studies showing that swimming and cycling seem to have no additional effect on bone growth (Olmedillas et al., 2011, Gomez-Bruton et al., 2015), which could be due to the low ground reaction forces produced during participation in the non-osteogenic environment. In regards to bone stiffness, the present study showed that footballers significantly increased bone stiffness compared to cyclists. The latter is in accordance with cross-sectional analysis from this cohort showing that footballers had significantly higher bone stiffness compared to swimmers and cyclists (Vlachopoulos et al., 2017c).

Football participation during adolescence may induce higher bone outcomes compared to leisure active controls according to cross-sectional evidence (Zouch et al., 2014, Ubago-Guisado et al., 2015a). However, evidence from a study in prepubescent boys found that footballers had non-significant but higher bone outcomes compared to active controls after 10 months of training (Zouch et al., 2008). These results are in line with our findings showing that footballers had higher (3.3 % to 8.4 %) but not significant bone outcomes compared to active controls after 12 months. It should be noted that the control group was physically active (MVPA= 64 min/day) and some controls engaged in other weight-bearing sports (< 3 hours per week) which might explain the nonsignificant difference compared to footballers. A previous cross-sectional study showed that footballers had significantly higher bone stiffness at lower extremities compared to active controls (Falk et al., 2010). The differences in bone outcomes between adolescent footballers and controls might increase in the future due to the previous findings showing that 3 years of football training exhibited significantly greater adjusted BMC in total body, legs and intertrochanteric sites compared to age-matched controls (Vicente-Rodriguez et al., 2004a).

Swimming is considered a non-osteogenic sport and does not promote positive changes on bone development above that observed due to growth. According to a recent meta-analysis, swimmers and sedentary controls have similar bone outcomes (Gomez-Bruton et al., 2016b). In addition, adolescent males that participated only in swimming had lower BMD and BMC at several sites of the skeleton compared to age-matched controls (Gomez-Bruton et al., 2015). In the present study swimmers had similar BMC gains with active controls after controlling for relevant covariates (including T0 BMC). Similarly, we found swimmers to have similar bone outcomes with controls at baseline after controlling for the same covariates (Vlachopoulos et al., 2017c). A possible explanation is that swimming has non-gravitational training characteristics and despite swimmers having augmented higher lean mass it was not enough to produce bone adaptations after 12-months of training (Maimoun et al., 2013b). Regarding bone stiffness, previous cross-sectional findings showed similar values between swimmers and controls (Gomez-Bruton et al., 2015).

Cycling is a widely practised sport that applies low mechanical forces to the skeleton during training (Olmedillas et al., 2012) and the present analysis showed that cyclists had lower but non-significant adjusted BMC and bone stiffness than controls. Previous evidence exist only from cross-sectional studies indicating that adolescent female cyclists had similar or lower bone outcomes compared to non-athletic controls(Ferry et al., 2013). Another cross-sectional study found that males cyclists (< 17 years) had significantly lower BMC at the legs compared to age-matched controls (Olmedillas et al., 2011). According to the baseline cross-sectional analysis of this cohort, cyclists had non-significantly higher adjusted BMC at the most skeletal sites (Vlachopoulos et al., 2017c). However, after one year cyclists had non-significant lower bone

development in BMC and bone stiffness than controls. The differences observed in the current study might be explained by the non-osteogenic environment of both swimming and cycling and by the mechanical loading produced by the sports-specific patterns. In addition, participation in plyometric training or other weight-bearing activities might explain the difference on bone outcomes between adolescent athletes and needs further investigation to quantify the impact of weight training on bone outcomes.

The strengths of the present study are 1) the investigation of bone outcomes across osteogenic and non-osteogenic male adolescent groups over 12 months; 2) the combination of DXA and QUS, which provides a comprehensive insight into BMC and bone stiffness outcomes and 3) the rigorous methodology that enabled the inclusion of a selection of specific confounders which increases the internal validity of the study. A limitation of the present study is the lack of nutrition-related covariates and the two time points of the longitudinal assessment. However, we have observed that dietary intakes (total energy, protein and calcium) were no different between the groups at T0 and T1 (data not reported). In addition, despite the two measurements completed, this is the first stud to assess the differences in bone development of these sports over 12 months. Also, it should be noted that all sport groups were very active, but cyclists trained less compared to footballers and swimmers.

6.6 Conclusions

In summary, this is the first study to investigate the 12-month development on BMC and bone stiffness in adolescent males engaged in osteogenic (football) and non-osteogenic sports (swimming and cycling). The findings of this study suggest that 12 months of football participation induces greater BMC and bone stiffness compared to cycling or swimming participation. In addition, footballers

had higher BMC although not significant compared to an active control group. Swimmers and cyclists had similar bone outcomes after 12 months, and both groups no significant differences in any of the bone outcomes compared to active controls. These findings suggest that participation in non-osteogenic sports during adolescence should be combined with weight-bearing activities in order to optimise bone development. Studies focusing on females and using specific interventions to improve bone mineralization in non-osteogenic sports during growth are needed.

7. Longitudinal adaptations of bone mass, geometry and metabolism in adolescent male athletes

7.1 Abstract

Adolescence is a crucial period for bone development and exercise can enhance bone acquisition during this period of life. However, it is not known how the different loading sports practised can affect bone acquisition in adolescent male athletes. Therefore, the purpose of the present study was to determine the 1 year longitudinal bone acquisition among adolescent males involved in osteogenic (football) and non-osteogenic sports (swimming and cycling) and compared to active controls. 116 adolescent males aged 12-14 years at baseline were followed for 1 year: 37 swimmers, 37 footballers, 28 cyclists and 14 active controls. Bone mineral content (BMC) assessed using dual-energy x-ray absorptiometry (DXA), cross-sectional area (CSA), crosssectional moment of inertia (CSMI) and section modulus (Z) at the femoral neck assessed using hip structural analysis (HSA), and bone texture of the lumbar spine using trabecular bone score (TBS). Serum N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] were analysed. Footballers had significantly higher adjusted BMC at the lumbar spine (7.0 %) and femoral neck (5.0 %) compared to cyclists, and significantly greater BMC at the lumbar spine (6.9 %) compared to swimmers. Footballers presented significantly greater TBS (4.3%) compared to swimmers, and greater CSMI (10.2 %), CSA (7.1 %), Z (8.9 %) and TBS (4.2 %) compared to cyclists. No differences were noted between cyclists and swimmers, and both groups had similar bone acquisition compared to controls. PINP was significantly higher in footballers and controls compared to cyclists and swimmers (3.3-6.0 %) and 25(OH)D was significantly higher in footballers and cyclists compared to swimmers and controls (9.9-13.1 %). These findings suggest that bone acquisition is higher in adolescent male footballers compared to swimmers and cyclists at the femoral neck and lumbar spine sites of the skeleton.

Keywords: adolescence, bone mineral content, exercise, hip structural analysis, trabecular bone score.

7.2 Introduction

Puberty is a period of life associated with a rapid increase in bone mass (Gordon et al., 2016, Theintz et al., 1992). Low bone mass during adolescence is associated with increased fracture risk and osteoporosis later in life (Davies et al., 2005, Goulding et al., 2001, Cooper et al., 2004a). The increasing prevalence of fractures for boys is expected to increase by 24 % (Donaldson et al., 2008), and the economic burden of fractures will cost £ 5,465 (€ 6,723) million per year by 2025 (Hernlund et al., 2013). The childhood and adolescent years are critical for bone acquisition with up to 43 % of peak bone mass (PBM) acquired during the 5-year period surrounding the peak height velocity (Baxter-Jones et al., 2011, Boreham and McKay, 2011). The factors affecting PBM during growth include non-modifiable factors, such as genetics (Bonjour et al., 2007), and modifiable factors, such as nutrition (e.g. calcium and vitamin D) (Chevalley et al., 2005, Moschonis et al., 2011) and physical activity (Janz et al., 2014, Gracia-Marco et al., 2012, Wilkinson et al., 2017). Exercise and sports participation can enhance BMC and bone mineral density (BMD) during youth

(Behringer et al., 2014, Maimoun et al., 2013a), and the benefits can be maintained into adulthood (Baxter-Jones et al., 2008, Kato et al., 2009). However, not all types of exercise and sports are beneficial for bone development. Participation in football can augment BMC at the loaded sites of the skeleton (Zouch et al., 2014, Maimoun et al., 2013b) while participation in swimming and cycling may have a negative or no impact on bone outcomes (Olmedillas et al., 2012, Gomez-Bruton et al., 2013), which may predispose to a sub- optimal PBM.

With football, cycling and swimming being among the most popular sports globally (Sport England, 2016), there is limited evidence comparing the impact of these sports on bone development (Vlachopoulos et al., 2017c). To date, only cross-sectional studies have evaluated the impact of these "osteogenic" and "non-osteogenic" sports on bone outcomes compared to controls in adolescent males (Nebigh et al., 2009, Ubago-Guisado et al., 2015a, Vlachopoulos et al., 2017c) showing higher bone outcomes in those participating in osteogenic sports. However, despite controlling for important confounders, the cross-sectional design precludes any determination of casual relationship between the differences observed between the groups (Lehtonen-Veromaa et al., 2000). Therefore, longitudinal studies that can identify sportspecific bone development patterns may be appropriate, but not available to date. Previous longitudinal studies comparing bone acquisition between sports focused only on females and include sports other than football, cycling and swimming (Ackerman et al., 2011, Maimoun et al., 2013a, Nickols-Richardson et al., 1999, Maimoun et al., 2010). A 1-year longitudinal study in adolescent female athletes found that the osteogenic effect of rhythmic gymnastics induced greater bone mass gain at load sites of the skeleton compared to swimming participation (Maimoun et al., 2013a). The investigation of females and specific weight-bearing sports cannot allow generalization of the bone acquisition in adolescent males or in other sports due to the fact that bone mass gain depends on specific mechanical stimuli that differs between the type of sports practised during adolescence (Greene and Naughton, 2006a) and due to the hormonal and body composition differences between sexes (Sayers et al., 2010). In addition, there are no longitudinal studies using a comprehensive analysis of important confounders, such as lean mass and objectively measured moderate-to-vigorous physical activity (MVPA) (Vlachopoulos et al., 2017d), comparing the effect of swimming, football and cycling participation on bone outcomes in adolescent male athletes.

Most studies have used dual-energy x-ray absorptiometry (DXA) to evaluate BMC, areal BMD and bone area (Behringer et al., 2014, Zouch et al., 2014) due to the low cost, radiation and availability (Crabtree et al., 2014). However, there are few studies using techniques such as hip structural analysis (HSA) to assess bone geometry estimates at the clinically relevant site of the femoral neck in adolescents (Maimoun et al., 2013b, Ferry et al., 2011). Previously, 8-months of football participation induced greater acquisition in cross-sectional area (CSA), cross-sectional moment of inertia (CSMI), section modulus and subperiosteal width compared to swimming participation in adolescent female athletes (Ferry et al., 2013). However there are no longitudinal studies available in adolescent males. Moreover, there are no studies in adolescent athletes using the recently developed trabecular bone score (TBS), which can predict fracture risk (Hans et al., 2011b) and fragility of the lumbar spine (Shawwa et al., 2016, Silva et al., 2014). Furthermore, there are no longitudinal studies on the effects of sport participation on bone turnover and nutrition markers, such as N-

terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] which can further explain bone formation and resorption in relation to the sports practised (Banfi et al., 2010, Gracia-Marco et al., 2010, Vaitkeviciute et al., 2016).

Therefore, the scope of the present investigation was to determine the longitudinal (1 year) differences on clinically relevant DXA-measured BMC sites, hip geometry estimates, TBS and bone turnover and nutritional markers in adolescent male athletes engaged in football, swimming, cycling and active controls aged 12-14 years at baseline.

7.3 Subjects and Methods

7.3.1 Cohort and study design

The present study shows a 12-month longitudinal analysis of sport participation as part of the longitudinal PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) study, whose purpose, methodology and inclusion/exclusion criteria have been described elsewhere (Vlachopoulos et al., 2015). For the present study, the measurements completed at baseline (T0) in autumn/winter 2014/15, and 1 year later (T1) in autumn/winter 2015/2016 (mean difference of visits = 1 year and 7 days) were used. Five participants were excluded in the present study because they did not complete the second visit (n=3) or they had missing data in any of the variables included (n=2). Therefore 116 adolescent males (13.1 years ± 1.0 at T0 and 14.1 years ± 1.0 at T1) were included: 37 swimmers, 37 footballers, 28 cyclists engaged in these sports more than 3 hour per week the

last 3 or more years and 14 active controls not engaged in these sports more than 3 hour per week the last 3 or more years.

7.3.2 Outcomes measures: DXA, HSA, TBS and biochemical analyses

DXA scanner (GE Healthcare Inc, Wisconsin, USA) was used to measure BMC (g), fat mass (g) and lean mass (g). The lumbar spine (LS, L1-L4) and bilateral proximal femora scans were used to assess BMC. All DXA scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the enCORE software version 14.10.022 (GE Healthcare Inc, Wisconsin, USA) and following previous guidelines (Crabtree et al., 2014). The coefficient of variation was not determined in the present study. Previous paediatric studies have shown that the DXA percentage coefficient of variation was between 1.0 % and 2.9 % depending on the region (Johnson and Dawson-Hughes, 1991).

The hip geometry estimates of the femoral neck were obtained and the following variables used: 1) the cross sectional area (CSA, mm²), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia (CSMI, mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; and 3) section modulus (Z, mm³), which is an indicator of maximum bending strength in a cross section. The coefficient of variations (CVs) of these variables previously found to be between 7.9 % and 11.7 % (Khoo et al., 2005).

TBS is a DXA based technological tool that provides an indirect textural index of trabecular microarchitecture in the lumbar spine and has been shown to significantly predict fracture risk independently of BMC (Hans et al., 2011a).

TBS assesses DXA images of the lumbar spine scans using a grey-level analysis as the slope at the origin of the log-log representation of the experimental variogram (Pothuaud et al., 2009). All TBS analyses were performed by the same trained researcher using the TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The calculation was performed at the lumbar spine region of interest as in the BMC measurement. The CVs of TBS in relation to BMC has been reported to be between 1.1 % to 1.9 % (Silva et al., 2014).

Capillary blood samples were collected at a non-training weekend day in the morning in heparin fluoride coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) and centrifuged at 3000rpm for 15 minutes at 4°C. Serum samples were stored at -80°C until analysis in a single session. Total serum levels of PINP, CTX-I, 25(OH)D and total calcium were analysed following guidelines (Vasikaran et al., 2011). ELISA kits (Abbexa Ltd., Cambridge, UK) for PINP (test range: 6-400 pg·mL⁻¹, sensitivity: 1.2 pg·mL⁻¹, inter and intra-assay CVs: 3.1 % and 8.2 % respectively), CTX-I (test range: 0.1-7.0 ng·mL⁻¹, sensitivity: 0.03 ng·mL⁻¹, inter and intra-assay CVs: 4.9 % and 6.8 % respectively), and 25(OH)D (test range: 3-80 ng·mL⁻¹, sensitivity: 1.2 ng·mL⁻¹, inter and intra-assay CVs: 6.1 % and 8.6 % respectively) were used. Total calcium serum was measured using direct colorimetric assay (Cayman Chemical Company, MI, U.S.A.) and had a sensitivity of 0.25 mg·dL⁻¹ and the absorbance was read at 570-590 nm (inter and intra-assay CVs: 5.1 % and 7.3 % respectively).

7.3.3 Other measures

Height (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair development (Tanner and Whitehouse, 1976).

Daily habitual physical activity was measured for seven consecutive days at T0 and T1 using wrist accelerometers (GENEA, Cambridgeshire, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (Phillips et al., 2013). Data were collected at 100 Hz and analysed at 1 s epoch intervals to establish time spent in MVPA using a cut-off point of ≥ 1140 counts per minute previously validated in youth (Phillips et al., 2013). Weekly training hours were obtained by face to face interviews at T0 at T1.

7.3.4 Statistical analyses

Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA). Data were checked for normality using Shapiro-Wilk's test, skewness and kurtosis values, and presented as mean and standard deviation. Data analysis was completed in two stages: 1) unadjusted data using one-way analysis of variance (ANOVA) with Bonferroni post hoc comparisons and Chi-Square tests used to detect between-group differences on bone outcomes (DXA and HSA, TBS) and biochemical markers, and 2) adjusted data using one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect the differences between the groups at T1 using age, height, lean mass, MVPA and the bone outcomes at T0 as covariates (Vicente-Rodriguez et al., 2005, Gracia-Marco et al., 2012, Vlachopoulos et al., 2017d,

Ubago-Guisado et al., 2017). Preliminary analyses showed that bone outcome results did not change when maturation stage was used as a confounder instead of age. Thus maturation was not included in the model. Percentages of differences between groups for all variables were calculated. Significance was set at p<0.05 and p<0.01.

7.4 Results

Table 7.1 presents the descriptive characteristics of the participants at T0 and T1. At T1 swimmers were older, taller, heavier and had more lean mass than the footballers. Swimmers were more mature than footballers and controls. Swimmers trained more hours and years than cyclists. Footballers spent more time doing MVPA than swimmers and controls. In addition, footballers trained more hours and years than cyclists and swimmers. Cyclists were older than controls. Cyclists spent more time doing MVPA compared to swimmers and controls. Controls had more body fat percentage than all the sport groups. From T0 to T1, all the descriptive characteristics for all groups increased significantly except body fat percentage which reduced significantly in the sport groups and MVPA that decreased significantly in all groups. Results of unadjusted bone outcomes are presented in Supplementary Table 7.1.

Table 7.1. Descriptive characteristics of the participants at baseline and after 1 year of sport

participation

_participation							
N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)			
Age (yrs)							
TÖ	13.5 (1.0) ^{b,dd} 14.6 (1.0) ^{b,dd,**}	12.9 (0.9)	13.2 (1.0) ^d	12.3 (0.5)			
T1	14.6 (1.0) ^{b,dd,**}	13.9 (0.9)**	14.2 (1.0) ^{d,**}	13.2 (0.5)**			
Height (cm)							
T0	165.1 (9.7) ^{bb,d}	155.2 (9.3)	160.7 (10)	154.5 (9.9)			
T1	171.6 (8.9) bb,dd,**	162.7 (10.3)**	166.6 (10.7)**	160.7 (10.5) ^{**}			
Body mass (kg)							
T0	51.9 (8.7) ^{bb}	44.3 (7.9)	49.3 (12.5)	48.3 (13.0)			
T1	58.9 (8.2) ^{b,**}	50.8 (9.7)**	54.7 (12.5)**	55.2 (15.6)**			
BMI (kg/m²)							
T0	18.9 (1.6)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)			
T1	19.9 (2.0)**	19.0 (1.8)**	21.0 (3.1)	21.0 (3.7)**			
Lean mass (kg)	h dd	>	()				
T0	41.1 (9.0) ^{b,dd} 47.8 (8.7) ^{b,dd,**}	35.4 (7.2)	37.5 (7.5)	31.7 (5.5)			
T1	47.8 (8.7) ^{5,44,}	41.2 (9.2)**	42.9 (8.2)**	36.8 (7.1)**			
Body fat (%)	47.0 (7.0)**	45.7 (5.0)**	40.0 (0.0)**	00 0 (40 E) 33 bb cc			
T0	17.3 (7.3)**	15.7 (5.6)**	18.0 (9.0)**	29.0 (10.5) ^{aa,bb,cc}			
T1	14.4 (6.4)	14.5 (6.0)	16.1 (9.2)	27.9 (10.9) ^{aa,bb,cc}			
Tanner stages							
(%)	(40/05/40/40/0)	(04/05/04/46/0)	(4.4/00/05/00/4)	(20/24/24/20/0)			
T0 T1	(16/25/16/43/0)	(24/35/24/16/0)	(14/28/25/28/4) (7/11/14/57/11)**	(29/21/21/29/0)			
Training (h/week)	(5/11/11/51/22) ^{b,d,**}	(6/16/35/43/0)	(7/11/14/57/11)	(0/21/43/36/0)			
To	9.4 (5.1) ^{cc}	10.0 (2.3) ^{cc}	5.2 (2.1)				
T1	8.9 (3.6) ^{cc}	9.4 (1.7)°°	5.6 (2.0)	_			
Years of training	0.9 (0.0)	3. 4 (1.7)	3.0 (2.0)				
TO	5.9 (2.5) ^{cc}	7.5 (2.3) ^{a,cc}	3.9 (1.3)	_			
T1	6.9 (2.5) ^{cc,**}	8.5 (2.3) ^{a,cc,**}	4.9 (1.3)**	_			
MVPA (min/day)	J.J (2.0)	0.0 (2.0)					
T0	85.0 (30.9)**	119.8 (29.7) ^{aa,dd,**}	106.5 (33.7) ^{a,**}	83.2 (26.8)*			
T1	62.9 (21.8)	92.4 (25.7) ^{aa,dd}	85.6 (21.8) ^{aa,d}	64.3 (18.1)			
Values presented as mean ± SD. BMI: Body mass index, MVPA: Moderate to vigorous physical							

Values presented as mean ± SD. BMI: Body mass index, MVPA: Moderate to vigorous physical activity. T0 = baseline values, T1 = 1 year values. Superscript letters denote a higher significant difference between sports: a (swimmers), b (footballers), c (cyclists), d (controls), a,b,c,d p<0.05, aa,bb,cc,dd p<0.001 and between T0 and T1 of each sport: p<0.05, p<0.001.

7.4.1 Longitudinal adjusted differences in BMC acquisition

BMC-adjusted results for lumbar spine and femoral neck are presented at Figure 7.1 and Table 7.2. At T1 footballers presented significantly greater BMC at the lumbar spine (7.0 %) and femoral neck (5.0 %) compared to cyclists. Also, footballers had significantly greater BMC at the lumbar spine (6.9 %) and a non-significant higher BMC at the femoral neck (2.3 %) compared to swimmers. Footballers had higher but not significant BMC at the lumbar spine (6.0 %) and femoral neck (2.7 %) compared to controls at T1. Cyclists had similar BMC at the lumbar spine (-1.0 %) and femoral neck (-2.3 %) compared to controls. Swimmers had similar BMC at the lumbar spine (-0.8 %) and femoral neck (0.4 %) compared to controls. In addition, swimmers had similar BMC at the lumbar spine (0.2 %) and femoral neck (2.7 %) compared to cyclists.

Supplementary table 7.1. Unadjusted bone outcomes using DXA, HSA and TBS at baseline and after 1

year of sports participation in adolescent males

N = 116	Swimmers	Footballers	Cyclists	Controls	
	(N = 37)	(N = 37)	(N = 28)	(N = 14)	
Lumbar spine (BMC, g)					
T0	42.97 (11.37) ^d	38.54 (8.93)	38.91 (10.78)	32.64 (8.67)	
T1	51.41 (11.52) ^{d,**}	46.84 (12.57)**	45.41 (12.48)**	38.68 (10.55) [*]	
Femoral Neck (BMC, g)	,	, ,	, ,	, ,	
T0	4.46 (0.66) ^{dd}	4.53 (0.74) ^{dd}	4.35 (0.76) ^d	3.52 (0.73)	
T1	5.04 (0.79) ^{d,**}	5.17 (0.89) ^{dd,**}	4.77 (0.89) ^{d,*}	4.00 (0.84)**	
CSA (mm²)					
T0	137.7 (20.9) ^{dd}	140.9 (20.4) ^{dd}	134.8 (22.4) ^d	109.8 (21.0)	
T1	152.5 (25.3) ^{d,**}	161.2 (25.1) ^{dd,**}	146.6 (26.4)*	124.2 (26.7)**	
Section modulus (mm ³)					
T0	557.5 (123.7) ^{dd}	548.1 (116.7) ^d	527.0 (122.8) ^d	405.7 (108.9)	
T1	664.4 (158.4) ^{d,**}	673.4 (182.5) ^{d,**}	596.6 (162.2)**	487.5 (140.5)**	
CSMI (mm ⁴)					
T0	8849.2 (2564.5) ^d	8471.6 (2606.6) ^d	8281.4 (2512.1) ^d	6020.7 (2071.9)	
T1	11217.6 (3536.3) ^{d,*}	11187.5 (3764.9)*	10064.1 (3428.2)*	7600.5 (2665.9) [*]	
Trabecular bone score					
T0	1.390 (0.065)	1.422 (0.092)°	1.362 (0.067)	1.385 (0.641)	
_T1	1.384 (0.065)	1.423 (0.712) ^{cc,d}	1.356 (0.073)	1.355 (0.068)	

Values are presented as mean \pm SD. BMC: Bone mineral content, CSA: Cross sectional area, CSMI: Cross sectional moment of inertia. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). ^{a,b,c,d} p<0.05 and ^{aa,bb,cc,dd} p<0.001, and ^{*} between T0 and T1 of each sport: $^{\circ}$ p<0.05, $^{\circ}$ p<0.001.

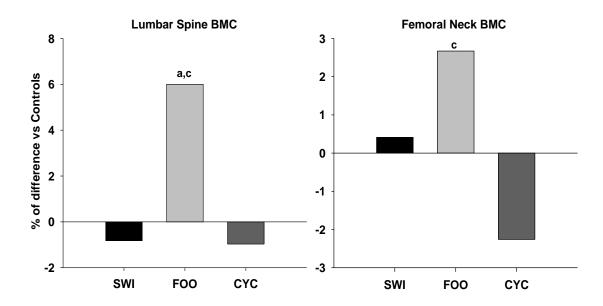


Figure 7.1. Difference (%) in adjusted bone mineral content (BMC) between the sports groups and controls after 1 year. The results are adjusted for age, height, lean mass, MVPA, and bone outcomes at baseline. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). ^{a,b,c,d} p<0.05.

Table 7.2. Adjusted bone outcomes using DXA, HSA and TBS at baseline and after 1 year of sports

participation in adolescent males

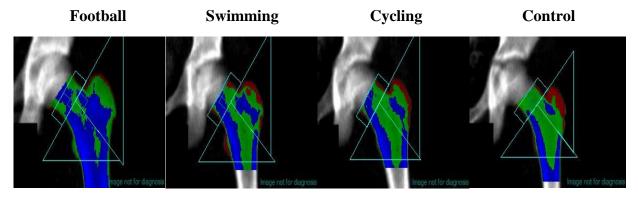
Curios res a re	Cooth all and	Oveliata	Caratrala
		•	Controls
(N = 37)	(N = 37)	(N = 28)	(N = 14)
38.16 (0.89)	40.78 (0.91)	39.67 (0.96)	39.88 (1.47)
46.10 (0.73)	49.27 (0.71) ^{a,c}	46.03 (0.77)	46.48 (1.18)
4.22 (0.08) ^{dd}	4.68 (0.08) ^{a,c,dd}	4.28 (0.08) ^d	3.92 (0.12)
4.89 (0.05)	5.00 (0.05) ^{cc}	4.76 (0.05)	4.87 (0.09)
131.4 (2.3)	157.0 (2.4) ^{aa,c,dd}	132.6 (2.4)	121.6 (3.7)
148.0 (2.4)	144.5 (2.3) ^c	146.6 (2.4)	147.0 (4.0)
521.5 (12.0)	567.0 (12.1) ^{c,d}	511.7 (12.6)	479.4 (19.1)
626.5 (12.8)	655.0 (13.1) ^c	601.4 (13.4)	626.5 (21.2)
8113.5 (252.1)	8854.9 (254) ^d	7993.8 (264.8)	7527.2 (399.2)
10301.7 (220.2)	11088.7 (219.5) ^c	10063 (229.4)	10284.3 (358.2)
1.372 (0.011)	1.428 (0.012) ^{a,c}	1.363 (0.013)	1.413 (0.019)
1.365 (0.010)	1.423 (0.010) ^{a,c}	1.366 (0.011)	1.387 (0.016)
	Swimmers (N = 37) 38.16 (0.89) 46.10 (0.73) 4.22 (0.08) ^{dd} 4.89 (0.05) 131.4 (2.3) 148.0 (2.4) 521.5 (12.0) 626.5 (12.8) 8113.5 (252.1) 10301.7 (220.2) 1.372 (0.011)	Swimmers (N = 37) Footballers (N = 37) 38.16 (0.89) 40.78 (0.91) 46.10 (0.73) 49.27 (0.71) ^{a,c} 4.22 (0.08) ^{dd} 4.68 (0.08) ^{a,c,dd} 4.89 (0.05) 5.00 (0.05) ^{cc} 131.4 (2.3) 157.0 (2.4) ^{aa,c,dd} 148.0 (2.4) 144.5 (2.3) ^c 521.5 (12.0) 567.0 (12.1) ^{c,d} 626.5 (12.8) 655.0 (13.1) ^c 8113.5 (252.1) 8854.9 (254) ^d 10301.7 (220.2) 1.428 (0.012) ^{a,c}	Swimmers (N = 37)Footballers (N = 37)Cyclists (N = 28) $38.16 (0.89)$ $46.10 (0.73)$ $40.78 (0.91)$ $49.27 (0.71)^{a,c}$ $39.67 (0.96)$ $46.03 (0.77)$ $4.22 (0.08)^{dd}$ $4.89 (0.05)$ $4.68 (0.08)^{a,c,dd}$ $5.00 (0.05)^{cc}$ $4.28 (0.08)^d$ $4.76 (0.05)$ $131.4 (2.3)$ $148.0 (2.4)$ $157.0 (2.4)^{aa,c,dd}$ $144.5 (2.3)^c$ $132.6 (2.4)$ $146.6 (2.4)$ $521.5 (12.0)$ $626.5 (12.8)$ $567.0 (12.1)^{c,d}$ $655.0 (13.1)^c$ $511.7 (12.6)$ $601.4 (13.4)$ $8113.5 (252.1)$ $10301.7 (220.2)$ $8854.9 (254)^d$ $11088.7 (219.5)^c$ $7993.8 (264.8)$ $10063 (229.4)$ $1.372 (0.011)$ $1.428 (0.012)^{a,c}$ $1.363 (0.013)$

Values are presented as mean ± SE. BMC: Bone mineral content, CSA: Cross sectional area, CSMI: Cross sectional moment of inertia. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). ^{a,b,c,d} p<0.05 and ^{aa,bb,cc,dd} p<0.001. At T0 bone outcomes were adjusted for age, height, MVPA and lean mass. At T1 bone outcomes were adjusted for age, height, MVPA, lean mass and for T0 bone outcomes.

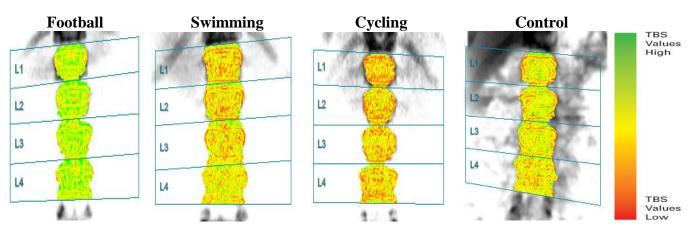
7.4.2 Longitudinal adjusted differences in HSA and TBS acquisition between groups

Results of adjusted bone geometry acquisition are presented at Figure 7.2 and Table 7.2. At T1 footballers presented significantly greater TBS (4.3%) compared to swimmers, greater CSMI (10.2 %), CSA (7.1 %), Z (8.9 %) and TBS (4.2 %) compared to cyclists and non-significant but higher CSMI, CSA, Z and TBS compared to controls (2.6 to 7.8 %). In addition, swimmers had greater but non-significant CSMI, CSA and Z (1.0 to 4.2%) and similar TBS (-0.1%) compared to cyclists. Swimmers had similar CSMI, CSA and Z (-0.1 to 0.7%) and non-significantly lower TBS (-1.6%) compared to controls. Finally, cyclists had lower but not significant CSMI, CSA, Z and TBS (-0.3 to -4.0%) compared to controls.

Hip Structural Analysis at the narrow neck of the femur region.



Trabecular bone score at the lumbar spine region.



	FOO vs SWI	FOO vs CYC	FOO vs CON	SWI vs CYC	SWI vs CON	CYC vs CON
СЅМІ	7.64%	10.19% *	7.82%	2.37%	0.17%	-2.15%
CSA	6.08%	7.09% *	6.80%	0.95%	0.68%	-0.27%
Z	4.63%	8.91% *	4.55%	4.17%	-0.08%	-4.01%
TBS	4.25%**	4.17%**	2.60%	-0.07%	-1.59%	-1.51%

Figure 7.2. Differences (%) in adjusted bone geometry estimates and trabecular bone score at the femoral neck and lumbar spine regions between the groups after 1 year of sports specific training. The results are adjusted for age, height, region specific lean mass, MVPA and baseline values of bone geometry estimates. CSMI: Cross sectional moment of inertia, CSA: cross-sectional area, TBS: Trabecular Bone score, Z: Section modulus. The figures represent unadjusted results of participants of the same age, height and training hours. Significance at * p<0.05 and ** p<0.01.

7.4.3 Longitudinal bone turnover and nutrition marker between groups

The biochemical markers are shown in Table 7.3 and Figure 7.3. At T0 there were no significant differences between the groups in any of the biochemical markers. After one year of sport participation (T1), bone formation (PINP) was significantly higher in footballers than swimmers (3.3 %) and cyclists (6.0 %). In addition, footballers had significantly higher 25(OH)D compared to swimmers (12.9 %) and controls (13.1 %). Cyclists had significantly lower PINP than controls (5.1 %), but they had significantly higher 25(OH)D compared to swimmers (9.9 %) and controls (11.1 %). CTX-I (14.8 %) and 25(OH)D (4.7 %) significantly increased from T0 to T1 in footballers while PINP did not change compared to the other groups. In swimmers there was a significant decrease in PINP (5.8 %) and a significant increase in CTX-I (9.8 %) from T0 to T1. Similarly, in cyclists PINP significantly decreased (7.2 %) and CTX-I non-significantly increased (4.3 %) from T0 to T1.

Table 7.3. Biochemical markers at baseline and after 1 year of sports participation in adolescent males

N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)
Serum calcium (mg/dl)				
T0	10.04 (0.44)	9.97 (0.40)	9.96 (0.41)	10.01 (0.35)
T1	9.91 (0.52)	9.96 (0.39)	9.89 (0.32)	9.74 (0.53)
25 (OH) D (ng/ml)				
TO , ,	13.73 (1.20)	14.44 (1.63)	14.39 (0.59)	13.92 (0.94)
T1	13.42 (0.89)	15.15 (1.17) ^{aa,dd,*}	14.89 (1.13) ^{aa,dd}	13.40 (0.93)
PINP (pg/ml)	, ,	, ,	, ,	, ,
T0	355.15 (10.07)**	352.04 (13.5)	350.83 (12.85)**	350.11 (16.76)
T1	335.67 (18.04)	346.62 (18.16) ^{a,cc}	327.15 (14.15)	344.81 (14.56) ^{cc}
CTX-I (ng/ml)				
TO TO	1.66 (0.25)	1.61 (0.29)	1.78 (0.19)	1.73 (0.23)
T1	1.84 (0.16)**	1.89 (0.08)**	1.86 (0.09)	1.85 (0.10)

Values are presented as mean ± SD. 25 (OH) D: 25(OH)D: 25-hydroxyvitamin D, CTX-I: Carboxiterminal telopeptide of type 1 collagen (CTX-1), PINP: N-terminal propeptide of procollagen type I. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and a,bb,cc,dd p<0.001, and between T0 and T1 of each sport: p<0.05, p<0.001.

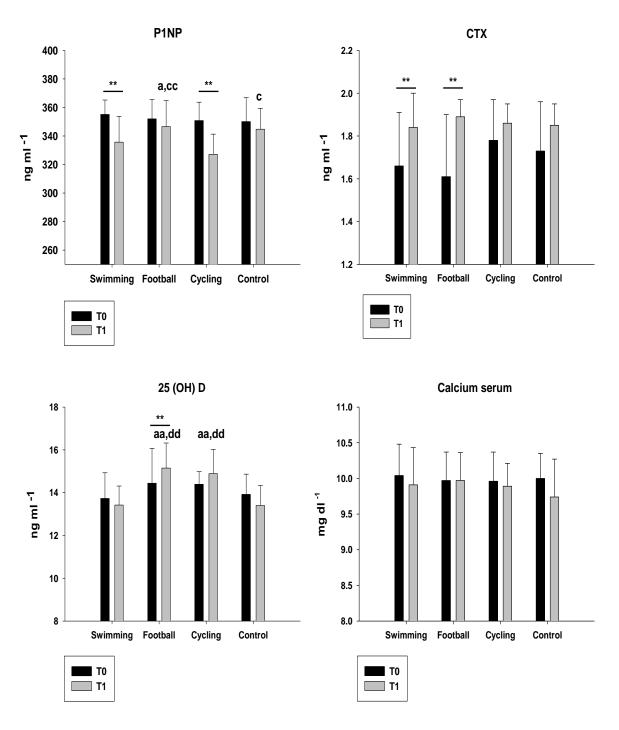


Figure 7.3. Differences in bone turnover and nutrition markers between the sports groups and controls after 1 year. 25 (OH) D: 25(OH)D: 25-hydroxyvitamin D, CTX-I: Carboxi-terminal telopeptide of type 1 collagen (CTX-1), PINP: N-terminal propeptide of procollagen type I. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls) and * denoted a significant difference between T0 and T1 measurement time. Significance at * p<0.05 and ** p<0.01.

7.5 Discussion

To the best of our knowledge, this is the first longitudinal study that has evaluated bone acquisition in adolescent male athletes and used comprehensive methodology to assess bone status by combining DXA, HSA, TBS and biochemical markers. The main findings of the present study show that: 1) the osteogenic responses of football participation over 1 year induced significantly greater bone acquisition in BMC, hip geometry estimates and TBS compared to that of swimming and cycling after accounting for relevant confounders; 2) participation in cycling and swimming did not induce significant gains in any bone outcomes and cyclists had non-significantly lower bone acquisition compared to controls; 3) footballers had significantly higher PINP than both non-osteogenic sports and significantly higher 25(OH)D concentration compared to swimmers and controls at T1, and PINP significantly decreased over time both in cyclists and swimmers, whereas CTX-I significantly increased in swimmers and footballers.

7.5.1 Longitudinal BMC acquisition between groups

In the present study footballers gained significantly higher BMC at the lumbar spine compared to cyclists and swimmers, and also gained significantly higher femoral neck BMC compared to cyclists. Also, footballers gained non-significant higher BMC than controls at the lumbar spine and femoral neck. Previous evidence in female footballers and swimmers aged 15.9 to 16.2 years, compared BMD gains after 8 months of training and found that BMD at lumbar spine and hip was significantly increased in footballers but not in swimmers (Ferry et al., 2013), which is in line with the findings of the present study. The differences observed in BMC acquisition among the sports groups in the

present longitudinal study might be explained by the loading patterns, such as plyometric exercises, included in the football training that can induce higher impact and bone adaptations in adolescent athletes (Gunter et al., 2008a). A different study in male footballers aged 12.9 years showed that 1 year of football training induced significantly higher gains in BMC at lumbar spine and total hip compared to active controls aged 12.5 years (Zouch et al., 2014), which is similar with the unadjusted findings of the present investigation. The similar BMC between the non-osteogenic groups (swimmers and cyclists) in the current study might be explained by the low repeated loading applied in the skeleton during participation in these sports, which might not enough to produce positive bone adaptations (Maimoun et al., 2013b).

7.5.2 Longitudinal HSA and TBS acquisition between groups

The present findings show that football participation for 1 year induced significantly greater acquisition in bone geometry estimates compared to cyclists and that participation in non-osteogenic sports induced non-significant lower or similar acquisition in bone geometry estimates compared to controls. Previous findings in adolescent female footballers and swimmers showed that 8 months of training in their respective sports induced a significant increase in CSA of the narrow neck in footballers compared to swimmers (Ferry et al., 2013). The geometrical differences between footballers and cyclists in the present study indicate that the continuous unloading environment of cycling may delay the corticalization of the bone structure despite the less hours of training in cyclists compared to footballers (5.6 vs 9.4 hours). In swimmers, the continuous unloading environment is present, but the forces applied to the wall in every change of direction during swimming may induce small adaptations to the femoral neck site of the skeleton (Gomez-Bruton et al., 2016a), which might

explain the non-significant difference between footballers and swimmers at the hip bone geometry estimates.

The present study also includes novel longitudinal findings using TBS in adolescent athletes showing that adolescent footballers had significantly higher improvement in TBS score compared to cyclists and swimmers. A recent cross-sectional evaluation in adults showed that participation in repeated moderate impact loading sports may result in lower TBS score and increased fracture risk compared to high impact loading sports (Heinio et al., 2015). The present study indicates that bone texture acquisition at the lumbar spine may be affected from the external loading environment after controlling for potential confounders that have been shown to influence TBS (Shawwa et al., 2016). It can be speculated that the high intensity loading impact during football participation can result in better trabecular structure at the lumbar spine due to the loading applied both in vertical and horizontal directions.

7.5.3 Longitudinal changes in biochemical markers between and within groups

PINP at T1was significantly higher in footballers compared to swimmers and cyclists while the concentrations of PINP significantly decreased in the non-osteogenic sports from T0 to T1. In addition, CTX-I significantly increased in footballers and swimmers from T0 to T1. The longitudinal changes in PINP and CTX-I suggest an increased bone turnover in footballers and increased bone resorption in swimmers which reflects the differences in bone acquisition between the groups and indicate a contribution of bone turnover on bone adaptations (Gracia-Marco et al., 2011b). A previous longitudinal study in females showed that gymnasts had significantly higher bone formation

(osteocalcin) levels than swimmers after 1 year of participation and that the osteocalcin levels decreased in swimmers but not in gymnasts (Maimoun et al., 2013a). The bone resorption findings of the present study are in line with a previous longitudinal study in peripubertal girls that did not find significant differences in bone resorption between gymnasts, runners, and controls after 1 year (Lehtonen-Veromaa et al., 2000). Also, it has been previously shown that bone remodelling might be increased due to participation in high intensity weight-bearing activities during puberty (Kambas et al., 2016, Jurimae et al., 2010), which might explain the increased bone remodelling in footballers.

Regarding the serum calcium and 25(OH)D, there were no differences between or within groups in serum calcium whereas 25(OH)D levels were significantly higher in footballers and cyclists compared to swimmers and controls at T1. In addition, 25(OH)D significantly increased in footballers from T0 to T1, which may partially contribute to the findings that bone resorption was not affected in this group as it was in the swimmers. All participants had insufficient 25(OH)D levels which agrees with low 25(OH)D levels previously reported in European adolescent population (Gonzalez-Gross et al., 2012a). The higher 25(OH)D levels in footballers and cyclists might be explained by the higher exposure to sunlight during training in these sports, although other parameters such as dietary intake and the sampling period have been reported to affect 25(OH)D levels (Ackerman et al., 2011).

7.5.4 Strengths and limitations

The present study includes the 1 year longitudinal evaluation of bone acquisition in adolescent male athletes and a control group that have never been compared before. In addition, the combination of three different methods

(DXA, HSA and TBS) to assess bone development adds novel findings to the literature by showing for first time that acquisition in HSA and TBS parameters differs between adolescent male athletes in osteogenic and non-osteogenic sports. Moreover, the serum bone turnover markers used in this study have been previously used in paediatric population (Gracia-Marco et al., 2011b, Gracia-Marco et al., 2010) and are recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry (IFCC) (Vasikaran et al., 2011) in order to provide with additional information regarding the bone metabolism changes due to the sports practised. A limitation of the present study might be the less hours of training in cyclists compared to footballers and swimmers. However, the study has strong internal validity due the comprehensive inclusion criteria and the objective control of potential confounders, such as baseline bone values, lean mass and MVPA measured by accelerometers. The findings of the present study strongly suggest that the type of exercise practised during adolescence can induce different bone acquisition on clinical relevant sites skeleton suggesting that weight-bearing activities should be incorporated during adolescence to optimize peak bone mass and reduce low bone status later in life.

7.6 Conclusions

This longitudinal study demonstrated for first time that bone acquisition at clinically relevant sites, assessed by BMC, HSA parameters and TBS is higher in adolescent male footballers compared to swimmers and cyclists. There was no difference between the swimmers and cyclists, and cyclists had non-significant lower bone acquisition compared to controls. Bone formation was higher in footballers compared to swimmers and cyclists and vitamin D levels were higher in footballers and cyclists compared to swimmers and controls.

Bone formation decreased over time in cyclists and swimmers but not in footballers or controls, whereas bone resorption increased in swimmers and footballers. These findings suggest that the osteogenic 1 year of football participation is beneficial for bone acquisition and that participation in non-osteogenic sports is not enough to induce positive bone adaptations. Therefore, programmes to improve bone outcomes of non-osteogenic athletes during adolescence are required. Further research with longer follow up needs to address whether the observed changes in this study translate into better bone health in footballers compared to swimmers and cyclists which could have important training implications for non-osteogenic sports.

8. The effect of a 9-month jumping intervention programme on bone and fitness outcomes in adolescent male athletes

8.1 Abstract

Objectives: To examine the effect of a 9-month jumping intervention on bone and fitness outcomes in adolescent male athletes.

Methods: 93 adolescent male swimmers (SWI), footballers (FOO) and cyclists (CYC) were randomised to intervention (INT) and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) or sport only control (CON-SWI =18, CON-FOO =15, CON-CYC =12) groups. The 9-month jumping intervention comprised of 3 levels using weight-adjustable vests (Level 1= 20 jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). Total body bone mineral content (BMC) was measured using dual-energy x-ray absorptiometry (DXA) and bone stiffness using quantitative ultrasound (QUS). Fitness was assessed using the 20 m shuttle run test, counter movement jump (CMJ) and standing long jump (SLJ) tests.

Results: INT-SWI had significantly higher gains in BMC legs and bone stiffness compared to CON-SWI (4.2-12.7 %). INT-CYC had significantly higher gains in BMC at TBLH and legs and bone stiffness compared to CON-CYC (5.0-12.3 %). There were no significant differences between INT-FOO and CON-FOO in any bone outcomes (0.9-3.9 %). INT-SWI, INT-CYC and INT-FOO significantly improved fitness outcomes (3.7-7.9 %) and CON-FOO significantly improved CMJ (4.0 %).

Conclusions: A 9-month jumping intervention improves bone outcomes only in male adolescents participating in SWI and CYC, while improvements in fitness were observed in all groups. This study highlights the importance of implementing weight-bearing activities, such as jumps, to improve bone health in adolescents involved in non-osteogenic sport athletes.

Keywords: adolescence, bone mass, plyometric jump training, football, cycling, swimming, fitness.

8.2 Introduction

Bone acquisition during the 5 years surrounding peak height velocity (PHV) accounts for up to 43 % of total peak bone mass (PBM) (Baxter-Jones et al., 2011, Boreham and McKay, 2011). External loading applied on the skeleton during adolescence, such as exercise, can increase bone mineral content (BMC) and bone mineral density (BMD) (Hind and Burrows, 2007, MacKelvie et al., 2002a) and tracks into adulthood (Baxter-Jones et al., 2008, Kato et al., 2009, Nikander et al., 2010). However, the bone gains are dependent on the loading characteristics of the sports practised (Maimoun et al., 2013a, Tervo et al., 2010).

Football, cycling and swimming are among the most popular sports performed around the world (Sport England, 2016). Previous studies conducted in adolescents indicate that participation in "osteogenic" sports, such us football, can augment BMC at the loaded sites of the skeleton (Zouch et al., 2014, Maimoun et al., 2013b, Ubago-Guisado et al., 2015a, Vlachopoulos et al., 2017a, Vlachopoulos et al., 2017b). However, participation in "non-osteogenic sports", such as swimming and cycling, may have a negative or no impact on

bone outcomes (Olmedillas et al., 2012, Gomez-Bruton et al., 2013), which may compromise the achievement of a high PBM and increase the risk of osteoporotic fractures in adulthood (Gordon et al., 2016, Chevalley et al., 2017). In a recent cross-sectional study we found that adolescent male swimmers and cyclists had lower adjusted BMC and BMD compared to footballers (Vlachopoulos et al., 2017c), with lean mass and fitness as important, significant predictors of bone outcomes (Vlachopoulos et al., 2017d). Despite the large number of adolescents involved in osteogenic and non-osteogenic sports, there is no evidence on the efficacy of jumping interventions to improve bone mineralization in adolescent athletes.

Previous jumping intervention studies were conducted in the school environment (MacKelvie et al., 2002b, Weeks et al., 2008, Petit et al., 2002) and have been shown to improve bone and fitness outcomes in non-athletic prepubertal and pubertal children (Fuchs et al., 2001, Larsen et al., 2016, McKay et al., 2005b). Adolescent footballers are thought to obtain the osteogenic stimulus needed to optimise their bone health through the sport specific weight-bearing training (Vicente-Rodriguez et al., 2004a). However, there is no evidence whether a jumping intervention can improve their bone health further. Adolescent swimmers and cyclists may not obtain the optimal bone mineralisation during this critical period due to the lack of osteogenic stimulus (Olmedillas et al., 2012, Gomez-Bruton et al., 2013, MacKelvie et al., 2002a), and therefore a jumping intervention with these groups represents a window of opportunity to optimise their bone health. To the best of our knowledge, such intervention has not been tested in adolescent athletic populations.

The purpose of the study was to examine the effects of a 9-month progressive jumping intervention programme on bone outcomes and fitness parameters in adolescent male swimmers (SWI), footballers (FOO) and cyclists (CYC). It was hypothesised that the intervention will induce significantly positive changes on bone outcomes in swimmers and cyclists but not in footballers.

8.3 Methods

8.3.1 Study design and participants

The present study represents a randomised controlled trial intervention as part of the longitudinal PRO-BONE study, whose methodology has been described elsewhere (Vlachopoulos et al., 2015). Inclusion criteria included: 1) Males 12-14 years old, engaged (≥ 3 h/week) in osteogenic (football) or non-osteogenic (swimming and cycling) sports in the last 3 years or more. Exclusion criteria included: 1) participation in another clinical trial; 2) any acute infection lasting until < 1 week before inclusion; 3) medical history of diseases or medications affecting bone metabolism or the presence of an injury (before inclusion) that may affect participation in their respective sports and/or any variable considered in the present study (4) non-Caucasian participants. For the present study, data obtained at pre (autumn/winter 2015/16) and post (summer/ autumn 2016) intervention programme (mean difference of visits = 289 days) were used. The Consolidated Standards of Reporting Trials (CONSORT) flow diagram is presented in Figure 8.1. A total of 93 adolescent male athletes (14.1±1.0 years at pre-intervention) completed all pre and post measurements. Each sport group was randomised by an independent researcher into two different groups: INT and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) and sport only

(without any additional intervention) (CON-SWI=18, CON-FOO=15, CON-CYC=12).

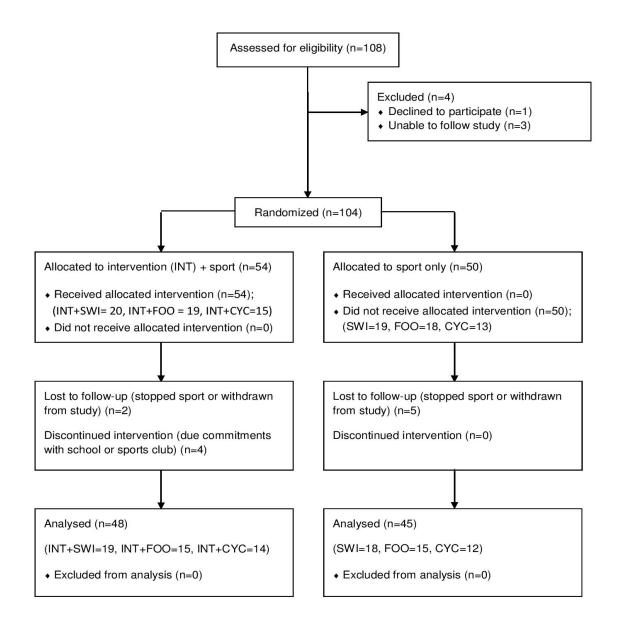


Figure 8.1. PRO-BONE study flow chart. CONSORT, Consolidated standards of reporting trials.

8.3.2 PRO-BONE study jump intervention programme

The 9-month progressive jump intervention programme (~10 min/day) consisted of CMJs and was performed by participants in the INT groups. The intervention consisted of 3 levels (12 weeks each) using adjustable weight vests (The Sports HQ, UK) and was performed on a hard surface. The intensity and the volume increased progressively by modifying the weight in the vests and the number of sets performed at each level (Level 1= 20 jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). A jump diary was used to record the number of jumps performed at each level and was returned to the research group every 3 months. Before the intervention, trained research assistants explained demonstrated the CMJ only to INT groups, and participants executed the CMJ to ensure proper technique. The CMJ was chosen for the intervention as it has a high rate of change in force (493 times body weight/s) and ground reaction forces (5 times body weight) in 8.3 - 11.7 years old boys and girls (McKay et al., 2005a). The reliability and validity of the CMJ has been previously reported (Acero et al., 2011).

Table 8.1. PRO BONE study plyometric jump intervention training progression and compliance									
Level	Exercise	Vest weights (kg)	Repetitions	² Sets / day (³ Rest)	⁴ Trainings / week	Jumps / week	⁵ Compliance in % and number (SD) of jumps completed		
	Exercise						INT-SWI (N=19)	INT-FOO (N=15)	INT-CYC (N=14)
1	¹ CMJ	-	20	3	3	180	90.3 %	95.3 %	91.3 %
Total level 1 (12 weeks)					180 x 12 =	2160	1949 (204)	2059 (155)	1971 (240)
2	¹ CMJ	2	20	4	3	240	75.0 %	83.9 %	83.1 %
Total level 2 (12 weeks)					240 x 12 =	2880	2159 (434)	2416 (444)	2393 (454)
3	¹ CMJ	5	20	4	4	320	46.0 %	56.8 %	47.1 %
Total level 3 (12 weeks)			320 x 12 =	3840	1765 (298)	2181 (434)	1807 (598)		
Lotal Intervention (36 Weeks)						69.5 % 6171 (1097)			

¹Countermovement jump. ²Sets = 20 CMJ. ³Rest between sets = 30 seconds. ⁴When 3 sets/day, jumps suggested to be performed in the morning before going to school (1 set), after school (1 set) and before going to bed (1 set). When 4 sets/day, jumps performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1 set).⁵No significant differences between the intervention groups at any level of the intervention.

8.3.3 Dual energy x-ray absorptiometry

A Lunar Prodigy DXA scanner (GE Healthcare Inc, Wisconsin, USA) was used to measure BMC (g), fat mass (g) and lean mass (g). The total body scan was used to obtain BMC at the arms (as a non-loaded site), legs, and total body less head (TBLH). All scans were undertaken by the same fully trained operator and following the International Society of Clinical Densitometry guidelines (Crabtree et al., 2014). The DXA percentage coefficient of variation has been reported between 1.0 % and 2.9 % at TBLH (Johnson and Dawson-Hughes, 1991).

8.3.4 Quantitative ultrasound

QUS measurements were performed with a Lunar Achilles Insight (GE Healthcare Inc., Wisconsin, USA). This portable device measured bone stiffness using ultrasound waves and measurements were always taken following manufacturer guidelines and by trained staff. QUS is considered a reliable, valid and radiation-free method to assess bone health in children (Sioen et al., 2016).

8.3.5 Anthropometry and maturity status

Height (cm) and body mass (kg) were measured by using standard procedures (Vlachopoulos et al., 2015). Somatic maturity status was assessed using predicted age at PHV. The age from age at PHV was predicted using age and height in a validated algorithm ($R^2 = 0.90$; standard error = 0.5) (Moore et al., 2015).

8.3.6 Physical activity, training characteristics and diet

Physical activity was measured for seven consecutive days at pre- and post-intervention using wrist accelerometers (GENEA, Cambridgeshire, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (Phillips et al., 2013). Data were collected at 100 Hz and analysed using 1 s epochs to establish time spent in MVPA using a validated cut-point (Phillips et al., 2013). Weekly training hours were obtained by face to face interviews at pre- and post-intervention.

Total energy, protein and calcium intake were assessed using a 24-h food recall. The validity and reliability of self-reported dietary intake has been previously reported in children (Weber et al., 2004). Total energy, calcium and protein intake were estimated using the CompEat Pro software (Nutrition systems, VIS Visual Information Systems Ltd., UK).

8.3.7 Cardiorespiratory and muscular fitness

Cardiorespiratory fitness was estimated using the 20 m shuttle run test (20mSRT) (Leger et al., 1988) completed in the same sports hall both pre-and post-intervention. The participants were encouraged to continue the test until they reached maximal effort. The test terminated when the participant failed to reach the line two consecutive times. The last completed shuttle determined the score of the test and the number of shuttles completed was taken as an indicator of cardiorespiratory fitness. The 20mSRT has been shown to be reliable and valid in adolescents (Castro-Pinero et al., 2010).

Muscular fitness was assessed using the SLJ test and the CMJ test at least 30 minutes before performing the 20mSRT and following a standardized warm up. For the SLJ, participants were advised to jump as far as possible in order to land with both feet and the distance (cm) measured between the starting line

and the participant's heels was recorded. The CMJ was assessed on a jump mat (Probotics Inc, Alabama, USA) which calculates jump height based on flight time. The participants performed the CMJ with their feet shoulder width apart. For both jump tests three maximal jumps were performed and the best score was used. The reliability of the CMJ and SLJ has been reported in adolescents (Ortega et al., 2008).

8.3.8 Statistical analyses

Statistical analyses were performed using the SPSS version 21.0 for Windows (IBM statistics, Chicago, IL, USA). Data were checked for normality and presented as mean and standard deviation. Data were analysed for each sport group separately using: 1) paired t-tests to detect the differences in characteristics and fitness parameters between pre- and post-intervention visits, and 2) after controlling for baseline bone status, change in lean mass and post maturity status (years from PHV), a one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect differences between the intervention and the non-intervention groups in 9-month adjusted gains (Δ BMC and Δ bone stiffness). The selection of the covariates was based on relevant predictors of bone outcomes in adolescents (Jackowski et al., 2011a, Wilkinson et al., 2017, Vlachopoulos et al., 2017d, Ubago-Guisado et al., 2017). Percentages of difference between the intervention and non-intervention groups were used to quantify the magnitude of the differences in adjusted bone outcome gains. Significance was set at p < 0.05.

8.4 Results

8.4.1 Descriptive characteristics

Table 8.2. Characteristics of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme

		SWI	MERS			FOOTE	BALLERS		CYCLISTS				
TOTAL (N=105)		INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		ONTROL (N=12)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	
Age (years)	14.5 ± 0.9	15.3 ± 0.9	14.7 ± 1.1	15.4 ± 1.1	13.8 ± 1.0	14.6 ± 1.0	13.7 ± 0.8	14.5 ± 0.8	14.1 ± 1.1	14.9 ± 1.1	14.1 ± 0.9	14.9 ± 0.9	
Height (cm)	170.3 ± 10.0	174.1 ± 9.6	172.8 ± 7.6	176.0 ± 6.9	160.5 ± 11.0	165.4 ± 11.1	163.3 ± 9.3	168.9 ± 9.1	168.2 ± 10.7	174.5 ± 8.2	162.7 ± 9.7	166.4 ± 9.6	
Body mass (kg)	57.2 ± 9.0	62.6 ± 7.8	60.6 ± 7.2	63.6 ± 7.7	49.3 ± 10.8	54.8 ± 11.6	49.6 ± 7.4	55.4 ± 7.1	57.7 ± 13.0	62.6 ± 12.2	51.0 ± 12.4	55.2 ± 13.9	
Lean mass (kg)	46.7 ± 9.4	51.2 ± 8.5	48.7 ± 8.2	51.9 ± 6.7	41.5 ± 10.0	46.1 ± 10.5	39.6 ± 7.4	44.9 ± 7.2	45.0 ± 7.9	49.5 ± 7.6	40.3 ± 8.6	44.0 ± 9.2	
Fat mass (kg)	7.6 ± 3.1	8.5 ± 2.9	8.2 ± 3.4	8.7 ± 3.8	5.4 ± 2.2	6.1 ± 2.3	7.1 ± 2.3	7.6 ± 3.5	9.9 ± 9.7	10.0 ± 9.7	8.1 ± 5.5	8.3 ± 6.5	
PHV (years)	1.0 ± 1.0	1.7 ± 1.0	1.2 ± 1.0	1.8 ± 0.9	0.0 ± 1.0	0.7 ± 1.0	0.1 ± 0.8	0.9 ± 0.8	0.7 ± 1.1	1.4 ± 1.0	0.3 ± 0.9	1.0 ± 1.0	
MVPA (min/day)	61.3 ± 19.6	67.5 ± 19.6	59.6 ± 25.2	60.0 ± 18.1	97.7 ± 18.8	83.3 ± 18.5	89.6 ± 33.5	76.3 ± 22.4	85.6 ± 22.1	93.9 ± 15.7	88.9 ± 21.8	78.7 ± 13.4	
Training volume (hrs/week)	7.9 ± 3.6	11.8 ± 5.4	10.2 ± 3.1	12.9 ± 5.4	10.2 ± 1.2	12.0 ± 2.8	8.8 ± 2.0	10.7 ± 1.8	5.3 ± 2.0	7.9 ± 3.8	5.3 ± 1.6	8.2 ± 2.8	
Energy ntake (kcal/day)	2534 ± 382	2465 ± 221	2603 ± 425	2386 ± 133	2237 ± 517	2379 ± 262	2419 ± 620	2309 ± 234	2320 ± 280	2244 ± 166	2221 ± 325	2226 ± 152	
Protein ntake g/day)	85.8 ± 30.9	89.8 ± 31.2	77.9 ± 28.5	82.8 ± 24.8	95.3 ± 21.6	97.1 ± 29.3	92.0 ± 35.3	90.1 ± 20.4	80.3 ± 13.3	88.0 ± 23.0	84.9 ± 28.9	86.4 ± 21.1	
Calcium ntake (mg/day)	1237 ± 280	1118 ± 289	1155 ± 257	1109 ± 189	1342 ± 350	1231 ± 167	1177 ± 378	1179 ± 178	1183 ± 370	1253 ± 196	1385 ± 280	1279 ± 149	

Values are mean ± standard deviation. Bold values indicate significant higher values between PRE and POST. MVPA: Moderate to vigorous physical activity; PHV: peak height velocity.

The mean total compliance of the intervention is shown in Table 8.1. Table 8.2 presents the descriptive characteristics of the participants pre and post intervention. In all INT groups, the studied variables significantly increased from pre- to post-intervention, except MVPA and fat mass in INT-CYC. Similarly, all variables significantly increased from pre to post in CON-SWI, CON-FOO and CON-CYC except MVPA, and fat mass. Total energy, protein and calcium intakes did not change pre and post for any of the groups.

8.4.2 Nine-month adjusted bone gains

Table 8.3. PRE and 9-month adjusted gain in bone mineral content (BMC, g) and bone stiffness of the intervention and control groups

TOTAL (N=105)		SWIN	MERS			FOOTE	BALLERS		CYCLISTS				
	INTERVENTION (N=19)		CONTROL (N=18)			INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=19)		NTROL l=18)	
	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	
TBLH	1892 ±	322	1952 ±	251	1730 ±	386	1769 ±	325	1848 ±	341	1582 ±	203	
BMC	339	(276-368)	325	(203-300)	479	(335-438)	398	(274-377)	379	(290-394)	401	(145-260)	
Legs	888 ±	141	922 ±	95	877 ±	150	886 ±	131	899 ±	148	759 ±	87	
BMC	147	(124-159)	135	(77-114)	246	(130-170)	201	(111-150)	170	(129-168)	168	(65-109)	
Arms	290 ±	44	303 ±	40	226 ±	46	228 ±	48	271 ±	49	233 ±	40	
BMC	68	(37-51)	67	(33-47)	76	(38-53)	61	(41-56)	68	(40-56)	74	(32-48)	
Bone stiffness	95.9 ±	10.3	94.5 ±	-1.8	104.4 ±	15.3	105.7 ±	10.9	95.6 ±	9.5	93.6 ±	-2.2	
	12.0	(7.2-13.5)	16.3	(-5.1-1.6)	11.9	(11.6-8.9)	12.4	(7.2-14.6)	15.6	(5.9-13.1)	14	(-6.0-1.7)	

Raw values at PRE are mean ± standard deviation. Values at 9-month were adjusted for pre bone values, change in lean mass and post peak height velocity, and presented as mean and 95% CI. BMC: Bone mineral content, TBLH: Total body less head. Bold values denote significant higher gains compared to the sport specific control group. p<0.05.

Table 8.3 shows pre-intervention BMC (g) and the 9-month adjusted gains in BMC (g) and bone stiffness in all groups. Figure 8.2 shows the percentage of difference on adjusted BMC and bone stiffness gains between each of the INT-SPORT and CON-SPORT groups. INT-SWI gained significantly higher legs BMC (5.6 %) and bone stiffness (12.6 %) than CON-SWI. INT-CYC gained significantly higher TBLH BMC (5.6%), legs BMC (5.0%) and bone stiffness (12.3%) than CON-CYC (all p<0.05). There were no significant differences between INT-FOO and CON-FOO for the bone outcomes (p>0.05).

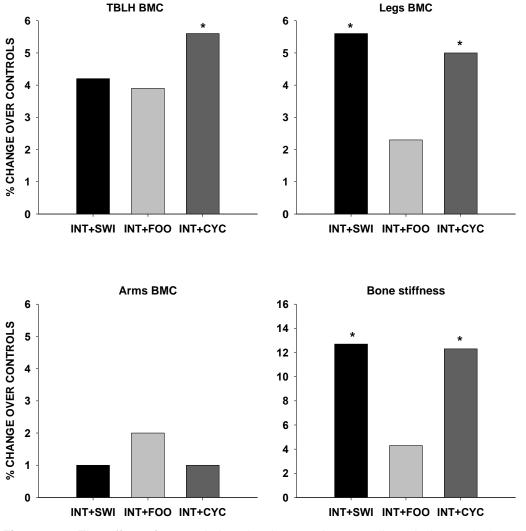


Figure 8.2. The effect of 9-month jumping intervention on adjusted change in bone mineral content (BMC, g) and bone stiffness presented as percentage (%) over control groups (0 lines). Results were adjusted for baseline (pre) bone outcomes, change in lean mass and post maturity status. Superscript * denotes significant higher change compared to the sport specific control group, p<0.05.

8.4.3 Physical fitness parameters

Table 8.4 presents physical fitness measurements of the groups pre- to post-intervention. INT-SWI and INT-CYC significantly increased CMJ (6.4-7.2 %), SLJ (3.7-4.8 %) and 20mSRT (7.0-7.9 %) from pre- to post-intervention (all p<0.05). In INT-FOO, CMJ (4.7 %) and SLJ (4.0 %) were significantly increased from pre- to post-intervention (all p<0.05), but 20mSRT did not (3.9 %). In CONSWI and CON-CYC none of the fitness parameters (1.5-2.5 %) improved (p>0.05). CON-FOO significantly increased CMJ (4.0 %) from pre- to post-intervention (p<0.05).

Table 8.4. Physical fitness measurements of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme

		SWI	MMERS			FOOTE	BALLERS		CYCLISTS			
TOTAL (N=105)	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=19)		CONTROL (N=18)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Counter movement jump (cm)	46.8 ± 7.2	49.9 ± 7.7	46.6 ± 9.4	45.9 ± 8.8	45.3 ± 6.1	47.5 ± 5.7	41.7 ± 5.8	43.4 ± 6.4	42.7 ± 5.9	45.9 ± 5.3	45.4 ± 7.8	46.3 ± 8.0
Standing Long Jump (cm)	195.8 ± 27.8	203.1 ± 27.9	191.1 ± 27.7	194.7 ± 28.1	188.1 ± 24.9	194.6 ± 23.4	184.1 ± 21.2	191.7 ± 22.3	173.8 ± 34.1	182.3 ± 31.8	180.7 ± 24.5	185.3 ± 28.4
20mSRT (shuttles)	79.2 ± 17.6	85.7 ± 17.3	74.2 ± 24.4	76.1 ± 18.0	94.5 ± 14.4	98.3 ± 16.1	92.0 ± 21.9	94.9 ± 25.0	82.4 ± 24.2	88.4 ± 22.6	82.8 ± 19.2	85.7 ± 25.1

20mSRT: 20 meter shuttle run test. Values are mean ± standard deviation. Bold values indicate significant higher values between PRE and POST.

8.5 Discussion

This is the first jumping intervention implemented in male adolescent athletes involved in osteogenic (football) and non-osteogenic (swimming and cycling) sports. The main findings of the present study were that a 9-month progressive jumping intervention programme: 1) induced significant improvements on clinically relevant bone outcomes in athletes engaged in the non-osteogenic sports, swimming and cycling, but not in the osteogenic sport, football; and 2) induced significant improvements on fitness outcomes in both osteogenic and non-osteogenic sports groups. Collectively, this study shows that a progressive jump training programme can be easily implemented by sport clubs to improve bone health and fitness outcomes in male adolescent athletes who participate in non-osteogenic sports such as swimming and cycling.

8.5.1 Effectiveness of the PRO-BONE jumping intervention on bone gains

Comparisons between jumping intervention studies focusing on athletes are difficult because there are no previous studies evaluating the effect of a jumping intervention on bone outcomes in athletes. The effect of the PRO-BONE jumping intervention in non-osteogenic sport athletes on bone outcomes was either greater or equivalent to that of previous jumping intervention studies in non-athletic children and adolescents (Weeks et al., 2008, McKay et al., 2005b, MacKelvie et al., 2002b). A previous 8-month school-based jumping intervention reported that non-athletic adolescent males had 4.3 % significantly higher gains at total body BMC and 5.0 % improvements at QUS outcome compared to controls (Weeks et al., 2008). In the present 9-month intervention, there was a greater magnitude in improvements at TBLH BMC (4.2 - 5.6 %), legs BMC (5.0 - 5.6 %), and bone stiffness (12.3 - 12.7 %) in INT-SWI and INT-CYC compared

to CON-SWI and CON-CYC. The greater improvements observed on bone outcomes in non-osteogenic sport athletes can be explained by the ground reaction forces and progressive skeletal stimulus applied on the unloaded skeleton during the jumping intervention (Gomez-Bruton et al., 2016a, Olmedillas et al., 2012). Previous 7 and 8-month jumping intervention studies in a school-based environment on non-athletic pubertal children and adolescents reported significant improvements on BMC (1.4 - 4.5 %) in the intervention groups compared to controls (MacKelvie et al., 2002b, McKay et al., 2005b, Fuchs et al., 2001). In these previous studies, the effect of the intervention was greater at the weight-bearing sites of the skeleton (4.5 %), which is equivalent with the higher improvements observed at legs BMC (5.0 - 5.6 %) in the present study. In addition, the greater bone adaptations in the non-osteogenic groups of the current study compared to previous work may be attributed to the ability of the skeleton to adapt better to the external stimulus after long-term (8 and 6 years for swimmers and cyclists, respectively) non-osteogenic sport participation (Maimoun et al., 2013b, Nikander et al., 2005). In contrast, but consistent with our hypothesis, the stimulus provided by the jumping intervention was not enough to induce significant bone gains in INT-FOO compared to CON-FOO, despite footballers showing better compliance to the intervention compared to cyclist and swimmers (75% vs. 69.5% and 66%, respectively, p>0.05). The compliance in the present study was equivalent or lower compared to other studies (70% vs 80-90%) (MacKelvie et al., 2002b, Fuchs et al., 2001, Gunter et al., 2008a) and this might be due to the longer duration of the present intervention (9 months vs 7-8 months) and due to the greater number of jumps performed in the present study (160 vs 90 jumps per week) (McKay et al., 2005b). According to the mechanostat theory the bones

adapt their strength and content to respond to the strain caused by external physiological loads up to a certain point (Schoenau, 2005, Ireland et al., 2014). Footballers may have reached a ceiling for bone improvements as we have previously shown to have greater bone outcomes compared to swimmers and cyclists after adjusting for lean mass among other confounders (Vlachopoulos et al., 2017c). A different intervention programme of longer duration and loading intensity may be needed to improve bone outcomes in osteogenic sports, such as football. The implemented jumping intervention significantly improved bone outcomes in non-osteogenic sports, such as swimming and cycling compared to their respective control groups indicating a window of opportunity to counteract the lack of osteogenic stimulus observed in adolescent athletes involved in non-osteogenic sports (Olmedillas et al., 2012).

8.5.2 Effectiveness of the PRO-BONE jumping intervention on physical fitness

Physical fitness has extensively been considered as a powerful marker of health (Baptista et al., 2016) and physical fitness is associated with lean mass and bone status during adolescence (Ubago-Guisado et al., 2017). The present intervention induced significant improvements in CMJ (6.2 - 7.0 %), SLJ (4.6 - 5.0 %) and 20mSRT (6.8 - 7.6 %) in INT-SWI and INT-CYC, while there were no improvements in CON-SWI and CON-CYC. Also, CMJ significantly improved in INT-FOO and CON-FOO (3.9 - 4.1 %) and FOO INT significantly improved SLJ (3.5 %). The 20mSRT did not improve in INT-FOO and CON-FOO which might be explained by our previous cross-sectional findings showing that footballers already have significantly higher 20mSRT score compared to swimmers and cyclists (Ubago-Guisado et al., 2017), therefore the potential to improve20mSRT score from the present jumping intervention programme is

greater in swimmers and cyclists than in footballers. The improvements observed for the fitness outcomes in the present study are likely to be related to the ground reaction forces applied on the skeleton during the jumping intervention, which acts as a mechanism to improve bone outcomes via the muscular contractions produced (McKay et al., 2005a, Ginty et al., 2005a). The findings of the present study are in accordance with a 10-month intervention programme that included small-sided ball games and circuit strength training groups and found 9-10 % higher improvements on CMJ distance of both non-athletic 8-10 year olds compared to controls (Larsen et al., 2016). The magnitude on fitness improvements in the present study might be explained by the dose-response of benefits of plyometric training on physical performance in adolescent athletes (Lesinski et al., 2016).

8.5.3 Strengths and limitations

The strengths of the present study are: 1) the evaluation for first time of a novel 9-month progressive jumping intervention programme in adolescent athletes participating in osteogenic and non-osteogenic sports; 2) the combination of DXA and QUS, which provides a comprehensive insight into the responses of the intervention on BMC and bone stiffness and 3) the low cost and relative ease jumping programme for young athletes that almost any sport club could implement with minimal training for the coach and the athlete. Despite DXA is a clinically relevant device to assess bone outcomes, the two-dimensional imaging technique cannot provide information regarding the structural adaptations that may be induced from the jumping intervention.

8.6 Conclusion

The present study is the first randomised control trial to investigate the musculoskeletal effects of a 9-month progressive jumping intervention programme in adolescent male athletes. The findings indicate that the jumping intervention programme can significantly improve bone mass at total body and legs, and bone stiffness in adolescent male athletes involved in non-osteogenic sports and induce significant improvements in muscular and cardiorespiratory fitness on both osteogenic and non-osteogenic groups. The intervention programme can be implemented by non-osteogenic sports clubs and athletes to improve bone health.

9. A 9-month jumping intervention to improve bone mass, geometry and metabolism in adolescent male athletes

9.1 Abstract

The objective of study was to examine the effects of a 9-month jumping intervention on bone mass, geometry, texture, bone turnover and nutritional markers in adolescent male athletes. Ninety three adolescent male swimmers (SWI), footballers (FOO) and cyclists (CYC) were randomized to an intervention (INT) and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) or sport only control (CON-SWI =18, CON-FOO =15, CON-CYC =12) groups. The 9-month jumping intervention consisted of 3 levels using weight vests (Level 1= 20 jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). Bone mineral content (BMC) was assessed using dual-energy x-ray absorptiometry. Cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and section modulus (Z) at the femoral neck were assessed using hip structural analysis, and trabecular texture of the lumbar spine using trabecular bone score (TBS). Serum Nterminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] were analysed. INT-CYC gained significantly higher lumbar spine BMC (4.6 %) and femoral neck BMC (9.8 %) than CON-CYC. INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There were no significant differences between INT-FOO and CON-FOO in any of the bone outcomes. PINP significantly decreased in CON-SWI, INT-FOO, CON-FOO and CON-

CYC. CTX-I significantly decreased in CON-SWI and CON-CYC. 25(OH)D significantly increased in INT-CYC, CON-CYC, INT-FOO and CON-FOO. This novel jumping intervention programme improved bone outcomes at lumbar spine and femoral neck of adolescent male athletes involved in non-osteogenic sports, such as swimming and cycling, but not in those involved in osteogenic sports, such as football.

Keywords: adolescence, bone gains, exercise, intervention, jumping, sports.

9.2 Introduction

Exercise during childhood and adolescence can improve bone mineral content (BMC) and areal bone mineral density (aBMD) (Hind and Burrows, 2007, Baxter-Jones et al., 2008, Ward et al., 2003) with benefits maintained into adulthood (Kato et al., 2009, Tervo et al., 2010, Weaver et al., 2016). Low bone mass during adolescence is associated with increased fracture risk and osteoporosis later in life (Christoffersen et al., 2017, Cooper et al., 2004a, Clark et al., 2006). The adolescent years are critical for bone development with up to 43 % of peak bone mass acquired during the 5-year period surrounding peak height velocity (PHV) (Baxter-Jones et al., 2011, MacKelvie et al., 2002a). Bone acquisition depends, in part, on the ground reaction forces applied to the skeleton during exercise, yet not all the types of sport can improve bone mass and structure (McKay et al., 2005a, Maimoun et al., 2013a, Vlachopoulos et al., 2017b). Football, cycling and swimming are among the most popular sports worldwide for adolescents and previous longitudinal studies show that weight-bearing sports, such us football, can augment BMC at the loaded sites of the

skeleton (Zouch et al., 2014, Maimoun et al., 2013b, Vlachopoulos et al., 2017a). However, participation in non-weight-bearing, such as swimming and cycling, may have a negative or no impact on bone development (Olmedillas et al., 2012, Gomez-Bruton et al., 2013, Vlachopoulos et al., 2017c), which may compromise the achievement of a higher peak bone mass.

A large number of adolescents are involved in football, swimming and cycling, but there is limited knowledge on the effectiveness of interventions to improve bone mineralization in athletes during this period of life. Previous intervention studies were conducted in the school environment (MacKelvie et al., 2002b, Weeks et al., 2008, Petit et al., 2002) and have shown that jumping can improve bone outcomes in non-athletic prepubertal and pubertal children (McKay et al., 2005b, Fuchs et al., 2001, Weeks et al., 2008). Adolescent footballers have been found to obtain the osteogenic stimulus needed to optimise their bone health through the sport specific weight-bearing training (Vicente-Rodriguez et al., 2004a, Vlachopoulos et al., 2017c), but there is no evidence whether a jumping intervention can improve further their bone health. In contrast, adolescent swimmers and cyclists may not obtain the optimal bone mineralisation during this critical period due to the lack of osteogenic stimulus (Olmedillas et al., 2012, Gomez-Bruton et al., 2013). However, it is not known whether a jumping intervention can counteract the lack of osteogenic stimulus in non-weight bearing adolescent athletes, such as swimmers and cyclists.

Changes in BMC and aBMD and bone area (Behringer et al., 2014, Zouch et al., 2014) due to external mechanical loading can be measured by dual-energy x-ray absorptiometry (DXA), but adaptations in strength, structure and geometry during growth or following an intervention such as jumping, may not be detected due to the two dimensional nature of DXA (Petit et al., 2002, MacKelvie et al.,

2004). However, there are studies using techniques such as hip structural analysis (HSA) to assess bone geometry estimates, such as cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and section modulus at the femoral neck in adolescents (Maimoun et al., 2013b, Ferry et al., 2013). In addition, the recently developed trabecular bone score (TBS), which can predict fracture risk (Hans et al., 2011b) and fragility of the lumbar spine (Shawwa et al., 2016, Silva et al., 2014), can provide an indirect textural index of trabecular microarchitecture in the lumbar spine (Hans et al., 2011a). Moreover, the assessment of bone turnover and nutrition markers, such as N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] can provide important information about bone formation and resorption in relation to the sports practised during adolescence (Banfi et al., 2010, Gracia-Marco et al., 2010, Vaitkeviciute et al., 2016). We recently found that footballers gained significantly higher HSA and TBS outcomes compared to swimmers and cyclists after one year of sport specific training, and footballers had significantly higher bone formation compared to both non-osteogenic sports (Vlachopoulos et al., 2017a).

The scope of the study was to examine the effects of a 9-month progressive jumping intervention programme on BMC, hip geometry estimates, TBS at the clinically relevant skeletal sites of lumbar spine and femoral neck, and bone turnover markers in adolescent male swimmers (SWI), footballers (FOO) and cyclists (CYC). It was hypothesised that the intervention will induce significantly positive changes on bone outcomes in swimmers and cyclists but not in footballers.

9.3 Methods

9.3.1 Cohort and study design

The present randomized controlled trial intervention is part of the longitudinal PRO-BONE study, whose methodology has been described elsewhere (Vlachopoulos et al., 2015). The inclusion criteria were adolescent males 12-14 years old, engaged (≥ 3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports in the last 3 years or more. The exclusion criteria were participation in another clinical trial, any acute infection lasting until < 1 week before inclusion, medical history of diseases or medications affecting bone metabolism or the presence of an injury (before inclusion) that may affect participation in their respective sports and/or any variable considered in the present study and non-Caucasian participants. Informed consent was obtained from all parents and participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics approval received from the following committees: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West - Cornwall & Plymouth, ref. number 14/SW/0060). For the present study, data obtained at pre (autumn/winter 2015/16) and post (summer/autumn 2016) the intervention programme (mean difference of visits = 289 days) are used. The Consolidated Standards of Reporting Trials (CONSORT) flow diagram is presented in Figure 9.1. A total of 93 adolescent males (14.1±1.0 years at pre-intervention) completed all pre and post measurements. The sports groups were simply

randomized by an independent researcher into two different groups: INT and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) and sport only (without any additional intervention) (CON-SWI=18, CON-FOO=15, CON-CYC=12).

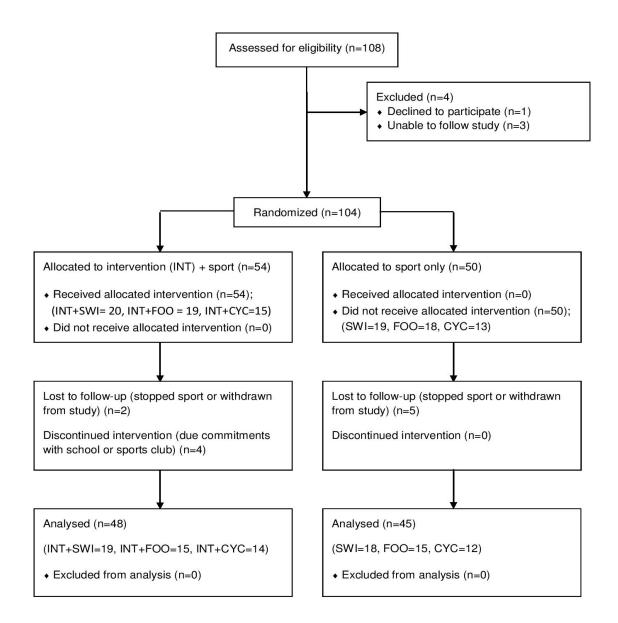


Figure 9.1. PRO-BONE study flow chart. CONSORT, Consolidated standards of reporting trials.

9.3.2 PRO-BONE study jump intervention programme

The 9-month progressive jump intervention programme (~10 min/day) consisted of counter movement jumps (CMJ) and was performed by participants in the INT groups. The intervention consisted of 3 levels (12 weeks each) using adjustable weight vests (The Sports HQ, UK) and was performed on a hard surface. The intensity and the volume increased progressively (Table 9.1) by modifying the weight in the vests and the number of sets performed at each level (Level 1= 20 jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). A jump diary was used to record the number of jumps performed at each level and was returned to the research group every 3 months. Before the intervention, trained research assistants explained and demonstrated the CMJ only to INT groups, and participants executed the CMJ to ensure proper technique. The CMJ was chosen for the intervention as it has a high rate of change in force (493 times body weight/s) and ground reaction forces (5 times body weight) in 8.3 - 11.7 years old boys and girls (McKay et al., 2005a). The reliability and validity of the CMJ has been previously reported (Acero et al., 2011).

Table 9.1.	Table 9.1. PRO BONE study plyometric jump intervention training progression and compliance												
Level	Exercise	Vest weights (kg)	Repetitions	² Sets / day	⁴Trainings /	Jumps /		mpliance in % SD) of jumps c					
Level	Exercise			(³ Rest)	week	week	INT-SWI (N=19)	INT-FOO (N=15)	INT-CYC (N=14)				
1	¹ CMJ	-	20	3	3	180	90.3 %	95.3 %	91.3 %				
Total level 1 (12 weeks)					180 x 12 =	2160	1949 (204)	2059 (155)	1971 (240)				
2	¹ CMJ	2	20	4	3	240	75.0 %	83.9 %	83.1 %				
Total leve	l 2 (12 weeks)				240 x 12 =	2880	2159 (434)	2416 (444)	2393 (454)				
3	¹ CMJ	5	20	4	4	320	46.0 %	56.8 %	47.1 %				
Total leve	l 3 (12 weeks)				320 x 12 =	3840	1765 (298)	2181 (434)	1807 (598)				
Total inter	rvention (36 weeks)					8880	66.0 % 5858 (1051)	75.0 % 6656 (1281)	69.5 % 6171 (1097)				

¹Countermovement jump. ²Sets = 20 CMJ. ³Rest between sets = 30 seconds. ⁴When 3 sets/day, jumps suggested to be performed in the morning before going to school (1 set), after school (1 set) and before going to bed (1 set). When 4 sets/day, jumps performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1 set). ⁵No significant differences between the intervention groups at any level of the intervention.

9.3.3 Bone outcomes: DXA, HSA, TBS and biochemical markers

A Lunar Prodigy DXA scanner (GE Healthcare Inc., Wisconsin, USA) was used to measure BMC (g), fat mass (g) and lean mass (g). The lumbar spine (LS, L1-L4) and bilateral proximal femora scans were used to assess BMC. All DXA scans and subsequent in-software analyses were completed by the same researcher using the same Lunar Prodigy DXA scanner and the enCORE software version 14.10.022 (GE Healthcare Inc, Wisconsin, USA) and following the International Society of Clinical Densitometry guidelines (Crabtree et al., 2014). The coefficient of variation was not determined in the present study, but previous paediatric studies have shown that the DXA percentage coefficient of variation (CV) was between 0.64 % and 1.16 % at femoral neck and lumbar spine regions (Shepherd et al., 2011).

The hip geometry estimates of the femoral neck were obtained and the following variables used: 1) the cross sectional area (CSA, mm²), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia (CSMI, mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; and 3) section modulus (Z, mm³), which is an indicator of maximum bending strength in a cross section. The CVs of these variables previously found to be between 7.9 % and 11.7 % (Khoo et al., 2005).

TBS is a DXA based technological tool that provides an indirect textural index of trabecular microarchitecture in the lumbar spine and has been shown to significantly predict fracture risk independent of BMC (Hans et al., 2011a). TBS assesses DXA images of the lumbar spine scans using a grey-level analysis as the slope at the origin of the log-log representation of the experimental

variogram (Pothuaud et al., 2009). All TBS analyses were performed by the same trained researcher using the TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The calculation was performed at the lumbar spine region of interest as in the BMC measurement. The CVs of TBS in relation to BMC has been reported to be 1.1 % to 1.9 % (Silva et al., 2014).

Capillary blood samples were collected in the morning of non-training weekends using heparin fluoride coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) and centrifuged at 3000 rpm for 15 minutes at 4°C. Serum samples were stored at -80°C until later analysis. Total serum levels of PINP, CTX-I, 25(OH)D and total calcium were analysed following guidelines (Vasikaran et al., 2011). ELISA kits (Abbexa Ltd., Cambridge, UK) for PINP (test range: 6-400 pg·mL⁻¹, sensitivity: 1.2 pg·mL⁻¹, inter and intra-assay CVs: 8.6 % and 9.1 % respectively), CTX-I (test range: 0.1-7.0 ng·mL⁻¹, sensitivity: 0.03 ng·mL⁻¹, inter and intra-assay CVs: 8.3 % and 9.2 % respectively), and 25(OH)D (test range: 3-80 ng·mL⁻¹, sensitivity: 1.2 ng·mL⁻¹, inter and intra-assay CVs: 6.4 % and 8.0 % respectively) were used. Total calcium serum was measured using direct colorimetric assay (Cayman Chemical Company, MI, U.S.A.) and had a sensitivity of 0.25 mg·dL⁻¹ and the absorbance was read at 570-590 nm (inter and intra-assay CVs: 7.9 % and 9.0 % respectively).

9.3.4 Other measures

Height (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Somatic maturity status was assessed using predicted age at peak height velocity (PHV) which is a somatic biological maturity indicator and reflects the maximum growth velocity during

adolescence. The age at PHV was predicted using age and height in validated algorithm showing how far an individual is from this maturity milestone (years from age at PHV). The coefficient of determination has been reported ($R^2 = 0.90$; standard error = 0.5) (Moore et al., 2015).

Physical activity was measured for seven consecutive days at pre- and post-intervention using wrist accelerometers (GENEA, Cambridgeshire, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (Phillips et al., 2013). Data were collected at 100 Hz and analysed at 1 s epoch intervals to establish time spent in MVPA using a validated cut-point (Phillips et al., 2013). Weekly training hours were obtained by face to face interviews at pre- and post-intervention.

9.3.5 Statistical analyses

Statistical analyses were performed using the SPSS version 21.0 for Windows (IBM Corp, New York, USA). Data were checked for normality and presented as mean and standard deviation (SD). Data were analysed for each sport group separately using: 1) paired t-tests to detect the differences in characteristics and blood markers between pre- and post-intervention visits, and 2) after controlling for baseline bone status, change in lean mass and post maturity status (years from PHV), a one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect differences between the intervention and the non-intervention groups in 9-month adjusted gains (Δ BMC, Δ HSA and Δ TBS). The selection of the covariates was based on relevant predictors of bone outcomes in adolescents (Jackowski et al., 2011a, Wilkinson et al., 2017, Vlachopoulos et al., 2017d, Ubago-Guisado et al., 2017). Percentages of difference between the

intervention and non-intervention groups were used to quantify the magnitude of the differences in adjusted bone outcome gains. Significance was set at p<0.05.

9.4 Results

9.4.1 Cohort characteristics

Table 9.1 shows the mean total compliance of the intervention. Table 9.2 presents the descriptive characteristics of the participants pre- and post-intervention. In all INT groups, all variables significantly increased from pre- to post-intervention, except fat mass in INT-CYC and MVPA in all groups. Similarly, all variables significantly increased from pre to post in CON-SWI, CON-FOO and CON-CYC except MVPA and fat mass.

Table 9.2. Characteristics of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme

		SWIN	MERS			FOOTE	BALLERS		CYCLISTS				
TOTAL (N=93)	INTERVENTION (N=19)		CONTROL (N=18)			INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		NTROL N=12)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	
Age (years)	14.5 ± 0.9	15.3 ± 0.9	14.7 ± 1.1	15.4 ± 1.1	13.8 ± 1.0	14.6 ± 1.0	13.7 ± 0.8	14.5 ± 0.8	14.1 ± 1.1	14.9 ± 1.1	14.1 ± 0.9	14.9 ± 0.9	
Height (cm)	170.3 ± 10.0	174.1 ± 9.6	172.8 ± 7.6	176.0 ± 6.9	160.5 ± 11.0	165.4 ± 11.1	163.3 ± 9.3	168.9 ± 9.1	168.2 ± 10.7	174.5 ± 8.2	162.7 ± 9.7	166.4 ± 9.6	
Body mass (kg)	57.2 ±	62.6 ±	60.6 ±	63.6 ±	49.3 ±	54.8 ±	49.6 ±	55.4 ±	57.7 ±	62.6 ±	51.0 ±	55.2 ±	
	9.0	7.8	7.2	7.7	10.8	11.6	7.4	7.1	13.0	12.2	12.4	13.9	
Lean mass (kg)	46.7 ±	51.2 ±	48.7 ±	51.9 ±	41.5 ±	46.1 ±	39.6 ±	44.9 ±	45.0 ±	49.5 ±	40.3 ±	44.0 ±	
	9.4	8.5	8.2	6.7	10.0	10.5	7.4	7.2	7.9	7.6	8.6	9.2	
Fat mass (kg)	7.6 ±	8.5 ±	8.2 ±	8.7 ±	5.4 ±	6.1 ±	7.1 ±	7.6 ±	9.9 ±	10.0 ±	8.1 ±	8.3 ±	
	3.1	2.9	3.4	3.8	2.2	2.3	2.3	3.5	9.7	9.7	5.5	6.5	
PHV	1.0 ±	1.7 ±	1.2 ±	1.8 ±	0.0 ±	0.7 ±	0.1 ±	0.9 ±	0.7 ±	1.4 ±	0.3 ±	1.0 ±	
(years)	1.0	1.0	1.0	0.9	1.0	1.0	0.8	0.8	1.1	1.0	0.9	1.0	
MVPA	61.3 ±	67.5 ±	59.6 ±	60.0 ±	97.7 ±	83.3 ±	89.6 ±	76.3 ± 22.4	85.6 ±	93.9 ±	88.9 ±	78.7 ±	
(min/day)	19.6	19.6	25.2	18.1	18.8	18.5	33.5		22.1	15.7	21.8	13.4	
Training volume (hrs/week)	7.9	11.8 ±	10.2 ±	12.9 ±	10.2 ±	12.0 ±	8.8 ±	10.7 ±	5.3 ±	7.9 ±	5.3 ±	8.2 ±	
	± 3.6	5.4	3.1	5.4	1.2	2.8	2.0	1.8	2.0	3.8	1.6	2.8	

Values are mean ± standard deviation. Bold values indicate significant higher values between PRE and POST, p<0.05. MVPA: Moderate to vigorous physical activity; PHV: peak height velocity.

9.4.2 Bone quantity, geometry and texture

Table 9.3 shows the 9-month adjusted changes in bone outcomes for lumbar spine and femoral neck. Figures 9.2 and 9.3 show the percentage of difference on adjusted bone outcomes gains between the groups INT-CYC gained significantly higher lumbar spine BMC (4.6 %) and femoral neck BMC (9.8 %) than CON-CYC. Also, INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There were no significant differences between INT-FOO and CON-FOO for any of the bone outcomes.

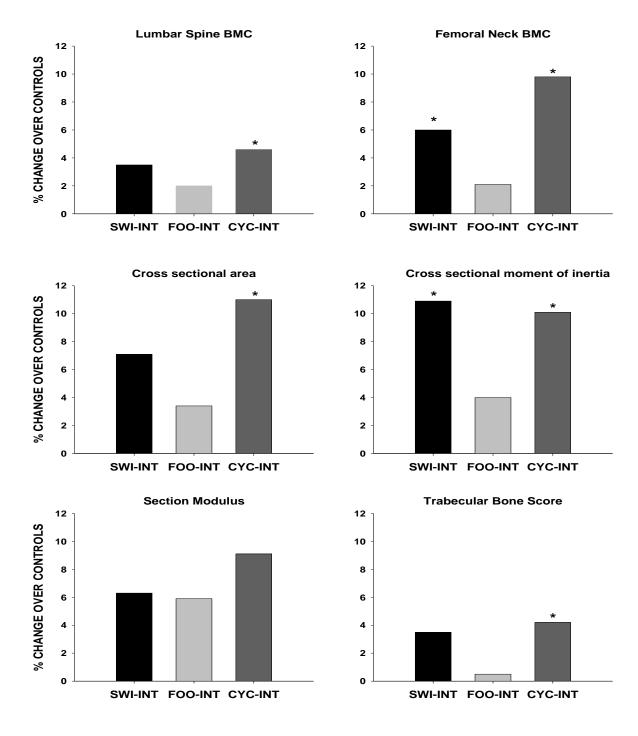


Figure 9.2. Nine-month adjusted changes in Bone Mineral Content (BMC), Hip Structural Analysis (HSA) parameters and Trabecular Bone Score. Results were adjusted for baseline bone outcomes, changes in lean mass and post peak height velocity. * denotes significant differences compared to the sport specific control group, p<0.05.

Table 9.3. PRE and 9-month adjusted change in bone mineral content (BMC, g) hip structural analysis parameters and trabecular bone score of the intervention and control groups

		SWI		FOOTB		CYCLISTS						
TOTAL (N=93)	INTERVENTION (N=19)		CONTRO L (N=18)	IN	ΓERVENT (N=15)	TION		ONTROL (N=15)	INTERVENTION (N=19)		CONTROL (N=18)	
(11–33)	PRE	9-month adjusted change Δ (95% CI)	PRE	9-month adjusted change Δ (95% CI)	PRE	9-month adjusted change Δ (95% CI)	PRE	9-month adjusted change Δ (95% CI)	PRE	9-month adjusted change Δ (95% CI)	PRE	9-month adjusted change ∆ (95% CI)
Lumbar Spine BMC	50.8 ± 11.0	7.8 (6.7-8.8)	51.9 ± 12.4	6.9 (5.8-8.0)	46.2 ± 14.4	9.2 (8.0-10.3)	47.5 ± 11.1	7.8 (6.6-9.0)	48.0 ± 12.8	8.5 (7.3-9.7)	41.9 ± 12.4	5.7 (4.4-7.0)
Femoral Neck BMC	5.0 ± 0.7	0.6 (0.4-0.7)	5.0 ± 0.9	0.3 (0.2-0.4)	5.1 ± 1.0	0.6 (0.5-0.8)	5.2 ± 0.8	0.5 (0.3-0.6)	5.0 ± 0.9	0.6 (0.5-0.8)	4.5 ± 0.9	0.1 (-0.1-0.3)
CSA	155.7 ± 23.3	20.9 (15.5-26.3)	149.3 ± 27.5	9.4 (3.9-14.9)	157.7 ± 26.8	25.2 (18.9-31.5)	159.3 ± 23.5	20.0 (13.7-26.3)	154.2 ± 27.2	23.1 (16.9-29.3)	140.0 ± 25.7	5.6 (-1.1-12.3)
CSMI	11106 ± 3592	2678 (2238-3119)	11349 ± 3565	1500 (1035-1965)	10893 ± 3988	2813 (2309-3316)	10998 ± 3492	2402 (1901-2903)	11220 ± 3715	2668 (2167-3169)	8840 ± 2917	1214 (665-1764)
Section Modulus	655.8 ± 152.5	125.5 (90.2-160.8)	673.2 ± 168.5	89.3 (52.0-126.5)	655.3 ± 176.1	161.1 (120.6- 201.6)	666.4 ± 184.8	122.1 (81.5-162.6)	658.7 ± 161.2	117.7 (77.7-157.7)	557.1 ± 139.4	48.8 (4.8-92.7)
Trabecular bone score	1.390 ± 0.058	0.102 (0.078- 0.125)	1.370 ± 0.072	0.054 (0.029- 0.080)	1.429 ± 0.075	0.122 (0.084- 0.141)	1.404 ± 0.050	0.113 (0.095- 0.148)	1.364 ± 0.073	0.103 (0.075- 0.129)	1.348 ± 0.081	0.042 (0.012- 0.071)

Raw values at PRE are mean ± standard deviation. Values at 9-month were adjusted for pre bone values, change in lean mass and post peak height velocity, and presented as mean and 95% CI. BMC: Bone mineral content. Bold values denote significant higher gains compared to the sport specific control group, p<0.05.

9.4.3 Bone turnover and nutrition markers

The biochemical markers of the participants pre- and post-intervention are presented in Table 9.4. Bone formation, as measured by PINP, was reduced in all CON sport groups (4.4 % in SWI, 3.3% in FOO and 4.2% in CYC). Interestingly, bone formation did not decline in INT-SWI and INT-CYC but it slightly did in INT-FOO (1.8 %).Bone resorption, as measured by CTX-I, was reduced by 3.8% in CON-SWI and CON-CYC. However, bone resorption did not vary in any of the INT groups or in CON-FOO. 25(OH)D significantly increased in INT-CYC (3.1 %), CON-CYC (3.7 %), INT-FOO (3.1 %) and CON-FOO (3.5 %), but not in INT-SWI and CON-SWI. In all groups serum calcium significantly increased from pre- to post-intervention.

Table 9.4. Biochemical markers of the of the intervention and control groups before (PRE) and after (POST) the 9-month intervention programme

	SWIMMERS					FOOTB	ALLERS		CYCLISTS			
TOTAL (N=93)	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		CONTROL (N=12)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
PINP (pg/ml)	338.06 ± 19.88	335.87 ± 12.39	335.33 ± 15.02	320.77 ± 10.54	342.01 ± 16.98	336.09 ± 11.27	346.54 ± 18.62	335.13 ± 11.34	327.50 ± 10.58	326.44 ± 9.18	324.94 ± 17.45	311.51 ± 8.88
CTX-I (ng/ml)	1.81 ± 0.21	1.80 ± 0.19	1.87 ± 0.10	1.77 ± 0.06	1.89 ± 0.09	1.88 ± 0.08	1.89 ± 0.08	1.88 ± 0.08	1.87 ±0.10	1.86 ± 0.09	1.86 ± 0.08	1.79 ± 0.06
25 (OH) D (ng/ml)	13.25 ± 0.83	13.33 ± 0.93	13.46 ± 0.88	13.53 ± 0.94	15.45 ± 1.16	15.73 ± 1.15	14.78 ± 1.19	15.41 ± 1.33	15.13 ± 1.12	15.61 ± 1.05	14.75 ± 1.08	15.48 ± 1.26
Serum calcium (mg/dl)	9.87 ± 0.38	10.39 ± 0.18	9.96 ± 0.65	10.41 ± 0.23	9.86 ± 0.42	10.25 ± 0.27	10.01 ± 0.40	10.28 ± 0.27	9.96 ±0.29	10.21 ± 0.24	9.84 ± 0.34	10.23 ± 0.23

Values are mean ± standard deviation. 25 (OH) D: 25-hydroxyvitamin D, CTX-I: Carboxi-terminal telopeptide of type 1 collagen, PINP: N-terminal propeptide of procollagen type I. Bold values indicate significant higher values between PRE and POST, p<0.05.

9.5 Discussion

This is the first study to examine the effect of jumping intervention on BMC at clinically relevant sites, hip geometry estimates, TBS and bone turnover markers in adolescent male athletes involved in in osteogenic (football) and non-osteogenic sports (swimming and cycling). The findings demonstrate that a 9-month progressive jumping intervention programme can significantly improve BMC, HSA and TBS bone outcomes at the clinically relevant skeletal sites of lumbar spine and femoral neck in non-osteogenic sport athletes, such as swimmers and cyclists, but not in the osteogenic sport athletes, such as footballers. In addition, bone formation (PINP) was maintained in the non-osteogenic INT sport groups while decreased in the CON non-osteogenic sport groups. Moreover, bone resorption (CTX-I) significantly decreased in the CON non-osteogenic sport groups, suggesting an increased bone turnover in these groups.

9.5.1 PRO-BONE jumping intervention effects on BMC gains at clinical sites

Currently, there are no jumping intervention studies conducted in an athletic population to improve bone outcomes, therefore the findings of the present study were compared with jumping interventions applied in non-athletic children and adolescents (Weeks et al., 2008, McKay et al., 2005b, MacKelvie et al., 2002b, Fuchs et al., 2001). The present jumping intervention significantly improved femoral neck BMC (6.0 – 9.8 %) in INT-SWI and INT-CYC compared to CON-SWI and CON-CYC, and lumbar spine BMC (4.6) % in INT-CYC compared to CON-CYC. Previously, an 8-month school-based jumping intervention reported that non-athletic adolescent males and females gained

6.0 % higher femoral neck BMC and 2.3 % higher lumbar spine BMC compared to controls (Weeks et al., 2008). Also, a 7-month school-based jumping intervention reported that non-athletic prepubescent children had 4.5 % and 3.1 % significantly higher gains at femoral neck and lumbar spine BMC respectively compared to age-matched controls (Fuchs et al., 2001). The greater magnitude of improvements observed in swimmers and cyclists in the current study may be explained by the ability of the previously unloaded skeletons of the nonosteogenic groups to respond better to the external stimulus of the jumping intervention (Maimoun et al., 2013b, Nikander et al., 2005). Another explanation might be the longer duration of the present intervention (9 months vs 7-8 months) and the greater number of jumps performed in the present study (160 vs 90 jumps per week) (McKay et al., 2005b) by increasing the ground reaction forces applied to the skeleton progressively using the weight vests. These improvements may indicate a window of opportunity to counteract the lack of osteogenic stimulus observed in adolescent swimmers and cyclists (Gomez-Bruton et al., 2016a, Olmedillas et al., 2012, Vlachopoulos et al., 2017c). In contrast, but consistent with our hypothesis, the stimulus provided by the jumping intervention was not enough to induce significant bone gains in INT-FOO compared to CON-FOO. This is in accordance with the mechanostat theory indicating that the bones adapt their strength and content to respond to the strain caused by external physiological loads up to a certain point (Schoenau, 2005, Ireland et al., 2014). Footballers may have reached a threshold for bone improvements as we have previously shown to have greater bone outcomes compared to swimmers and cyclists (Vlachopoulos et al., 2017c). However, a longer duration jumping intervention programme may be needed to improve further bone outcomes in osteogenic sports, such as football.

9.5.2 PRO-BONE jumping intervention effects on HSA and TBS gains

In addition to BMC adaptations, the present 9-month jumping intervention significantly improved HSA and TBS bone parameters. More specifically, INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) compared to CON-CYC, and INT-SWI gained significantly higher CSMI (10.9 %) compared to CON-SWI. Previously, only two studies previously used HSA to describe bone geometry and structural strength adaptations from a jumping intervention in non-athletic populations (Petit et al., 2002, McKay et al., 2005b). Petit et al (Petit et al., 2002) reported that a 7-month jumping intervention induced significantly greater increase in CSA (2.3 %) and section modulus (4.0 %) in the intervention group compared to an age-matched non-athletic control group. McKay et al (McKay et al., 2005b) did not find significant improvements in HSA parameters after an 8-month jumping intervention in nonathletic pubertal children, but section modulus (3.3 %) and CSA (2.0 %) had similar magnitude of increase with the study of Petit et al. In the present study, the greater improvements in bone outcomes of swimmers and cyclists compared to footballers may be explained by mechanoadaptation that converts the external stimulus of the jumping intervention to greater structural adaptations of previously unloaded bones (Scott et al., 2008). The present study is the first to present findings on TBS adaptations after a jumping intervention in adolescent athletes. Currently, there are no jumping intervention studies using TBS and only a recent cross-sectional study in adults reported that moderate impact loading sports was associated with a lower TBS score and increased fracture risk compared to high impact loading sports (Heinio et al., 2015). The present study indicates that trabecular structure at the lumbar spine may be adapted to the forces produced from the jumping intervention after

controlling for potential confounders (Shawwa et al., 2016). The compliance in the present study was a bit lower compared to other studies (70% vs 80-90%) (MacKelvie et al., 2002b, Fuchs et al., 2001, Gunter et al., 2008a) and this might be due to the longer duration of the present intervention (9 months vs 7-8 months) (Weeks et al., 2008, MacKelvie et al., 2002b). However, the present jumping intervention had 1-2 months greater duration and progressive loading compared to previous studies, which might be responsible for the higher gains observed. The latter is in accordance with a 20-month exercise randomized control trial in prepubertal non-athletic males that found greater magnitude of improvements in CSMI (12.3 %) and section modulus (7.4 %) in the intervention group compared to age-matched controls (MacKelvie et al., 2004).

9.5.3 PRO-BONE jumping intervention effects on biochemical markers

The analysis of biochemical markers in the present study showed that the jumping intervention prevented the significant decline of bone formation (PINP) and resorption (CTX-I) markers in INT-SWI and INT-CYC. In contrast, bone formation significantly decreased in INT-FOO and all CON-SPORT groups, and bone resorption significantly decreased in CON-SWI and CON-CYC. Previous studies have shown that bone turnover markers are associated with bone outcomes during growth and can provide important information about bone remodelling (Gracia-Marco et al., 2010, Gracia-Marco et al., 2011b). In addition, the intensity of physical activity and the type of sports practised may be potent regulators of bone remodelling (Vlachopoulos et al., 2017a, Kambas et al., 2016). Recently, a study has shown that one session of plyometric jumping exercises can stimulate bone formation in in boys and young men, with boy's response to be more pronounced (Kish et al., 2015). However, there are no studies investigating the response of bone turnover markers after a longer

jumping intervention in combination with clinically relevant bone outcomes. The findings of the present study suggest that the cellular activity of bone turnover markers (both formation and resorption) in INT-SWI and INT-CYC was protected from declining due to the jumping intervention. In addition, serum calcium significantly increased from pre- to post-intervention, and 25(OH)D significantly increased in INT-CYC, CON-CYC, INT-FOO and CON-FOO, but not in INT-SWI and CON-SWI. There is an expected increase in serum calcium levels by age in adolescents (Norman et al., 2011), and the significant increase of 25(OH)D in cyclists and footballers might be explained by the higher exposure to sunlight during training in these sports, although other parameters such as dietary intake and the sampling period have been reported to affect 25(OH)D levels (Ackerman et al., 2011).

9.5.4 Strengths and limitations

The strengths of the present study include the evaluation for first time of a novel 9-month progressive jumping intervention programme in adolescent athletes participating in osteogenic and non-osteogenic sports. In addition, the combination of DXA, HSA, TBS and biochemical markers can provide novel and clinically relevant findings regarding the bone changes induced from a jumping intervention programme in adolescent male athletes. The low cost and relative ease jumping programme for young athletes represent another strength, and almost any sport club could implement the programme with minimal training for the coach and the athlete. In this study genetic information was not obtained and this may play a role on bone adaptations from the intervention, representing an area for further research.

9.6 Conclusions

This is the first randomized control trial to investigate the effects of a 9-month progressive jumping intervention programme on bone mass, geometry, texture and biochemical markers in adolescent male athletes. The findings indicate that the jumping intervention programme can significantly improve bone quantity, geometry and TBS bone outcomes at the femoral neck and lumbar spine, and maintain the bone turnover in adolescent male athletes involved in swimming and cycling, but not in football. The present jumping intervention programme can be implemented by non-osteogenic sports clubs and athletes to improve bone health.

10. Summary of findings, practical implications and future directions

The purpose of this thesis was to investigate differences in bone outcomes in adolescent males participating in osteogenic (football) and non-osteogenic (swimming and cycling) sports using cross-sectional (Chapter4) and longitudinal (Chapters 6 and 7) research designs. Also, the thesis aimed to identify the determinants affecting bone outcomes (Chapter 5) as well as to investigate the effectiveness of a 9-montth jumping intervention programme to improve bone development in adolescent male footballers, swimmers and cyclists (Chapters 8 and 9). This chapter aims to summarise, synthesize, discuss and highlight the practical implications of the findings of this thesis. In addition, the thesis limitations are highlighted and future research directions are provided.

10.1 Summary of experimental chapters

10.1.1 Chapter 4

Chapter 4 examined bone mass and geometry in adolescent males who were involved in football, swimming and cycling for 3 or more hours per week for the last 3 or more years in comparison with an active control group. Findings from this study showed that footballers had significantly higher BMD at total body less head, total hip and legs compared to all groups (7-21%). In addition, footballers had significantly higher bone geometry estimates (8-21%) and bone stiffness (10-20%) compared to all groups. This is the first study to compare bone outcomes between adolescent males involved in osteogenic and non-osteogenic sports. These findings indicate that the bone status of adolescent male footballers is significantly better than swimmers, cyclists and controls.

10.1.2 Chapter 5

Chapter 5 aimed to identify the determinants of bone outcomes, as there was no previous evidence assessing the contribution of different factors affecting bone status of male adolescent athletes. The study examined the contribution of the sports practised, height, lean and fat mass, serum calcium and vitamin D, MVPA, vertical jump and CRF with aBMD and hip geometry estimates using multiple linear regressions. The results of this study showed that region specific lean mass was the strongest positive predictor of aBMD (β=0.614-0.931) and football participation was the next strongest predictor (β =0.304-0.579). In addition, height (β =0.235-0.380), fat mass (β =0.189), serum calcium (β =0.103), serum vitamin D (β =0.104-0.139) and vertical jump (β =0.146-0.203) were positively associated with aBMD across various specific sites. Similarly, hip geometry estimates were positively associated with lean mass (β =0.370-0.568) and height (β=0.338-0.430). Football participation was associated with CSA (β =0.322) and CSMI (β =0.140-0.142). CRF (β =0.183-0.207) was associated with Z and CSMI. The findings of this study indicate that region specific lean mass is the strongest determinant of aBMD and hip geometry estimates in adolescent male athletes, followed by football participation and height while the contribution of the other predictors was site specific.

10.1.3 Chapter 6

There are a lack of longitudinal studies comparing the effects of osteogenic and non-osteogenic sports on bone quantity and quality in adolescent athletes. Therefore, Chapter 6 assessed the effect of 12-month participation in adolescent males engaged in football, swimming and cycling on bone development in comparison to an active control group. Bone outcomes at 12

months were assessed after adjustments for baseline bone status, age, height, lean mass and MVPA. Footballers had higher improvements in adjusted BMC at the total body, total hip, shaft, Ward's triangle, legs and bone stiffness compared to cyclists (6.3 to 8.0 %). Footballers had significantly higher adjusted BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %). There were no significant differences between swimmers and cyclists for any bone outcomes. Swimming and cycling participation resulted in non-significant lower bone development at most sites of the skeleton compared to controls (-4.3 to -0.6 %). The results of this study suggest that football participation induces significantly greater improvements in BMC and bone stiffness over 12 months compared to non-osteogenic sports and controls.

10.1.4 Chapter 7

Chapter 7 aimed to examine changes in bone mass, hip geometry and lumbar spine texture after 1 year of training among adolescent males involved in osteogenic (football) and non-osteogenic sports (swimming and cycling) and compared to active controls. A second aim was to assess the biochemical markers of bone metabolism and nutrition. Bone outcomes were adjusted for baseline bone status, age, height, lean mass and MVPA and showed that footballers had significantly higher adjusted BMC at the lumbar spine (7.0 %) and femoral neck (5.0 %) compared to cyclists, and significantly greater BMC at the lumbar spine (6.9 %) compared to swimmers. Footballers presented significantly greater TBS (4.3%) compared to swimmers, and greater CSMI (10.2 %), CSA (7.1 %), Z (8.9 %) and TBS (4.2 %) compared to cyclists. There were no differences between cyclists and swimmers, and both groups had similar bone status compared to controls. In addition to bone outcomes, PINP

was significantly higher in footballers and controls compared to cyclists and swimmers (3.3-6.0 %) and 25(OH)D was significantly higher in footballers and cyclists compared to swimmers and controls (9.9-13.1 %). Collectively, these findings suggest that bone mass and geometry is higher in adolescent male footballers compared to swimmers and cyclists at the femoral neck and lumbar spine sites of the skeleton. Bone formation was lower in cyclists and swimmers compared to footballers and the active control group, but further investigation on the index of bone remodelling is needed to understand the individual biomarker changes by examining the whole turnover result.

10.1.5 Chapter 8

Cross-sectional and longitudinal evidence from Chapters 4, 6 and 7 demonstrate that bone status and development is significantly different in adolescent males involved in osteogenic and non-osteogenic sports. Therefore, Chapter 8 aimed to examine, for first time, the effect of a 9-month jumping intervention programme on bone and fitness outcomes in adolescent male athletes. A total of 93 adolescent male athletes (37 swimmers, 30 footballers and 26 cyclists) were randomly allocated to intervention (INT) and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) or sport only (CON-SWI =18, CON-FOO =15, CON-CYC =12) groups. Findings showed that INT-SWI had significantly higher gains in BMC legs and bone stiffness compared to CON-SWI (4.2-12.7%). INT-CYC had significantly higher gains in BMC at TBLH and legs and bone stiffness compared to CON-CYC (5.0-12.3%). There were no significant differences between INT-FOO and CON-FOO in bone gains (0.9-3.9%). INT-SWI, INT-CYC and INT-FOO significantly improved fitness outcomes (3.7-7.9%) and CON-FOO significantly improved CMJ (4.0%). These novel findings

show that a 9-month jumping intervention can improve bone outcomes only in male adolescents participating in SWI and CYC, but not in FOO, while improvements in fitness were observed in all groups.

10.1.6 Chapter 9

Chapter 9 examined the effects of a 9-month jumping intervention on bone mass, geometry and texture at femoral neck and lumbar spine, and bone turnover and nutritional markers in adolescent male athletes. The results showed that INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There were no significant differences between INT-FOO and CON-FOO in any of the bone outcomes. PINP significantly decreased in CON-SWI, INT-FOO, CON-FOO and CON-CYC. CTX-I significantly decreased in CON-SWI and CON-CYC. 25(OH)D significantly increased in INT-CYC, CON-CYC, INT-FOO and CON-FOO. This novel jumping intervention programme improved bone mass, geometry and texture at the lumbar spine and femoral neck of adolescent male athletes involved in non-osteogenic sports, such as swimming and cycling, but not in those involved in osteogenic sports, such as football. Whether the positive effects on bone status from the intervention will be maintained after the cessation of the intervention requires further longitudinal research.

10.2 Synthesis of findings

10.2.1 Sport participation and bone adaptations

Collectively, the present thesis investigated how bone development differs among adolescent males involved in osteogenic and non-osteogenic sports and how to improve bone development in these sports. In an attempt to combine cross-sectional, longitudinal and RCT data, Figure 10.1 was designed to show bone development in each group (sport-intervention, sport only and control) over the 21 months (12 months follow-up + 9 months intervention). Results show that TBLH BMC was higher in footballers at baseline compared to all the other groups after adjusting for lean mass and PHV. The higher BMC in footballers remained and increased at month 12, while there were no differences between the other groups at baseline and month 12. At month 21, swimmers and cyclists who performed the 9-month jumping intervention notably improved BMC compared to sport only swimmers and cyclists and controls, but the intervention did not augment bone status in footballers. The differences in BMC between the groups at baseline and 12-monhts can be explained the weight-bearing characteristics of football that stimulate higher bone adaptations on the skeleton due to the greater ground reaction forces applied on the skeleton during football participation (McKay et al., 2005a, Plaza-Carmona et al., 2014). In addition, the improvements observed in swimmers and cyclists who performed the intervention indicate that the BMC of non-osteogenic sports athletes can be improved if stimulated adequately via a jumping programme. These summarised findings indicate that the bone development is different between the osteogenic and non-osteogenic sports practised during adolescence and that a jumping intervention programme can improve bone development in non-osteogenic sports athletes.

Changes in TBLH BMC during 21 months

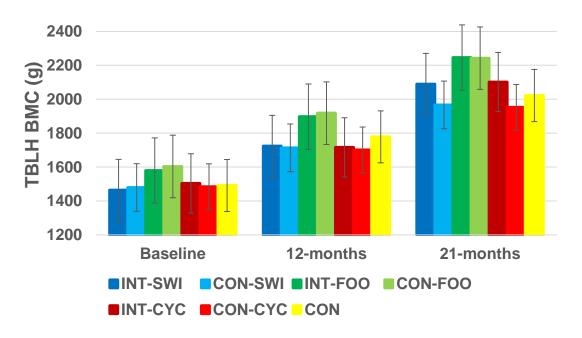


Figure 10.1. Changes in adjusted TBLH BMC during the studied period in all groups. Values were adjusted for lean mass and peak height velocity at each time point.

Previous research in prepubescent boys found that footballers had non-significant but higher bone outcomes compared to active controls after 10 months of training (Zouch et al., 2008). These results are in line with the findings of Chapters 6 and 7 showing that footballers had higher but not significant bone outcomes compared to active controls after 12 months. It should be noted that in the present thesis the control group was physically active (MVPA= 64 min/day) and some controls engaged in other weight-bearing sports (< 3 hours per week) which might explain the non-significant difference compared to footballers. Findings in hip geometry estimates of female footballers and swimmers showed that 8 months of training in their respective sports induced a significant increase in CSA of the narrow neck in footballers compared to swimmers (Ferry et al., 2013). A possible explanation is that

swimming has non-gravitational training characteristics and despite swimmers having augmented lean mass it was not enough to produce bone adaptations after 12-months of training (Maimoun et al., 2013b).

According to the "Functional Muscle-Bone-Unit" the largest physiological loads are caused by muscle contractions, which might be increased in highly active adolescents due to the greater muscle forces produced during participation in activities (Rauch et al., 2004). Football training and competition requires increased muscle activation (Seabra et al., 2012, Nebigh et al., 2009) and lean mass has been strongly associated with BMC and hip geometry estimates throughout puberty (Jackowski et al., 2014, Baptista et al., 2012, Wilkinson et al., 2017, Gracia-Marco et al., 2012). The movement characteristics of football include mechanical forces applied on the skeleton that are generated either through ground impact or muscle contractions (Kohrt et al., 2009). Muscle strength and the type of sports practised have been found as predictors of bone quality at the distal radius and distal tibia in elite athletes (Schipilow et al., 2013). The eccentric component of the movement characteristics involved in football has an important role and previous research in children has shown that jumps produce ground reaction forces of 3.5 to 5 times body weight of approximately 500 times body weight per second (McKay et al., 2005a). These training characteristics can improve lean mass which can lead to bone adaptations (Gracia-Marco, 2016).

The previous differences observed in bone gains among the sports groups might be explained by the plyometric exercises, accelerations, breakings and changes of direction that are part of the football training and can induce higher

bone mass in adolescent athletes despite the reduced lean mass in footballers compared to swimmers (Gunter et al., 2008a). In this regard, Larsen et al. found that a 10-month programme that included small-sided ball games in children improved BMD at the legs and total body compared to controls, and BMD at the legs compared to a circuit strength training (Larsen et al., 2016). A 12 year follow up study measured BMD 5 times starting from the age of 17 years of age in 19 badminton players, 48 ice hockey players and 25 controls (Tervo et al., 2010). During the active career, badminton players gained significantly more BMD compared to ice hockey players at all sites of the skeleton. At the final follow-up, badminton players had significantly higher BMD at the femoral neck, humerus, lumbar spine, and legs compared to hockey players and controls. It should be noted that after reducing or stopping the participation in badminton and ice hockey, both groups lost significantly more BMD at the femoral neck and lumbar spine compared to the control group. However, the benefits of participation in osteogenic sports during adolescent were maintained at the age of 29 years. The findings of the previous study indicate that the PBM attained might differ based on sports practised.

10.2.2 Determinants affecting bone outcomes

Chapter 5 indicated that the sports practised, height, lean and fat mass, serum calcium and vitamin D, MVPA, vertical jump and CRF explained a significant variance of aBMD and hip geometrical estimates at different skeletal sites (49.0 %-77.8 %) (Figure 10.2). Previous findings in a non-athletic population reporting that a similar model of determinants explained 40%-83 % of the variance in BMC in prepubertal girls (Daly et al., 2008), but there was no evidence examining the determinants of HSA outcomes in adolescent athletes.

The contribution of lean mass as the most important determinant of bone outcomes in adolescent athletes might be explained by the mechanostat theory that indicates the importance of functional muscle-bone-unit (Schoenau, 2005).

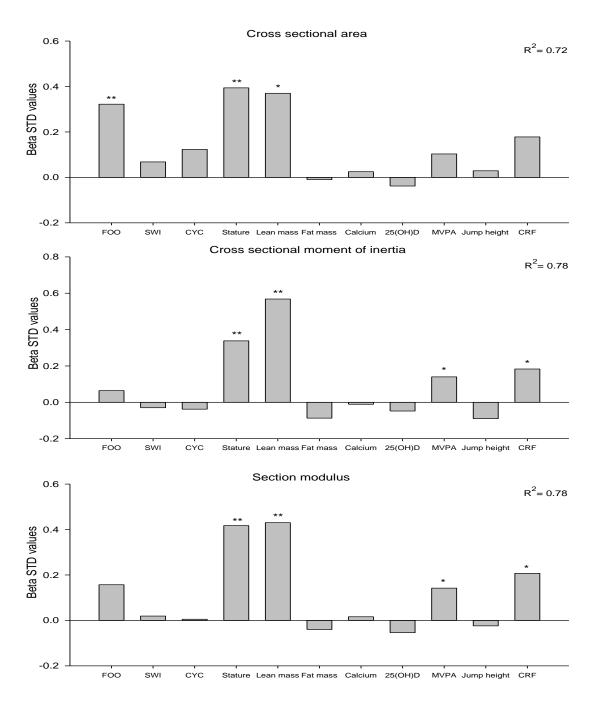


Figure 10.2. Determinants of bone geometry estimates using HAS. Adapted from Chapter 5 (Vlachopoulos et al., 2017d).

A previous study in non-athletic boys and girls reported that total lean mass was the best predictor of total body and lumbar spine aBMD (El Hage et al., 2009).

Another study in non-athletic children found that total lean mass was the strongest predictor of the aBMD at total body, and lumbar spine (Hrafnkelsson et al., 2010). However, the study did not distinguish the site-specific relationship of lean and bone mass, which was considered in the present study. It is of great interest to understand the region-specific relationship of lean mass and aBMD due to the site-specific adaptation of the skeleton during external loading, specifically in athletic populations (Baxter-Jones et al., 2008). Despite the lack of significant association between region specific lean mass and aBMD at the femoral neck and total hip, all the geometrical parameters of the narrow neck of the femur were significantly associated with region specific lean mass. This may reflect previous work in children and adolescents showing that HSA can provide more in depth geometrical evaluation at the hip site compared to BMD outcomes (Petit et al., 2005). In addition, studies using pQCT found that muscle cross sectional area was the strongest predictor of bone strength parameters in early pubertal boys and girls (Macdonald et al., 2006). The latter study highlighted the importance of using site specific lean mass to evaluate its contribution to hip geometry estimates.

The contribution of football on aBMD was independent of lean mass and is likely to be explained by the intermittent and high-intensity characteristics of football that can produce high strains on the skeleton and stimulate bone mineral acquisition (Ubago-Guisado et al., 2015a). The concentric contractions during football generate greater forces compared to cycling and swimming and this might explain the increased skeletal loading in this group (Soderman et al., 2000). The movement characteristics of the sports practised seems to be important for bone acquisition and the present study found that football

participation is one of the most important determinants, possibly because it includes high intensity concentric contractions that can enhance aBMD in adolescents. Regarding the contribution of nutrition on bone outcomes, dietary calcium and 25(OH)D can have a weak, but significant contribution on specific sites of the skeleton in adolescents (Macdonald et al., 2006). The sites of the skeleton, such as arms, are less loaded through sport and nutritional factors may have a potential influence. The site specific relationship between nutrition and bone outcomes can be attributed to the interactions of nutrients in relation to bone health (Ilich and Kerstetter, 2000). In relation to the contribution of MVPA and fitness on aBMD, we found that vertical jump height was the only significant predictor of aBMD at TBLH and LS. These findings show that overall MVPA does not appear to be important once participation in a particular sport is considered in the regression model. This suggests the characteristics of the sport practised and the contribution of lean mass mediates the relationship between fitness and bone outcomes (Vicente-Rodriguez et al., 2008).

10.2.3 Reversing the non-osteogenic effects on bone growth

Chapters 8 and 9 showed that the non-osteogenic sports such as swimming and cycling can benefit from a 9-month jumping intervention programme to improve bone development. The present section will synthesize the findings of Chapters 8 and 9 by comparing the effects of the intervention on bone outcomes between the groups (Figure 10.3).

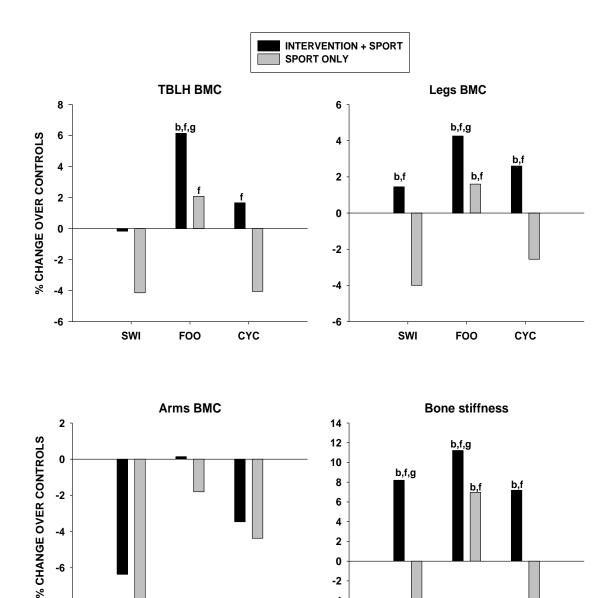


Figure 10.3. Effect of 9-month plyometric intervention programme on Bone Mineral Content (BMC, g) and Quantitative Ultrasound (QUS) acquisition. Adjusted percent change on bone outcomes between the sport group that performed the intervention and those that continued their sport specific trading. Results were adjusted for initial bone outcomes and change in lean mass. Superscript letters denote a higher significant difference compared to: a (CONTROL SWIMMERS), (INTERVENTION SWIMMERS), b С (INTERVENTION FOOTBALLERS), d (CONTROL FOOTBALLERS), e (INTERVENTION CYCLISTS), f (CONTROL CYCLISTS), g (CONTROLS). a,b,c,d,e,f,g p<0.05.

-2 -4

-6

-8

SWI

F₀0

CYC

-8

-10

SWI

F₀0

CYC

There was a greater difference at TBLH BMC (6.1 %) and bone stiffness (11.2 %) in FOO-INT compared to CON, which may be explained by the additional ground reaction forces applied on the skeleton due to football participation in addition to the intervention effects (McKay et al., 2005a, Plaza-265 Carmona et al., 2014). Although, there are no previous jumping interventions focusing on bone outcomes of adolescent athletes, evidence of 7 and 8-month school-based jumping intervention studies in non-athletic pubertal children and adolescents reported significant improvements on bone outcomes of the intervention groups compared to non-athletic controls (MacKelvie et al., 2002b, McKay et al., 2005b, Fuchs et al., 2001). In these previous studies, the effect of the intervention was greater at the weight-bearing sites of the skeleton, which is equivalent with the higher improvements observed at legs BMC in the present study and the non-significant bone differences at the arms BMC. The present thesis showed that a greater magnitude of bone improvements was found in the SWI-INT and CYC-INT, which might be explained by the previously unloaded skeleton of swimmers and cyclists (Maimoun et al., 2013b, Nikander et al., 2005). The non-weight bearing nature of swimming was reported in a longitudinal study comparing bone outcomes of female adolescent swimmers and footballers, showing that 8-months of sport-specific training can increase BMD at total body by 2.9 % in footballers, whereas BMD remained constant or non-significantly reduced in swimmers (Ferry et al., 2013). It should be noted that the effect of the intervention was greater in SWI-INT and CYC-INT than FOO-INT, inducing non-significant higher gains (1.6-8.4 %). This intervention might counteract the negative or neutral effects of swimming and cycling on bone outcomes (Vlachopoulos et al., 2017c, Gomez-Bruton et al., 2016b, Olmedillas et al., 2012), suggesting a window of opportunity for further research in adolescent athletes involved in non-osteogenic sports.

10.3 Strengths and limitations

The strength of this thesis is the combination of cross-sectional and longitudinal investigations to compare, for the first time, the effects of three of the most popular sports worldwide in male adolescents on bone development. Also, the examination of a jumping intervention programme using a RCT design provides unique evidence on how bone development of adolescent athletes can be improved with low cost and relative ease applicability for the athletes. The combination of the studies conducted is more likely to support cause and effect than previously conducted studies on the specific topic. The combination of DXA, HSA, TBS and QUS outcomes provides a thorough insight of the differences in BMD, BMC, bone geometry, texture and bone stiffness among groups. In addition, the assessment of biochemical and nutritional markers provides with additional information regarding bone metabolism changes due to sport participation. Finally, the rigorous methodology and strong internal validity used across the studies to control for potential confounders represents advancement within the published literature.

In cohort studies, a threat to the internal validity of the findings are the confounding factors, both measured and unmeasured. Confounding is a distortion of the estimated effect of an exposure on an outcome caused by the presence of an extraneous factor associated with both the exposure and the outcome (Samet et al., 2009). The most likely candidates for potential unmeasured confounders in the present study include hormonal factors, such as IGF-1, growth hormone, leptin and genetic polymorphisms that affect fat mass, lean mass and bone mass (Mitchell et al., 2015). A limitation of the intervention studies might be the fact that the non-athletic control group did not perform the 9-month jumping protocol, as performed in previous studies. However, the scope of the studies was to compare and improve bone status of

adolescent athletes, and therefore comparisons were established within groups and not between groups. In this regard, each sport had its own control group.

Bias or misclassification can also affect the validity of the results. Bias is a deviation of the results from the truth and information bias is a flaw in measuring exposure or outcome data (Samet et al., 2009). Bias in the measurement of the main exposure variable of bone outcomes is unlikely to have occurred because the DXA scans were always performed by the same researcher with appropriate quality assurance and following theoretical and practical bone densitometry training. Selection bias or misclassification of the participants is unlikely due to the strict inclusion criteria of the study that verified by parents, coaches and teachers. Bias in the collection, answering and recording the answers to the questions about the confounding variables used such as dietary intakes, may threaten the validity of the studies. It should be noted that pubertal status was self-reported by the participants and using stages of sexual development, which may induce some bias in adolescents (Koziel and Malina, 2017, Malina and Koziel, 2014). Therefore, age and PHV were used as covariates to control for pubertal status. Objectively measured data on anthropometry and body composition is unlikely to be biased as they were collected at fasted state and at the same time of the day in all participants' visits by trained researches. Recall bias in the answering, collection and recording of answers to the questions about training hours, fracture incidence and dietary intake may also threaten the validity of these results. However, any error of these confounding factors is likely to be random.

Error in objectively physical activity collection of data (underestimation) should also be mentioned as accelerometers do not properly measure physical activity in cyclists as bouts of activity are not detected (Slootmaker et al., 2009). Also,

GENEActiv accelerometers are supposed to be waterproof to assess physical activity of swimmers in and out of training, but some of them presented technical faults when immersed and therefore physical activity levels may have been underestimated. Earlier versions of GENEActiv and Actigraph (GTX2 and 3) devices were primarily designed to measure general body movement, and externally calibrated to energy consumption to be associated with obesity-related outcomes (Mattocks et al., 2007). Ideally, future studies should isolate the intensity and duration of physical activity during and outside of training and relate it with bone outcomes across different sports.

Limitations using the DXA technique relate to its two-dimensional projection images. Due to this, DXA cannot determine true vBMD and this is a compromise. However, to directly measure vBMD in children, a pQCT scan would be needed. Several mathematical formulas to calculate threedimensional densities have been developed, for correcting the effect of size and the known cylinder shape of human vertebrae (Carter et al., 1992). The BMC of a given area is measured, and aBMD is calculated by dividing BMC with area (g/cm²). Thus, no consideration is given to the depth or volume of the bone, which means that a smaller bone might be falsely interpreted as having a low BMD in children (Gordon et al., 2008). However, we adjusted for height and body composition in all the studies to remove the differences in size between the participants as suggested in the literature (Zemel et al., 2010). Measurement errors also occur because of the heterogeneity in soft tissue composition in different persons (accuracy error). The precision errors are smaller than the accuracy errors because when a person is rescanned, the effect of adipose tissue on the measurement is the same. Also, results and

radiation dose can differ in children when using different manufacturers of DXA scanners (Lee et al., 2004).

10.4 Practical implications and future directions

The studies conducted can be used by athletes and coaches as discipline specific evidence regarding the impact of sports participation and the effect of a jumping intervention on bone health in adolescent male athletes. From an epidemiological and paediatric bone health perspective the findings should not be used in isolation to formulate policies, but they can be part of the evidence to update current sports medicine policies and recommendations. Adolescent athletes engaged in other osteogenic sports might have similar enhanced bone status compared to non-osteogenic sports. Indeed, there is evidence that other osteogenic sports, such as basketball and handball can enhance bone status compared to participation in non-osteogenic sports (Agostinete et al., 2016, Vicente-Rodriguez et al., 2004b). To date, similar findings regarding the longitudinal effects of sports on bone status exist for female adolescent athletes involved in football, swimming and cycling (Ferry et al., 2013, Sherk et al., 2014, Maimoun et al., 2013a), but not in other sports. Regarding the determinants of bone outcomes, it would be interesting to expand the determinants evaluated by including hormonal levels, such as leptin and IGF-I, and measure functional genetic polymorphisms, as well as other variables such as gender, ethnicity and pubertal status on larger population engaged in more sporting activities. The jumping programme tested in the present thesis can be used (or adapted) in future studies aiming to improve bone mass in participants who might be at risk of low bone mass. Future RCTs in paediatric population and using techniques such as pQCT and markers of bone strength in these populations are needed.

10.5 Conclusion

The present thesis is the first to provide evidence on how different sports affect bone status and development, and to examine the effect of an intervention to improve bone health in male adolescent athletes. The findings indicate that adolescent male footballers have better bone status than swimmers, cyclists and controls, and the longitudinal findings show that bone mass and geometry is higher in adolescent male footballers compared to swimmers and cyclists after one year of sport specific training. Lean mass is the strongest determinant of bone outcomes, followed by football participation and height, while the contribution of the other predictors is site specific. A 9-month jumping intervention programme can improve bone development only in swimmers and cyclists, but not in footballers. Given the large number of adolescents involved in these sports globally, the present thesis can be used to inform sports science and medical professionals and direct future research to improve athletes' bone health during growth.

- ABRAMS, S. A. & STUFF, J. E. 1994. Calcium metabolism in girls: current dietary intakes lead to low rates of calcium absorption and retention during puberty. *Am J Clin Nutr*, 60, 739-43.
- ABU, E. O., HORNER, A., KUSEC, V., TRIFFITT, J. T. & COMPSTON, J. E. 1997. The localization of androgen receptors in human bone. *Journal of Clinical Endocrinology & Metabolism*, 82, 3493-3497.
- ACERO, R. M., FERNANDEZ-DEL OLMO, M., SANCHEZ, J. A., OTERO, X. L., AGUADO, X. & RODRIGUEZ, F. A. 2011. Reliability of squat and countermovement jump tests in children 6 to 8 years of age. *Pediatr Exerc Sci*, 23, 151-60.
- ACKERMAN, K. E., NAZEM, T., CHAPKO, D., RUSSELL, M., MENDES, N., TAYLOR, A. P., BOUXSEIN, M. L. & MISRA, M. 2011. Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. *J Clin Endocrinol Metab*, 96, 3123-33.
- ACKERMAN, K. E., PIERCE, L., GUERECA, G., SLATTERY, M., LEE, H., GOLDSTEIN, M. & MISRA, M. 2013. Hip Structural Analysis in Adolescent and Young Adult Oligoamenorrheic and Eumenorrheic Athletes and Nonathletes. *Journal of Clinical Endocrinology & Metabolism*, 98, 1742-1749.
- AGOSTINETE, R. R., LYNCH, K. R., GOBBO, L. A., LIMA, M. C., ITO, I. H., LUIZ-DE-MARCO, R., RODRIGUES-JUNIOR, M. A. & FERNANDES, R. A. 2016. Basketball Affects Bone Mineral Density Accrual in Boys More Than Swimming and Other Impact Sports: 9-mo Follow-Up. *J Clin Densitom*.
- AHMED, L. A., EMAUS, N., BERNTSEN, G. K., BJORNEREM, A., FONNEBO, V., JORGENSEN, L., SCHIRMER, H., STORMER, J. & JOAKIMSEN, R. M. 2010. Bone loss and the risk of non-vertebral fractures in women and men: the Tromso study. *Osteoporosis International*, 21, 1503-1511.
- ALWIS, G., KARLSSON, C., STENEVI-LUNDGREN, S., ROSENGREN, B. E. & KARLSSON, M. K. 2012. Femoral neck bone strength estimated by hip structural analysis (HSA) in Swedish Caucasians aged 6-90 years. *Calcif Tissue Int*, 90, 174-85.
- ANDREOLI, A., CELI, M., VOLPE, S. L., SORGE, R. & TARANTINO, U. 2012. Long-term effect of exercise on bone mineral density and body composition in post-menopausal ex-elite athletes: a retrospective study. *Eur J Clin Nutr*, 66, 69-74.
- ARA, I., VICENTE-RODRIGUEZ, G., PEREZ-GOMEZ, J., JIMENEZ-RAMIREZ, J., SERRANO-SANCHEZ, J. A., DORADO, C. & CALBET, J. A. 2006. Influence of extracurricular sport activities on body composition and physical fitness in boys: a 3-year longitudinal study. *Int J Obes* (Lond), 30, 1062-71.
- ARABI, A., NABULSI, M., MAALOUF, J., CHOUCAIR, M., KHALIFE, H., VIETH, R. & EL-HAJJ FULEIHAN, G. 2004. Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. *Bone*, 35, 1169-79. ASPEN INSTITUTE 2013. Sports & Fitness Industry Association.
- BACHRACH, L. K. 2001. Acquisition of optimal bone mass in childhood and adolescence. *Trends Endocrinol Metab*, 12, 22-8.
- BACHRACH, L. K., GORDON, C. M. & SECTION ON, E. 2016. Bone Densitometry in Children and Adolescents. *Pediatrics*, 138.
- BACHRACH, L. K., HASTIE, T., WANG, M. C., NARASIMHAN, B. & MARCUS, R. 1999. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab*, 84, 4702-12.
- BAILEY, D. A., MCKAY, H. A., MIRWALD, R. L., CROCKER, P. R. & FAULKNER, R. A. 1999. A sixyear longitudinal study of the relationship of physical activity to bone mineral accrual

- in growing children: the university of Saskatchewan bone mineral accrual study. *J Bone Miner Res,* 14, 1672-9.
- BANFI, G., LOMBARDI, G., COLOMBINI, A. & LIPPI, G. 2010. Bone metabolism markers in sports medicine. *Sports Med*, 40, 697-714.
- BAPTISTA, F., BARRIGAS, C., VIEIRA, F., SANTA-CLARA, H., HOMENS, P. M., FRAGOSO, I., TEIXEIRA, P. J. & SARDINHA, L. B. 2012. The role of lean body mass and physical activity in bone health in children. *Journal of Bone and Mineral Metabolism*, 30, 100-108.
- BAPTISTA, F., MIL-HOMENS, P., CARITA, A. I., JANZ, K. & SARDINHA, L. B. 2016. Peak Vertical Jump Power as a Marker of Bone Health in Children. *Int J Sports Med*, 37, 653-8.
- BARONCELLI, G. I. 2008. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. *Pediatr Res*, 63, 220-8.
- BARRY, D. W. & KOHRT, W. M. 2008. BMD decreases over the course of a year in competitive male cyclists. *J Bone Miner Res*, 23, 484-91.
- BASS, S. L., SAXON, L., DALY, R. M., TURNER, C. H., ROBLING, A. G., SEEMAN, E. & STUCKEY, S. 2002. The effect of mechanical loading on the size and shape of bone in pre-, peri-, and postpubertal girls: a study in tennis players. *J Bone Miner Res*, 17, 2274-80.
- BAUER, J. J. S., C.M. 2003. What is the prescription for healthy bones? *J. Musculoskelet. Neuronal Interact*, **3**, 352-355.
- BAXTER-JONES, A. D., FAULKNER, R. A., FORWOOD, M. R., MIRWALD, R. L. & BAILEY, D. A. 2011.

 Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Res*, 26, 1729-39.
- BAXTER-JONES, A. D., KONTULAINEN, S. A., FAULKNER, R. A. & BAILEY, D. A. 2008. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*, 43, 1101-7.
- BECK, B. R. 2009. Muscle forces or gravity--what predominates mechanical loading on bone? Introduction. *Med Sci Sports Exerc*, 41, 2033-6.
- BECK, T. J. 2007. Extending DXA beyond bone mineral density: understanding hip structure analysis. *Curr Osteoporos Rep*, 5, 49-55.
- BECK, T. J., RUFF, C. B., WARDEN, K. E., SCOTT, W. W., JR. & RAO, G. U. 1990. Predicting femoral neck strength from bone mineral data. A structural approach. *Invest Radiol*, 25, 6-18.
- BEHRINGER, M., GRUETZNER, S., MCCOURT, M. & MESTER, J. 2014. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. *J Bone Miner Res*, 29, 467-78.
- BERG, K. M., KUNINS, H. V., JACKSON, J. L., NAHVI, S., CHAUDHRY, A., HARRIS, K. A., MALIK, R. & ARNSTEN, J. H. 2008. Association between alcohol consumption and both osteoporotic fracture and bone density. *American Journal of Medicine*, 121, 406-418.
- BIELEMANN, R. M., MARTINEZ-MESA, J. & GIGANTE, D. P. 2013. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. *BMC Musculoskelet Disord*, 14, 77.
- BINKLEY, T. L., BERRY, R. & SPECKER, B. L. 2008. Methods for measurement of pediatric bone. *Rev Endocr Metab Disord*, 9, 95-106.
- BLAIR, H. C. & ATHANASOU, N. A. 2004. Recent advances in osteoclast biology and pathological bone resorption. *Histol Histopathol*, 19, 189-99.
- BLIMKIE, C. J., RICE, S., WEBBER, C. E., MARTIN, J., LEVY, D. & GORDON, C. L. 1996. Effects of resistance training on bone mineral content and density in adolescent females. *Can J Physiol Pharmacol*, 74, 1025-33.
- BONJOUR, J.-P. & CHEVALLEY, T. 2007. Pubertal timing, peak bone mass and fragility fracture risk. *BoneKEy-Osteovision*, **4**, 30-48.
- BONJOUR, J. P., BENOIT, V., PAYEN, F. & KRAENZLIN, M. 2013. Consumption of yogurts fortified in vitamin D and calcium reduces serum parathyroid hormone and markers of bone

- resorption: a double-blind randomized controlled trial in institutionalized elderly women. *J Clin Endocrinol Metab*, 98, 2915-21.
- BONJOUR, J. P., CARRIE, A. L., FERRARI, S., CLAVIEN, H., SLOSMAN, D., THEINTZ, G. & RIZZOLI, R. 1997. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *J Clin Invest*, 99, 1287-94.
- BONJOUR, J. P. & CHEVALLEY, T. 2014. Pubertal timing, bone acquisition, and risk of fracture throughout life. *Endocr Rev*, 35, 820-47.
- BONJOUR, J. P., CHEVALLEY, T., FERRARI, S. & RIZZOLI, R. 2009. The importance and relevance of peak bone mass in the prevalence of osteoporosis. *Salud Publica Mex*, 51 Suppl 1, S5-17.
- BONJOUR, J. P., CHEVALLEY, T., RIZZOLI, R. & FERRARI, S. 2007. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. *Med Sport Sci*, 51, 64-80
- BONJOUR, J. P., THEINTZ, G., LAW, F., SLOSMAN, D. & RIZZOLI, R. 1994. Peak bone mass. *Osteoporos Int*, 4 Suppl 1, 7-13.
- BOOT, A. M., DERIDDER, M. A. J., POLS, H. A. P., KRENNING, E. P. & KEIZERSCHRAMA, S. M. P. F. D. 1997. Bone mineral density in children and adolescents: Relation to puberty, calcium intake, and physical activity. *Journal of Clinical Endocrinology & Metabolism*, 82, 57-62.
- BOREHAM, C. A. & MCKAY, H. A. 2011. Physical activity in childhood and bone health. *Br J Sports Med*, 45, 877-9.
- BOUSSON, V., BERGOT, C., SUTTER, B., LEVITZ, P., CORTET, B. & SCIENTIFIC COMMITTEE OF THE GROUPE DE RECHERCHE ET D'INFORMATION SUR LES, O. 2012. Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects. *Osteoporos Int*, 23, 1489-501.
- BOUSSON, V., BERGOT, C., SUTTER, B., THOMAS, T., BENDAVID, S., BENHAMOU, C. L., BLAIN, H., BRAZIER, M., BREUIL, V., BRIOT, K., CHAPURLAT, R., CHAPUIS, L., COHEN SOLAL, M., FARDELLONE, P., FERON, J. M., GAUVAIN, J. B., LAROCHE, M., LEGRAND, E., LESPESSAILLES, E., LINGLART, A., MARCELLI, C., ROUX, C., SOUBERBIELLE, J. C., TREMOLLIERES, F., WERYHA, G., CORTET, B. & GROUPE DE RECHERCHE ET D'INFORMATION SUR LES, O. 2015. Trabecular Bone Score: Where are we now? *Joint Bone Spine*, 82, 320-5.
- BOYDEN, L. M., MAO, J. H., BELSKY, J., MITZNER, L., FARHI, A., MITNICK, M. A., WU, D. Q., INSOGNA, K. & LIFTON, R. P. 2002. High bone density due to a mutation in LDL-receptor-related protein 5. *New England Journal of Medicine*, 346, 1513-1521.
- BROWN, J. P., ALBERT, C., NASSAR, B. A., ADACHI, J. D., COLE, D., DAVISON, K. S., DOOLEY, K. C., DON-WAUCHOPE, A., DOUVILLE, P., HANLEY, D. A., JAMAL, S. A., JOSSE, R., KAISER, S., KRAHN, J., KRAUSE, R., KREMER, R., LEPAGE, R., LETENDRE, E., MORIN, S., OOI, D. S., PAPAIOAONNOU, A. & STE-MARIE, L. G. 2009. Bone turnover markers in the management of postmenopausal osteoporosis. *Clin Biochem*, 42, 929-42.
- BRUDVIK, C. & HOVE, L. M. 2003. Childhood fractures in Bergen, Norway: identifying high-risk groups and activities. *J Pediatr Orthop*, 23, 629-34.
- BUCK, D. W. & DUMANIAN, G. A. 2012. Bone Biology and Physiology: Part I. The Fundamentals. *Plastic and Reconstructive Surgery*, 129, 1314-1320.
- BULLEN, D. B., O'TOOLE, M. L. & JOHNSON, K. C. 1999. Calcium losses resulting from an acute bout of moderate-intensity exercise. *Int J Sport Nutr*, 9, 275-84.
- CALBET, J. A., DORADO, C., DIAZ-HERRERA, P. & RODRIGUEZ-RODRIGUEZ, L. P. 2001. High femoral bone mineral content and density in male football (soccer) players. *Med Sci Sports Exerc*, 33, 1682-7.
- CAMERON, J. R. & SORENSON, J. 1963. Measurement of Bone Mineral in Vivo: An Improved Method. *Science*, 142, 230-2.

- CAMERON, M. A., PATON, L. M., NOWSON, C. A., MARGERISON, C., FRAME, M. & WARK, J. D. 2004. The effect of calcium supplementation on bone density in premenarcheal females: a co-twin approach. *J Clin Endocrinol Metab*, 89, 4916-22.
- CAPULLI, M., PAONE, R. & RUCCI, N. 2014. Osteoblast and osteocyte: Games without frontiers. *Archives of Biochemistry and Biophysics*, 561, 3-12.
- CARTER, D. R., BOUXSEIN, M. L. & MARCUS, R. 1992. New approaches for interpreting projected bone densitometry data. *J Bone Miner Res*, 7, 137-45.
- CASTRO-PINERO, J., ARTERO, E. G., ESPANA-ROMERO, V., ORTEGA, F. B., SJOSTROM, M., SUNI, J. & RUIZ, J. R. 2010. Criterion-related validity of field-based fitness tests in youth: a systematic review. *Br J Sports Med*, 44, 934-43.
- CHENG S, L. A., KRÖGER H, LAMBERG-ALLARDT C, ALÉN M, KOISTINEN A, WANG QJ, SUURINIEMI M, SUOMINEN H, MAHONEN A, NICHOLSON PH, IVASKA KK, KORPELA R, OHLSSON C, VÄÄNÄNEN KH, TYLAVSKY F. 2005. Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. *Am J Clin Nutr*, 82, 1115-26.
- CHESI, A., MITCHELL, J. A., KALKWARF, H. J., BRADFIELD, J. P., LAPPE, J. M., MCCORMACK, S. E., GILSANZ, V., OBERFIELD, S. E., HAKONARSON, H., SHEPHERD, J. A., KELLY, A., ZEMEL, B. S. & GRANT, S. F. 2015. A trans-ethnic genome-wide association study identifies gender-specific loci influencing pediatric aBMD and BMC at the distal radius. *Hum Mol Genet*, 24, 5053-9.
- CHEVALLEY, T., BONJOUR, J. P., AUDET, M. C., MERMINOD, F., VAN RIETBERGEN, B., RIZZOLI, R. & FERRARI, S. 2017. Fracture Prospectively Recorded From Prepuberty to Young Adulthood: Are They Markers of Peak Bone Mass and Strength in Males? *J Bone Miner Res*.
- CHEVALLEY, T., BONJOUR, J. P., FERRARI, S., HANS, D. & RIZZOLI, R. 2005. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebo-controlled trial in prepubertal boys. *J Clin Endocrinol Metab*, 90, 3342-9.
- CHEVALLEY, T., BONJOUR, J. P., FERRARI, S. & RIZZOLI, R. 2009. The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche. *J Clin Endocrinol Metab*, 94, 3424-31.
- CHEVALLEY, T., BONJOUR, J. P., FERRARI, S. & RIZZOLI, R. 2011. Pubertal timing and body mass index gain from birth to maturity in relation with femoral neck BMD and distal tibia microstructure in healthy female subjects. *Osteoporosis International*, 22, 2689-2698.
- CHOPIN, F., BIVER, E., FUNCK-BRENTANO, T., BOUVARD, B., COIFFIER, G., GARNERO, P. & THOMAS, T. 2012. Prognostic interest of bone turnover markers in the management of postmenopausal osteoporosis. *Joint Bone Spine*, 79, 26-31.
- CHRISTOFFERSEN, T., AHMED, L. A., DALTVEIT, A. K., DENNISON, E. M., EVENSEN, E. K., FURBERG, A. S., GRACIA-MARCO, L., GRIMNES, G., NILSEN, O. A., SCHEI, B., TELL, G. S., VLACHOPOULOS, D., WINTHER, A. & EMAUS, N. 2017. The influence of birth weight and length on bone mineral density and content in adolescence: The Tromso Study, Fit Futures. *Arch Osteoporos*, 12, 54.
- CLARK, E. M., NESS, A. R., BISHOP, N. J. & TOBIAS, J. H. 2006. Association between bone mass and fractures in children: a prospective cohort study. *J Bone Miner Res*, 21, 1489-95.
- CLARKE, B. 2008. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol*, 3 Suppl 3, S131-9.
- COLE, Z. A., HARVEY, N. C., KIM, M., NTANI, G., ROBINSON, S. M., INSKIP, H. M., GODFREY, K. M., COOPER, C., DENNISON, E. M. & G, S. W. S. S. S. 2012. Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. *Bone*, 50, 562-567.
- COMPSTON, J., COOPER, A., COOPER, C., FRANCIS, R., KANIS, J. A., MARSH, D., MCCLOSKEY, E. V., REID, D. M., SELBY, P., WILKINS, M. & NATIONAL OSTEOPOROSIS GUIDELINE, G. 2009. Guidelines for the diagnosis and management of osteoporosis in

- postmenopausal women and men from the age of 50 years in the UK. *Maturitas*, 62, 105-8.
- CONSTANTINI, N. W., DUBNOV-RAZ, G., CHODICK, G., ROZEN, G. S., GILADI, A. & ISH-SHALOM, S. 2010. Physical activity and bone mineral density in adolescents with vitamin D deficiency. *Med Sci Sports Exerc*, 42, 646-50.
- COOPER, C. 1999. Epidemiology of osteoporosis. Osteoporos Int, 9 Suppl 2, S2-8.
- COOPER, C., CAMPION, G. & MELTON, L. J., 3RD 1992. Hip fractures in the elderly: a world-wide projection. *Osteoporos Int*, 2, 285-9.
- COOPER, C., DENNISON, E. M., LEUFKENS, H. G., BISHOP, N. & VAN STAA, T. P. 2004a. Epidemiology of childhood fractures in Britain: a study using the general practice research database. *J Bone Miner Res*, 19, 1976-81.
- COOPER, C., DENNISON, E. M., LEUFKENS, H. G. M., BISHOP, N. & VAN STAA, T. P. 2004b. Epidemiology of childhood fractures in Britain: A study using the General Practice Research Database. *Journal of Bone and Mineral Research*, 19, 1976-1981.
- COURTEIX, D., JAFFRE, C., LESPESSAILLES, E. & BENHAMOU, L. 2005. Cumulative effects of calcium supplementation and physical activity on bone accretion in premenarchal children: a double-blind randomised placebo-controlled trial. *Int J Sports Med*, 26, 332-8
- COURTEIX, D., LESPESSAILLES, E., PERES, S. L., OBERT, P., GERMAIN, P. & BENHAMOU, C. L. 1998. Effect of physical training on bone mineral density in prepubertal girls: A comparative study between impact-loading and non-impact-loading sports. *Osteoporosis International*, 8, 152-158.
- CRABTREE, N. J., ARABI, A., BACHRACH, L. K., FEWTRELL, M., EL-HAJJ FULEIHAN, G., KECSKEMETHY, H. H., JAWORSKI, M., GORDON, C. M. & INTERNATIONAL SOCIETY FOR CLINICAL, D. 2014. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. *J Clin Densitom*, 17, 225-42.
- CUMMINGS, S. R., BLACK, D. M., NEVITT, M. C., BROWNER, W., CAULEY, J., ENSRUD, K., GENANT, H. K., PALERMO, L., SCOTT, J. & VOGT, T. M. 1993. Bone-Density at Various Sites for Prediction of Hip-Fractures. *Lancet*, 341, 72-75.
- CUMMINGS, S. R. & MELTON, L. J. 2002. Epidemiology and outcomes of osteoporotic fractures. *Lancet*, 359, 1761-1767.
- DALY, R. M., STENEVI-LUNDGREN, S., LINDEN, C. & KARLSSON, M. K. 2008. Muscle determinants of bone mass, geometry and strength in prepubertal girls. *Med Sci Sports Exerc*, 40, 1135-41.
- DAMILAKIS, J., SOLOMOU, G., MANIOS, G. E. & KARANTANAS, A. 2013. Pediatric radiation dose and risk from bone density measurements using a GE Lunar Prodigy scanner. Osteoporos Int, 24, 2025-31.
- DATTA, H. K., NG, W. F., WALKER, J. A., TUCK, S. P. & VARANASI, S. S. 2008. The cell biology of bone metabolism. *J Clin Pathol*, 61, 577-87.
- DAVIES, J. H., EVANS, B. A. & GREGORY, J. W. 2005. Bone mass acquisition in healthy children. *Arch Dis Child*, 90, 373-8.
- DE LAET, C., KANIS, J. A., ODEN, A., JOHANSON, H., JOHNELL, O., DELMAS, P., EISMAN, J. A., KROGER, H., FUJIWARA, S., GARNERO, P., MCCLOSKEY, E. V., MELLSTROM, D., MELTON, L. J., MEUNIER, P. J., POLS, H. A. P., REEVE, J., SILMAN, A. & TENENHOUSE, A. 2005. Body mass index as a predictor of fracture risk: A meta-analysis. *Osteoporosis International*, 16, 1330-1338.
- DE SMET, S., MICHELS, N., POLFLIET, C., D'HAESE, S., ROGGEN, I., DE HENAUW, S. & SIOEN, I. 2015. The influence of dairy consumption and physical activity on ultrasound bone measurements in Flemish children. *J Bone Miner Metab*, 33, 192-200.
- DEPARTMENT OF HEALTH 2013. Statistics on obesity, physical activity and diet. *Chief Medical Officers*,, 1, 22-44.

- DIAS QUITERIO, A. L., CARNERO, E. A., BAPTISTA, F. M. & SARDINHA, L. B. 2011. Skeletal mass in adolescent male athletes and nonathletes: relationships with high-impact sports. *J Strength Cond Res*, 25, 3439-47.
- DIB, L., ARABI, A., MAALOUF, J., NABULSI, M. & EL-HAJJ FULEIHAN, G. 2005. Impact of anthropometric, lifestyle, and body composition variables on ultrasound measurements in school children. *Bone*, 36, 736-42.
- DIBBA, B., PRENTICE, A., CEESAY, M., STIRLING, D. M., COLE, T. J. & POSKITT, E. M. 2000. Effect of calcium supplementation on bone mineral accretion in gambian children accustomed to a low-calcium diet. *Am J Clin Nutr*, 71, 544-9.
- DIMAI, H. P. 2016. Use of dual-energy X-ray absorptiometry (DXA) for diagnosis and fracture risk assessment; WHO-criteria, T- and Z-score, and reference databases. *Bone*.
- DONALDSON, L. J., RECKLESS, I. P., SCHOLES, S., MINDELL, J. S. & SHELTON, N. J. 2008. The epidemiology of fractures in England. *J Epidemiol Community Health*, 62, 174-80.
- DOWTHWAITE, J. N., ROSENBAUM, P. F. & SCERPELLA, T. A. 2012. Site-specific advantages in skeletal geometry and strength at the proximal femur and forearm in young female gymnasts. *Bone*, 50, 1173-83.
- DUMITH, S. C., GIGANTE, D. P., DOMINGUES, M. R. & KOHL, H. W., 3RD 2011. Physical activity change during adolescence: a systematic review and a pooled analysis. *Int J Epidemiol*, 40, 685-98.
- DUNCAN, C. S., BLIMKIE, C. J., COWELL, C. T., BURKE, S. T., BRIODY, J. N. & HOWMAN-GILES, R. 2002a. Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. *Med Sci Sports Exerc*, 34, 286-94.
- DUNCAN, C. S., BLIMKIE, C. J., KEMP, A., HIGGS, W., COWELL, C. T., WOODHEAD, H., BRIODY, J. N. & HOWMAN-GILES, R. 2002b. Mid-femur geometry and biomechanical properties in 15- to 18-yr-old female athletes. *Med Sci Sports Exerc*, 34, 673-81.
- DUNCAN, E. L. & BROWN, M. A. 2010. Clinical review 2: Genetic determinants of bone density and fracture risk--state of the art and future directions. *J Clin Endocrinol Metab*, 95, 2576-87.
- DUREN, D. L., SHERWOOD, R. J., CHOH, A. C., CZERWINSKI, S. A., CHUMLEA, W. C., LEE, M., SUN, S. S., DEMERATH, E. W., SIERVOGEL, R. M. & TOWNE, B. 2007. Quantitative genetics of cortical bone mass in healthy 10-year-old children from the Fels Longitudinal Study. *Bone*, 40, 464-70.
- EL HAGE, R. 2013. Geometric indices of hip bone strength in young female football players. *Journal of Musculoskeletal & Neuronal Interactions*, 13, 206-212.
- EL HAGE, R., MOUSSA, E. & JACOB, C. 2010. Bone Mineral Content and Density in Obese, Overweight, and Normal-Weighted Sedentary Adolescent Girls. *Journal of Adolescent Health*, 47, 591-595.
- EL HAGE, R. P., COURTEIX, D., BENHAMOU, C. L., JACOB, C. & JAFFRE, C. 2009. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol*, 105, 759-64.
- EMAUS, N., WILSGAARD, T. & AHMED, L. A. 2014. Impacts of Body Mass Index, Physical Activity, and Smoking on Femoral Bone Loss: The Tromso Study. *Journal of Bone and Mineral Research*, 29, 2080-2089.
- ERLANDSON, M. C., KONTULAINEN, S. A., CHILIBECK, P. D., ARNOLD, C. M., FAULKNER, R. A. & BAXTER-JONES, A. D. 2012. Higher premenarcheal bone mass in elite gymnasts is maintained into young adulthood after long-term retirement from sport: a 14-year follow-up. *J Bone Miner Res*, 27, 104-10.
- ESLIGER, D. W., ROWLANDS, A. V., HURST, T. L., CATT, M., MURRAY, P. & ESTON, R. G. 2011. Validation of the GENEA Accelerometer. *Med Sci Sports Exerc*, 43, 1085-93.
- ESTRADA, K., STYRKARSDOTTIR, U., EVANGELOU, E., HSU, Y. H., DUNCAN, E. L., NTZANI, E. E., OEI, L., ALBAGHA, O. M., AMIN, N., KEMP, J. P., KOLLER, D. L., LI, G., LIU, C. T., MINSTER, R. L., MOAYYERI, A., VANDENPUT, L., WILLNER, D., XIAO, S. M., YERGES-ARMSTRONG, L. M., ZHENG, H. F., ALONSO, N., ERIKSSON, J., KAMMERER, C. M., KAPTOGE, S. K., LEO, P.

- J., THORLEIFSSON, G., WILSON, S. G., WILSON, J. F., AALTO, V., ALEN, M., ARAGAKI, A. K., ASPELUND, T., CENTER, J. R., DAILIANA, Z., DUGGAN, D. J., GARCIA, M., GARCIA-GIRALT, N., GIROUX, S., HALLMANS, G., HOCKING, L. J., HUSTED, L. B., JAMESON, K. A., KHUSAINOVA, R., KIM, G. S., KOOPERBERG, C., KOROMILA, T., KRUK, M., LAAKSONEN, M., LACROIX, A. Z., LEE, S. H., LEUNG, P. C., LEWIS, J. R., MASI, L., MENCEJ-BEDRAC, S., NGUYEN, T. V., NOGUES, X., PATEL, M. S., PREZELJ, J., ROSE, L. M., SCOLLEN, S., SIGGEIRSDOTTIR, K., SMITH, A. V., SVENSSON, O., TROMPET, S., TRUMMER, O., VAN SCHOOR, N. M., WOO, J., ZHU, K., BALCELLS, S., BRANDI, M. L., BUCKLEY, B. M., CHENG, S., CHRISTIANSEN, C., COOPER, C., DEDOUSSIS, G., FORD, I., FROST, M., GOLTZMAN, D., GONZALEZ-MACIAS, J., KAHONEN, M., KARLSSON, M., KHUSNUTDINOVA, E., KOH, J. M., KOLLIA, P., LANGDAHL, B. L., LESLIE, W. D., LIPS, P., LJUNGGREN, O., LORENC, R. S., MARC, J., MELLSTROM, D., OBERMAYER-PIETSCH, B., OLMOS, J. M., PETTERSSON-KYMMER, U., REID, D. M., RIANCHO, J. A., RIDKER, P. M., ROUSSEAU, F., SLAGBOOM, P. E., TANG, N. L., et al. 2012. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet*, 44, 491-501.
- FALK, B., BRAID, S., MOORE, M., YAO, M., SULLIVAN, P. & KLENTROU, N. 2010. Bone properties in child and adolescent male hockey and soccer players. *J Sci Med Sport*, 13, 387-91.
- FALK, B., BRONSHTEIN, Z., ZIGEL, L., CONSTANTINI, N. W. & ELIAKIM, A. 2003. Quantitative ultrasound of the tibia and radius in prepubertal and early-pubertal female athletes. *Arch Pediatr Adolesc Med*, 157, 139-43.
- FARR, J. N., CHEN, Z., LISSE, J. R., LOHMAN, T. G. & GOING, S. B. 2010. Relationship of total body fat mass to weight-bearing bone volumetric density, geometry, and strength in young girls. *Bone*, 46, 977-84.
- FAULKNER, K. G., WACKER, W. K., BARDEN, H. S., SIMONELLI, C., BURKE, P. K., RAGI, S. & DEL RIO, L. 2006. Femur strength index predicts hip fracture independent of bone density and hip axis length. *Osteoporos Int*, 17, 593-9.
- FAULKNER, R. A. & BAILEY, D. A. 2007. Osteoporosis: a pediatric concern? *Med Sport Sci*, 51, 1-12.
- FAULKNER, R. A., FORWOOD, M. R., BECK, T. J., MAFUKIDZE, J. C., RUSSELL, K. & WALLACE, W. 2003. Strength indices of the proximal femur and shaft in prepubertal female gymnasts. *Med Sci Sports Exerc*, 35, 513-8.
- FERRY, B., DUCLOS, M., BURT, L., THERRE, P., LE GALL, F., JAFFRE, C. & COURTEIX, D. 2011. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. *J Bone Miner Metab*, 29, 342-51.
- FERRY, B., LESPESSAILLES, E., ROCHCONGAR, P., DUCLOS, M. & COURTEIX, D. 2013. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. *Joint Bone Spine*, 80, 57-63.
- FROST, H. M. 1987. Bone "mass" and the "mechanostat": A proposal. *The Anatomical Record,* 219, 1-9.
- FROST, H. M. 2000. The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue organs. *J Bone Miner Metab*, 18, 305-16.
- FROST, H. M. 2001. From Wolff's law to the Utah paradigm: Insights about bone physiology and its clinical applications. *Anatomical Record*, 262, 398-419.
- FROST, H. M. 2003. Bone's mechanostat: a 2003 update. *Anat Rec A Discov Mol Cell Evol Biol*, 275, 1081-101.
- FROST, H. M. & SCHONAU, E. 2000. The "muscle-bone unit" in children and adolescents: a 2000 overview. *J Pediatr Endocrinol Metab*, 13, 571-90.
- FROST, M. L., BLAKE, G. M. & FOGELMAN, I. 1999. Contact quantitative ultrasound: an evaluation of precision, fracture discrimination, age-related bone loss and applicability of the WHO criteria. *Osteoporos Int*, 10, 441-9.
- FRUSH, T. J. & LINDENFELD, T. N. 2009. Peri-epiphyseal and Overuse Injuries in Adolescent Athletes. *Sports Health*, 1, 201-11.

- FUCHS, R. K., BAUER, J. J. & SNOW, C. M. 2001. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. *J Bone Miner Res*, 16, 148-56.
- FUCHS, R. K. & SNOW, C. M. 2002. Gains in hip bone mass from high-impact training are maintained: a randomized controlled trial in children. *J Pediatr*, 141, 357-62.
- GENANT, H. K., GRAMPP, S., GLUER, C. C., FAULKNER, K. G., JERGAS, M., ENGELKE, K., HAGIWARA, S. & VAN KUIJK, C. 1994. Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. *J Bone Miner Res*, 9, 1503-14.
- GEORGOPOULOS, N. A., MARKOU, K. B., THEODOROPOULOU, A., VAGENAKIS, G. A., MYLONAS, P. & VAGENAKIS, A. G. 2004. Growth, pubertal development, skeletal maturation and bone mass acquisition in athletes. *Hormones (Athens)*, 3, 233-43.
- GINTY, F., RENNIE, K. L., MILLS, L., STEAR, S., JONES, S. & PRENTICE, A. 2005a. Positive, site-specific associations between bone mineral status, fitness, and time spent at high-impact activities in 16- to 18-year-old boys. *Bone*, 36, 101-10.
- GINTY, F., RENNIE, K. L., MILLS, L., STEAR, S., JONES, S. & PRENTICE, A. 2005b. Positive, sitespecific associations between bone mineral status, fitness, and time spent at highimpact activities in 16-to 18-year-old boys. *Bone*, 36, 101-110.
- GOMEZ-BRUTON, A., GONZALEZ-AGUERO, A., GOMEZ-CABELLO, A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2013. Is bone tissue really affected by swimming? A systematic review. *PLoS One*, 8, e70119.
- GOMEZ-BRUTON, A., GONZALEZ-AGUERO, A., GOMEZ-CABELLO, A., MATUTE-LLORENTE, A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2014. The effects of swimming training on bone tissue in adolescence. *Scand J Med Sci Sports*.
- GOMEZ-BRUTON, A., GONZALEZ-AGUERO, A., GOMEZ-CABELLO, A., MATUTE-LLORENTE, A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2015. The effects of swimming training on bone tissue in adolescence. *Scand J Med Sci Sports*, 25, e589-602.
- GOMEZ-BRUTON, A., GONZALEZ-AGUERO, A., GOMEZ-CABELLO, A., MATUTE-LLORENTE, A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2016a. Swimming and bone: Is low bone mass due to hypogravity alone or does other physical activity influence it? *Osteoporos Int*, 27, 1785-93.
- GOMEZ-BRUTON, A., MONTERO-MARIN, J., GONZALEZ-AGUERO, A., GARCIA-CAMPAYO, J., MORENO, L. A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2016b. The Effect of Swimming During Childhood and Adolescence on Bone Mineral Density: A Systematic Review and Meta-Analysis. Sports Med, 46, 365-79.
- GONZALEZ-GROSS, M., VALTUENA, J., BREIDENASSEL, C., MORENO, L. A., FERRARI, M., KERSTING, M., DE HENAUW, S., GOTTRAND, F., AZZINI, E., WIDHALM, K., KAFATOS, A., MANIOS, Y., STEHLE, P. & GROUP, H. S. 2012a. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr*, 107, 755-64.
- GONZALEZ-GROSS, M., VALTUENA, J., BREIDENASSEL, C., MORENO, L. A., FERRARI, M., KERSTING, M., DE HENAUW, S., GOTTRAND, F., AZZINI, E., WIDHALM, K., KAFATOS, A., MANIOS, Y., STEHLE, P. & GRP, H. S. 2012b. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *British Journal of Nutrition*, 107, 755-764.
- GORDON, C. M., BACHRACH, L. K., CARPENTER, T. O., CRABTREE, N., EL-HAJJ FULEIHAN, G., KUTILEK, S., LORENC, R. S., TOSI, L. L., WARD, K. A., WARD, L. M. & KALKWARF, H. J. 2008. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. *J Clin Densitom*, 11, 43-58.
- GORDON, C. M., LEONARD, M. B., ZEMEL, B. S. & INTERNATIONAL SOCIETY FOR CLINICAL, D. 2014. 2013 Pediatric Position Development Conference: executive summary and reflections. *J Clin Densitom*, 17, 219-24.

- GORDON, C. M., ZEMEL, B. S., WREN, T. A., LEONARD, M. B., BACHRACH, L. K., RAUCH, F., GILSANZ, V., ROSEN, C. J. & WINER, K. K. 2016. The Determinants of Peak Bone Mass. *J Pediatr*.
- GOULDING, A., JONES, I. E., TAYLOR, R. W., WILLIAMS, S. M. & MANNING, P. J. 2001. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *J Pediatr*, 139, 509-15.
- GRACIA-MARCO, L. 2016. Physical activity, bone mass and muscle strength in children. *Acta Paediatr*, 105, 1127-8.
- GRACIA-MARCO, L., MORENO, L. A., ORTEGA, F. B., LEON, F., SIOEN, I., KAFATOS, A., MARTINEZ-GOMEZ, D., WIDHALM, K., CASTILLO, M. J., VICENTE-RODRIGUEZ, G. & GROUP, H. S. 2011a. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. *Am J Prev Med*, 40, 599-607.
- GRACIA-MARCO, L., ORTEGA, F. B., JIMENEZ-PAVON, D., RODRIGUEZ, G., CASTILLO, M. J., VICENTE-RODRIGUEZ, G. & MORENO, L. A. 2012. Adiposity and bone health in Spanish adolescents. The HELENA study. *Osteoporos Int*, 23, 937-47.
- GRACIA-MARCO, L., ORTEGA, F. B., JIMENEZ-PAVON, D., RODRIGUEZ, G., VALTUENA, J., DIAZ-MARTINEZ, A. E., GONZALEZ-GROSS, M., CASTILLO, M. J., VICENTE-RODRIGUEZ, G. & MORENO, L. A. 2011b. Contribution of bone turnover markers to bone mass in pubertal boys and girls. *J Pediatr Endocrinol Metab*, 24, 971-4.
- GRACIA-MARCO, L., VICENTE-RODRIGUEZ, G., CASAJUS, J. A., MOLNAR, D., CASTILLO, M. J. & MORENO, L. A. 2011c. Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study. *Eur J Appl Physiol*, 111, 2671-80.
- GRACIA-MARCO, L., VICENTE-RODRIGUEZ, G., VALTUENA, J., REY-LOPEZ, J. P., DIAZ MARTINEZ, A. E., MESANA, M. I., WIDHALM, K., RUIZ, J. R., GONZALEZ-GROSS, M., CASTILLO, M. J., MORENO, L. A. & GROUP, H. S. 2010. Bone mass and bone metabolism markers during adolescence: The HELENA Study. *Horm Res Paediatr*, 74, 339-50.
- GRANT, S. F., REID, D. M., BLAKE, G., HERD, R., FOGELMAN, I. & RALSTON, S. H. 1996. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet*, 14, 203-5.
- GREENE, D. A. & NAUGHTON, G. A. 2006a. Adaptive skeletal responses to mechanical loading during adolescence. *Sports Med*, 36, 723-32.
- GREENE, D. A. & NAUGHTON, G. A. 2006b. Adaptive skeletal responses to mechanical loading during adolescence. *Sports Medicine*, 36, 723-732.
- GREENWAY, K. G., WALKLEY, J. W. & RICH, P. A. 2012. Does long-term swimming participation have a deleterious effect on the adult female skeleton? *Eur J Appl Physiol*, 112, 3217-25.
- GULLBERG, B., JOHNELL, O. & KANIS, J. A. 1997. World-wide projections for hip fracture. *Osteoporos Int*, 7, 407-13.
- GUNTER, K., BAXTER-JONES, A. D., MIRWALD, R. L., ALMSTEDT, H., FUCHS, R. K., DURSKI, S. & SNOW, C. 2008a. Impact exercise increases BMC during growth: an 8-year longitudinal study. *J Bone Miner Res*, 23, 986-93.
- GUNTER, K., BAXTER-JONES, A. D., MIRWALD, R. L., ALMSTEDT, H., FULLER, A., DURSKI, S. & SNOW, C. 2008b. Jump starting skeletal health: a 4-year longitudinal study assessing the effects of jumping on skeletal development in pre and circum pubertal children. *Bone*, 42, 710-8.
- GUNTER, K. B., ALMSTEDT, H. C. & JANZ, K. F. 2012. Physical activity in childhood may be the key to optimizing lifespan skeletal health. *Exerc Sport Sci Rev*, 40, 13-21.
- HAAPASALO, H., KONTULAINEN, S., SIEVANEN, H., KANNUS, P., JARVINEN, M. & VUORI, I. 2000. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: A peripheral quantitative computed tomography study of the upper arms of male tennis players. *Bone*, 27, 351-357.
- HADJIDAKIS, D. J. & ANDROULAKIS, II 2006. Bone remodeling. Ann N Y Acad Sci, 1092, 385-96.

- HALABA, Z. P., KONSTANTYNOWICZ, J., PLUSKIEWICZ, W., KACZMARSKI, M. & PIOTROWSKA-JASTRZEBSKA, J. 2005. Comparison of phalangeal ultrasound and dual energy X-ray absorptiometry in healthy male and female adolescents. *Ultrasound Med Biol*, 31, 1617-22
- HANS, D., GOERTZEN, A. L., KRIEG, M. A. & LESLIE, W. D. 2011a. Bone microarchitecture assessed by TBS predicts osteoporotic fractures independent of bone density: the Manitoba study. *J Bone Miner Res*, 26, 2762-9.
- HANS, D., GOERTZEN, A. L., KRIEG, M. A. & LESLIE, W. D. 2011b. Bone Microarchitecture Assessed by TBS Predicts Osteoporotic Fractures Independent of Bone Density: The Manitoba Study. *Journal of Bone and Mineral Research*, 26, 2762-2769.
- HARVEY, N. C., COLE, Z. A., CROZIER, S. R., KIM, M., NTANI, G., GOODFELLOW, L., ROBINSON, S. M., INSKIP, H. M., GODFREY, K. M., DENNISON, E. M., WAREHAM, N., EKELUND, U. & COOPER, C. 2012. Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. *Osteoporos Int*, 23, 121-30.
- HEDSTROM, E. M., SVENSSON, O., BERGSTROM, U. & MICHNO, P. 2010. Epidemiology of fractures in children and adolescents Increased incidence over the past decade: a population-based study from northern Sweden. *Acta Orthopaedica*, 81, 148-153.
- HEINIO, L., NIKANDER, R. & SIEVANEN, H. 2015. Association between long-term exercise loading and lumbar spine trabecular bone score (TBS) in different exercise loading groups. *Journal of Musculoskeletal & Neuronal Interactions*, 15, 279-285.
- HEINONEN, A., SIEVANEN, H., KANNUS, P., OJA, P., PASANEN, M. & VUORI, I. 2000. High-impact exercise and bones of growing girls: A 9-month controlled trial. *Osteoporosis International*, 11, 1010-1017.
- HELEN L LAMBERT, R. E., KAVITA KARNIK, JEAN M RUSSELL, AND MARGO E BARKER 2008. Calcium supplementation and bone mineral accretion in adolescent girls: an 18-mo randomized controlled trial with 2-y follow-up. *Am J Clin Nutr*.
- HENRY, Y. M., FATAYERJI, D. & EASTELL, R. 2004. Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: relative contributions of bone size and volumetric bone mineral density. *Osteoporos Int*, 15, 263-73.
- HERNANDEZ, C. J., BEAUPRE, G. S. & CARTER, D. R. 2003. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporos Int*, 14, 843-7.
- HERNLUND, E., SVEDBOM, A., IVERGARD, M., COMPSTON, J., COOPER, C., STENMARK, J., MCCLOSKEY, E. V., JONSSON, B. & KANIS, J. A. 2013. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos*, 8, 136.
- HIND, K. & BURROWS, M. 2007. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. *Bone*, 40, 14-27.
- HIND, K., GANNON, L., WHATLEY, E. & COOKE, C. 2012a. Sexual dimorphism of femoral neck cross-sectional bone geometry in athletes and non-athletes: a hip structural analysis study. *J Bone Miner Metab*, 30, 454-60.
- HIND, K., GANNON, L., WHATLEY, E., COOKE, C. & TRUSCOTT, J. 2012b. Bone cross-sectional geometry in male runners, gymnasts, swimmers and non-athletic controls: a hipstructural analysis study. *Eur J Appl Physiol*, 112, 535-41.
- HOY, C. L., MACDONALD, H. M. & MCKAY, H. A. 2013. How Does Bone Quality Differ Between Healthy-weight and Overweight Adolescents and Young Adults? *Clinical Orthopaedics and Related Research*, 471, 1214-1225.
- HRAFNKELSSON, H., SIGRUDSSON, G., MAGNUSSON, K. T., JOHANNSSON, E. & SIGURDSSON, E. L. 2010. Factors associated with bone mineral density and content in 7-year-old children. *Bone*, 46, 1058-1062.

- HUNCHAREK, M., MUSCAT, J. & KUPELNICK, B. 2008. Impact of dairy products and dietary calcium on bone-mineral content in children: Results of a meta-analysis. *Bone*, 43, 312-321.
- HUYBRECHTS, I., LIN, Y., DE KEYZER, W., SIOEN, I., MOURATIDOU, T., MORENO, L. A., SLIMANI, N., JENAB, M., VANDEVIJVERE, S., DE BACKER, G. & DE HENAUW, S. 2011. Dietary sources and sociodemographic and economic factors affecting vitamin D and calcium intakes in Flemish preschoolers. *Eur J Clin Nutr*, 65, 1039-47.
- ILICH, J. Z. & KERSTETTER, J. E. 2000. Nutrition in bone health revisited: a story beyond calcium. *J Am Coll Nutr*, 19, 715-37.
- IRELAND, A., FERRETTI, J. L. & RITTWEGER, J. 2014. Imaging of the muscle-bone relationship. *Curr Osteoporos Rep,* 12, 486-95.
- IRELAND, A., MADEN-WILKINSON, T., MCPHEE, J., COOKE, K., NARICI, M., DEGENS, H. & RITTWEGER, J. 2013. Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players. *Med Sci Sports Exerc*, 45, 1749-58.
- ISHIKAWA, S., KIM, Y., KANG, M. & MORGAN, D. W. 2013. Effects of weight-bearing exercise on bone health in girls: a meta-analysis. *Sports Med*, 43, 875-92.
- JACKOWSKI, S. A., ERLANDSON, M. C., MIRWALD, R. L., FAULKNER, R. A., BAILEY, D. A., KONTULAINEN, S. A., COOPER, D. M. & BAXTER-JONES, A. D. 2011a. Effect of maturational timing on bone mineral content accrual from childhood to adulthood: evidence from 15 years of longitudinal data. *Bone*, 48, 1178-85.
- JACKOWSKI, S. A., KONTULAINEN, S. A., COOPER, D. M., LANOVAZ, J. L. & BAXTER-JONES, A. D. 2011b. The timing of BMD and geometric adaptation at the proximal femur from childhood to early adulthood in males and females: a longitudinal study. *J Bone Miner Res*, 26, 2753-61.
- JACKOWSKI, S. A., LANOVAZ, J. L., VAN OORT, C. & BAXTER-JONES, A. D. G. 2014. Does lean tissue mass accrual during adolescence influence bone structural strength at the proximal femur in young adulthood? *Osteoporosis International*, 25, 1297-1304.
- JANZ, K. F., LETUCHY, E. M., BURNS, T. L., EICHENBERGER GILMORE, J. M., TORNER, J. C. & LEVY, S. M. 2014. Objectively measured physical activity trajectories predict adolescent bone strength: Iowa Bone Development Study. *Br J Sports Med*, 48, 1032-6.
- JANZ, K. F., LETUCHY, E. M., EICHENBERGER GILMORE, J. M., BURNS, T. L., TORNER, J. C., WILLING, M. C. & LEVY, S. M. 2010. Early physical activity provides sustained bone health benefits later in childhood. *Med Sci Sports Exerc*, 42, 1072-8.
- JAWORSKI, M., LEBIEDOWSKI, M., LORENC, R. S. & TREMPE, J. 1995. Ultrasound bone measurement in pediatric subjects. *Calcif Tissue Int*, 56, 368-71.
- JEE, W. S. 2000. Principles in bone physiology. J Musculoskelet Neuronal Interact, 1, 11-3.
- JOERIS, A., LUTZ, N., WICKI, B., SLONGO, T. & AUDIGE, L. 2014. An epidemiological evaluation of pediatric long bone fractures a retrospective cohort study of 2716 patients from two Swiss tertiary pediatric hospitals. *BMC Pediatr*, 14, 314.
- JOHANSSON, H., ODEN, A., KANIS, J. A., MCCLOSKEY, E. V., MORRIS, H. A., COOPER, C., VASIKARAN, S. & TURNOVER, I.-I. J. W. G. O. S. O. B. M. O. B. 2014. A meta-analysis of reference markers of bone turnover for prediction of fracture. *Calcif Tissue Int*, 94, 560-7.
- JOHNELL, O. & KANIS, J. A. 2006. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int*, 17, 1726-33.
- JOHNELL, O., KANIS, J. A., ODEN, A., JOHANSSON, H., DE LAET, C., DELMAS, P., EISMAN, J. A., FUJIWARA, S., KROGER, H., MELLSTROM, D., MEUNIER, P. J., MELTON, L. J., 3RD, O'NEILL, T., POLS, H., REEVE, J., SILMAN, A. & TENENHOUSE, A. 2005. Predictive value of BMD for hip and other fractures. *J Bone Miner Res*, 20, 1185-94.
- JOHNSON, J. & DAWSON-HUGHES, B. 1991. Precision and stability of dual-energy X-ray absorptiometry measurements. *Calcif Tissue Int*, 49, 174-8.
- JONES, I. E., WILLIAMS, S. M., DOW, N. & GOULDING, A. 2002. How many children remain fracture-free during growth? a longitudinal study of children and adolescents

- participating in the Dunedin Multidisciplinary Health and Development Study. *Osteoporos Int,* 13, 990-5.
- JURIMAE, J. 2010. Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr*, 22, 494-500.
- JURIMAE, J., MAESTU, J. & JURIMAE, T. 2010. Bone turnover markers during pubertal development: relationships with growth factors and adipocytokines. *Med Sport Sci*, 55, 114-27.
- KALKWARF, H. J., LAOR, T. & BEAN, J. A. 2011. Fracture risk in children with a forearm injury is associated with volumetric bone density and cortical area (by peripheral QCT) and areal bone density (by DXA). *Osteoporosis International*, 22, 607-616.
- KAMBAS, A., LEONTSINI, D., AVLONITI, A., CHATZINIKOLAOU, A., STAMPOULIS, T., MAKRIS, K., DRAGANIDIS, D., JAMURTAS, A. Z., TOURNIS, S. & FATOUROS, I. G. 2016. Physical activity may be a potent regulator of bone turnover biomarkers in healthy girls during preadolescence. *Journal of Bone and Mineral Metabolism*, 1-10.
- KANIS, J. A., BURLET, N., COOPER, C., DELMAS, P. D., REGINSTER, J. Y., BORGSTROM, F. & RIZZOLI, R. 2008. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int*, 19, 399-428.
- KANIS, J. A., JOHANSSON, H., JOHNELL, O., ODEN, A., DE LAET, C., EISMAN, J. A., POLS, H. & TENENHOUSE, A. 2005. Alcohol intake as a risk factor for fracture. *Osteoporosis International*, 16, 737-742.
- KANIS, J. A., JOHANSSON, H., ODEN, A., JOHNELL, O., DE LAET, C., EISMAN, J. A., MCCLOSKEY, E. V., MELLSTROM, D., MELTON, L. J., 3RD, POLS, H. A., REEVE, J., SILMAN, A. J. & TENENHOUSE, A. 2004a. A family history of fracture and fracture risk: a meta-analysis. *Bone*, 35, 1029-37.
- KANIS, J. A. & JOHNELL, O. 2005a. Requirements for DXA for the management of osteoporosis in Europe. *Osteoporosis International*, 16, 229-238.
- KANIS, J. A. & JOHNELL, O. 2005b. Requirements for DXA for the management of osteoporosis in Europe. *Osteoporos Int*, 16, 229-38.
- KANIS, J. A., JOHNELL, O., DE LAET, C., JOHANSSON, H., ODEN, A., DELMAS, P., EISMAN, J., FUJIWARA, S., GARNERO, P., KROGER, H., MCCLOSKEY, E. V., MELLSTROM, D., MELTON, L. J., POLS, H., REEVE, J., SILMAN, A. & TENENHOUSE, A. 2004b. A meta-analysis of previous fracture and subsequent fracture risk. *Bone*, 35, 375-82.
- KANIS, J. A., JOHNELL, O., ODEN, A., JONSSON, B., DE LAET, C. & DAWSON, A. 2000a. Risk of hip fracture according to the World Health Organization criteria for osteopenia and osteoporosis. *Bone*, 27, 585-90.
- KANIS, J. A., JOHNELL, O., ODEN, A., SERNBO, I., REDLUND-JOHNELL, I., DAWSON, A., DE LAET, C. & JONSSON, B. 2000b. Long-term risk of osteoporotic fracture in Malmo. *Osteoporosis International*, **11**, 669-674.
- KANIS, J. A., ODEN, A., JOHANSSON, H., BORGSTROM, F., STROM, O. & MCCLOSKEY, E. 2009. FRAX (R) and its applications to clinical practice. *Bone*, 44, 734-743.
- KAPTOGE, S., BECK, T. J., REEVE, J., STONE, K. L., HILLIER, T. A., CAULEY, J. A. & CUMMINGS, S. R. 2008. Prediction of incident hip fracture risk by femur geometry variables measured by hip structural analysis in the study of osteoporotic fractures. *J Bone Miner Res*, 23, 1892-904.
- KATO, T., YAMASHITA, T., MIZUTANI, S., HONDA, A., MATUMOTO, M. & UMEMURA, Y. 2009. Adolescent exercise associated with long-term superior measures of bone geometry: a cross-sectional DXA and MRI study. *Br J Sports Med*, 43, 932-5.
- KAUFMAN, J. M., REGINSTER, J. Y., BOONEN, S., BRANDI, M. L., COOPER, C., DERE, W., DEVOGELAER, J. P., DIEZ-PEREZ, A., KANIS, J. A., MCCLOSKEY, E., MITLAK, B., ORWOLL, E., RINGE, J. D., WERYHA, G. & RIZZOLI, R. 2013. Treatment of osteoporosis in men. *Bone*, 53, 134-44.
- KEMPER HC, T. J., VAN MECHELEN W, POST GB, ROOS JC, LIPS P. 2000. A fifteen-year longitudinal study in young adults on the relation of physical activity and fitness with

- the development of the bone mass: The Amsterdam Growth And Health Longitudinal Study. *Bone*, 27, 847-853.
- KHOO, B. C., BECK, T. J., QIAO, Q. H., PARAKH, P., SEMANICK, L., PRINCE, R. L., SINGER, K. P. & PRICE, R. I. 2005. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multi-center clinical trials. *Bone*, 37, 112-21.
- KISH, K., MEZIL, Y., WARD, W. E., KLENTROU, P. & FALK, B. 2015. Effects of plyometric exercise session on markers of bone turnover in boys and young men. *Eur J Appl Physiol*, 115, 2115-24
- KITCHIN, B. & MORGAN, S. L. 2007. Not just calcium and vitamin D: other nutritional considerations in osteoporosis. *Curr Rheumatol Rep*, 9, 85-92.
- KLESGES, R. C., WARD, K. D., SHELTON, M. L., APPLEGATE, W. B., CANTLER, E. D., PALMIERI, G. M., HARMON, K. & DAVIS, J. 1996. Changes in bone mineral content in male athletes. Mechanisms of action and intervention effects. *JAMA*, 276, 226-30.
- KOHRT, W. M., BARRY, D. W. & SCHWARTZ, R. S. 2009. Muscle Forces or Gravity: What Predominates Mechanical Loading on Bone? *Medicine and Science in Sports and Exercise*, 41, 2050-2055.
- KORHONEN, M. T., HEINONEN, A., SIEKKINEN, J., ISOLEHTO, J., ALEN, M., KIVIRANTA, I. & SUOMINEN, H. 2012. Bone Density, Structure and Strength, and Their Determinants in Aging Sprint Athletes. *Medicine and Science in Sports and Exercise*, 44, 2340-2349.
- KOZIEL, S. M. & MALINA, R. M. 2017. Modified Maturity Offset Prediction Equations: Validation in Independent Longitudinal Samples of Boys and Girls. *Sports Med*.
- KRIEG, M. A., BARKMANN, R., GONNELLI, S., STEWART, A., BAUER, D. C., DEL RIO BARQUERO, L., KAUFMAN, J. J., LORENC, R., MILLER, P. D., OLSZYNSKI, W. P., POIANA, C., SCHOTT, A. M., LEWIECKI, E. M. & HANS, D. 2008. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. *J Clin Densitom*, 11, 163-87.
- KRUSTRUP, P., HANSEN, P. R., ANDERSEN, L. J., JAKOBSEN, M. D., SUNDSTRUP, E., RANDERS, M. B., CHRISTIANSEN, L., HELGE, E. W., PEDERSEN, M. T., SOGAARD, P., JUNGE, A., DVORAK, J., AAGAARD, P. & BANGSBO, J. 2010. Long-term musculoskeletal and cardiac health effects of recreational football and running for premenopausal women. *Scand J Med Sci Sports*, 20 Suppl 1, 58-71.
- KULAR, J., TICKNER, J., CHIM, S. M. & XU, J. K. 2012. An overview of the regulation of bone remodelling at the cellular level. *Clinical Biochemistry*, 45, 863-873.
- LANGTON, C. M., PALMER, S. B. & PORTER, R. W. 1984. The measurement of broadband ultrasonic attenuation in cancellous bone. *Eng Med*, 13, 89-91.
- LANOU, A. J., BERKOW, S. E. & BARNARD, N. D. 2005. Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. *Pediatrics*, 115, 736-43.
- LAPPE, J. M., WATSON, P., GILSANZ, V., HANGARTNER, T., KALKWARF, H. J., OBERFIELD, S., SHEPHERD, J., WINER, K. K. & ZEMEL, B. 2014. The Longitudinal Effects of Physical Activity and Dietary Calcium on Bone Mass Accrual Across Stages of Pubertal Development. *J Bone Miner Res*.
- LARSEN, M. N., NIELSEN, C. M., HELGE, E. W., MADSEN, M., MANNICHE, V., HANSEN, L., HANSEN, P. R., BANGSBO, J. & KRUSTRUP, P. 2016. Positive effects on bone mineralisation and muscular fitness after 10 months of intense school-based physical training for children aged 8-10 years: the FIT FIRST randomised controlled trial. *Br J Sports Med*.
- LAW, M. R. & HACKSHAW, A. K. 1997. A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. *BMJ*, 315, 841-6.
- LEE, V. R., BLEW, R. M., FARR, J. N., TOMAS, R., LOHMAN, T. G. & GOING, S. B. 2013. Estimation of whole body fat from appendicular soft tissue from peripheral quantitative computed tomography in adolescent girls. *Int J Body Compos Res,* 11, 1-8.

- LEE, W. T. K., CHEUNG, A. Y. K., LAU, J., LEE, S. K. M., QIN, L. & CHENG, J. C. Y. 2004. Bone densitometry: which skeletal sites are best predicted by bone mass determinants? *Journal of Bone and Mineral Metabolism*, 22, 447-455.
- LEGER, L. A., MERCIER, D., GADOURY, C. & LAMBERT, J. 1988. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*, 6, 93-101.
- LEHTONEN-VEROMAA, M., MOTTONEN, T., IRJALA, K., NUOTIO, I., LEINO, A. & VIIKARI, J. 2000. A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. *Journal of Clinical Endocrinology & Metabolism*, 85, 3726-3732.
- LEONARD, M. B., SHULTS, J., WILSON, B. A., TERSHAKOVEC, A. M. & ZEMEL, B. S. 2004. Obesity during childhood and adolescence augments bone mass and bone dimensions. *American Journal of Clinical Nutrition*, 80, 514-523.
- LERNER, U. H. 2000. Osteoclast formation and resorption. Matrix Biol, 19, 107-20.
- LESINSKI, M., PRIESKE, O. & GRANACHER, U. 2016. Effects and dose-response relationships of resistance training on physical performance in youth athletes: a systematic review and meta-analysis. *British Journal of Sports Medicine*, 50, 781-795.
- LESLIE, W. D., PAHLAVAN, P. S., TSANG, J. F., LIX, L. M. & MANITOBA BONE DENSITY, P. 2009. Prediction of hip and other osteoporotic fractures from hip geometry in a large clinical cohort. *Osteoporos Int*, 20, 1767-74.
- LIU, X. S., COHEN, A., SHANE, E., YIN, P. T., STEIN, E. M., ROGERS, H., KOKOLUS, S. L., MCMAHON, D. J., LAPPE, J. M., RECKER, R. R., LANG, T. & GUO, X. E. 2010. Bone density, geometry, microstructure, and stiffness: Relationships between peripheral and central skeletal sites assessed by DXA, HR-pQCT, and cQCT in premenopausal women. *J Bone Miner Res*, 25, 2229-38.
- MACDONALD, H., KONTULAINEN, S., PETIT, M., JANSSEN, P. & MCKAY, H. 2006. Bone strength and its determinants in pre- and early pubertal boys and girls. *Bone*, 39, 598-608.
- MACKELVIE, K. J., KHAN, K. M. & MCKAY, H. A. 2002a. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. *British Journal of Sports Medicine*, 36, 250-257.
- MACKELVIE, K. J., MCKAY, H. A., KHAN, K. M. & CROCKER, P. R. 2001. A school-based exercise intervention augments bone mineral accrual in early pubertal girls. *J Pediatr*, 139, 501-8.
- MACKELVIE, K. J., MCKAY, H. A., PETIT, M. A., MORAN, O. & KHAN, K. M. 2002b. Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. *J Bone Miner Res*, 17, 834-44.
- MACKELVIE, K. J., PETIT, M. A., KHAN, K. M., BECK, T. J. & MCKAY, H. A. 2004. Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. *Bone*, 34, 755-64.
- MAGKOS, F., KAVOURAS, S. A., YANNAKOULIA, M., KARIPIDOU, M., SIDOSSI, S. & SIDOSSIS, L. S. 2007. The bone response to non-weight-bearing exercise is sport-, site-, and sexspecific. *Clinical Journal of Sport Medicine*, 17, 123-128.
- MAIMOUN, L., COSTE, O., GALTIER, F., MURA, T., MARIANO-GOULART, D., PARIS, F. & SULTAN, C. 2010. Bone mineral density acquisition in peripubertal female rhythmic gymnasts is directly associated with plasma IGF1/IGF-binding protein 3 ratio. *European Journal of Endocrinology*, 163, 157-164.
- MAIMOUN, L., COSTE, O., MURA, T., PHILIBERT, P., GALTIER, F., MARIANO-GOULART, D., PARIS, F. & SULTAN, C. 2013a. Specific bone mass acquisition in elite female athletes. *J Clin Endocrinol Metab*, 98, 2844-53.
- MAIMOUN, L., COSTE, O., PHILIBERT, P., BRIOT, K., MURA, T., GALTIER, F., MARIANO-GOULART, D., PARIS, F. & SULTAN, C. 2013b. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. *Metabolism*, 62, 1088-98.

- MALINA, R. M., BOUCHARD, C. & BAR-OR, O. 2004. *Growth, Maturation and Physical Activity.* 2nd edition., Champaign, IL, Human Kinetics.
- MALINA, R. M. & KOZIEL, S. M. 2014. Validation of maturity offset in a longitudinal sample of Polish girls. *J Sports Sci*, 32, 1374-82.
- MANOLAGAS, S. C. 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Journal of Aging and Physical Activity*, 8, 248-248.
- MARKOVIC, G., DIZDAR, D., JUKIC, I. & CARDINALE, M. 2004. Reliability and factorial validity of squat and countermovement jump tests. *J Strength Cond Res*, 18, 551-5.
- MARTIN, R. B. & BURR, D. B. 1984. Non-invasive measurement of long bone cross-sectional moment of inertia by photon absorptiometry. *J Biomech*, 17, 195-201.
- MATKOVIC V, G. P., BADENHOP-STEVENS NE, LANDOLL JD, LI B, ILICH JZ, SKUGOR M, NAGODE LA, MOBLEY SL, HA EJ, HANGARTNER TN, CLAIRMONT A 2005. Calcium supplementation and bone mineral density in females from childhood to young adulthood: a randomized controlled trial. *Am J Clin Nutr*.
- MATTOCKS, C., LEARY, S., NESS, A., DEERE, K., SAUNDERS, J., TILLING, K., KIRKBY, J., BLAIR, S. N. & RIDDOCH, C. 2007. Calibration of an accelerometer during free-living activities in children. *Int J Pediatr Obes*, 2, 218-26.
- MAYORGA-VEGA, D., AGUILAR-SOTO, P. & VICIANA, J. 2015. Criterion-Related Validity of the 20-M Shuttle Run Test for Estimating Cardiorespiratory Fitness: A Meta-Analysis. *J Sports Sci Med*, 14, 536-47.
- MCKAY, H., TSANG, G., HEINONEN, A., MACKELVIE, K., SANDERSON, D. & KHAN, K. M. 2005a. Ground reaction forces associated with an effective elementary school based jumping intervention. *Br J Sports Med*, 39, 10-4.
- MCKAY, H. A., MACLEAN, L., PETIT, M., MACKELVIE-O'BRIEN, K., JANSSEN, P., BECK, T. & KHAN, K. M. 2005b. "Bounce at the Bell": a novel program of short bouts of exercise improves proximal femur bone mass in early pubertal children. *Br J Sports Med*, 39, 521-6.
- MELTON, L. J., CHRISCHILLES, E. A., COOPER, C., LANE, A. W. & RIGGS, B. L. 1992. How Many Women Have Osteoporosis. *Journal of Bone and Mineral Research*, 7, 1005-1010.
- METCALF, B. S., HENLEY, W. & WILKIN, T. J. 2012. The Effectiveness of Intervention on the Physical Activity of Children: Systematic Review and Meta-Analysis of Controlled Trials With Objectively-Measured Outcomes. *Diabetes*, 61, A183-A183.
- MICHALOPOULOU, M., KAMBAS, A., LEONTSINI, D., CHATZINIKOLAOU, A., DRAGANIDIS, D., AVLONITI, A., TSOUKAS, D., MICHOPOULOU, E., LYRITIS, G. P., PAPAIOANNOU, N., TOURNIS, S. & FATOUROS, I. G. 2013. Physical activity is associated with bone geometry of premenarcheal girls in a dose-dependent manner. *Metabolism-Clinical and Experimental*, 62, 1811-1818.
- MILLIKEN, L. A., FAIGENBAUM, A. D., LOUD, R. L. & WESTCOTT, W. L. 2008. Correlates of Upper and Lower Body Muscular Strength in Children. *Journal of Strength and Conditioning Research*, 22, 1339-1346.
- MIRWALD, R. L., BAXTER-JONES, A. D., BAILEY, D. A. & BEUNEN, G. P. 2002. An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc*, 34, 689-94.
- MITCHELL, J. A., CHESI, A., ELCI, O., MCCORMACK, S. E., KALKWARF, H. J., LAPPE, J. M., GILSANZ, V., OBERFIELD, S. E., SHEPHERD, J. A., KELLY, A., ZEMEL, B. S. & GRANT, S. F. 2015. Genetics of Bone Mass in Childhood and Adolescence: Effects of Sex and Maturation Interactions. *J Bone Miner Res*, 30, 1676-83.
- MITCHELL, J. A., CHESI, A., MCCORMACK, S. E., ROY, S. M., COUSMINER, D. L., KALKWARF, H. J., LAPPE, J. M., GILSANZ, V., OBERFIELD, S. E., SHEPHERD, J. A., KELLY, A., ZEMEL, B. S. & GRANT, S. F. 2016. Rare EN1 Variants and Pediatric Bone Mass. *J Bone Miner Res,* 31, 1513-7.
- MOORE, S. A., MCKAY, H. A., MACDONALD, H., NETTLEFOLD, L., BAXTER-JONES, A. D., CAMERON, N. & BRASHER, P. M. 2015. Enhancing a Somatic Maturity Prediction Model. *Med Sci Sports Exerc*, 47, 1755-64.

- MORRISON, N. A., QI, J. C., TOKITA, A., KELLY, P. J., CROFTS, L., NGUYEN, T. V., SAMBROOK, P. N. & EISMAN, J. A. 1994. Prediction of bone density from vitamin D receptor alleles. *Nature*, 367, 284-7.
- MOSCHONIS, G., KANELLAKIS, S., PAPAIOANNOU, N., SCHAAFSMA, A. & MANIOS, Y. 2011. Possible site-specific effect of an intervention combining nutrition and lifestyle counselling with consumption of fortified dairy products on bone mass: the Postmenopausal Health Study II. *Journal of Bone and Mineral Metabolism*, 29, 501-506.
- MOURATIDOU, T., VICENTE-RODRIGUEZ, G., GRACIA-MARCO, L., HUYBRECHTS, I., SIOEN, I., WIDHALM, K., VALTUENA, J., GONZALEZ-GROSS, M., MORENO, L. A. & GROUP, H. S. 2013. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin Densitom*, 16, 110-7.
- NATTIV, A., LOUCKS, A. B., MANORE, M. M., SANBORN, C. F., SUNDGOT-BORGEN, J., WARREN, M. P. & AMERICAN COLLEGE OF SPORTS, M. 2007. American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc*, 39, 1867-82.
- NEBIGH, A., ABED, M. E., BORJI, R., SAHLI, S., SELLAMI, S., TABKA, Z. & REBAI, H. 2017. Bone Turnover Markers and Lean Mass in Pubescent Boys: Comparison Between Elite Soccer Players and Controls. *Pediatr Exerc Sci*, 29, 513-519.
- NEBIGH, A., REBAI, H., ELLOUMI, M., BAHLOUS, A., ZOUCH, M., ZAOUALI, M., ALEXANDRE, C., SELLAMI, S. & TABKA, Z. 2009. Bone mineral density of young boy soccer players at different pubertal stages: relationships with hormonal concentration. *Joint Bone Spine*, 76, 63-9.
- NEVILL, A. M., HOLDER, R. L., MAFFULLI, N., CHENG, J. C., LEUNG, S. S., LEE, W. T. & LAU, J. T. 2002. Adjusting bone mass for differences in projected bone area and other confounding variables: an allometric perspective. *J Bone Miner Res*, 17, 703-8.
- NEVILLE, C. E., ROBSON, P. J., MURRAY, L. J., STRAIN, J. J., TWISK, J., GALLAGHER, A. M., MCGUINNESS, M., CRAN, G. W., RALSTON, S. H. & BOREHAM, C. A. 2002. The effect of nutrient intake on bone mineral status in young adults: the Northern Ireland young hearts project. *Calcif Tissue Int*, 70, 89-98.
- NICKOLS-RICHARDSON, S. M., O'CONNOR, P. J., SHAPSES, S. A. & LEWIS, R. D. 1999. Longitudinal bone mineral density changes in female child artistic gymnasts. *J Bone Miner Res*, 14, 994-1002.
- NIH CONSENSUS DEVELOPMENT PANEL ON OSTEOPOROSIS PREVENTION, D. & THERAPY 2001. Osteoporosis prevention, diagnosis, and therapy. *JAMA*, 285, 785-95.
- NIKANDER, R., SIEVANEN, H., HEINONEN, A., DALY, R. M., UUSI-RASI, K. & KANNUS, P. 2010. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. *BMC Med*, 8, 47.
- NIKANDER, R., SIEVANEN, H., HEINONEN, A. & KANNUS, P. 2005. Femoral neck structure in adult female athletes subjected to different loading modalities. *J Bone Miner Res*, 20, 520-8.
- NILSSON, O., CHRYSIS, D., PAJULO, O., BOMAN, A., HOLST, M., RUBINSTEIN, J., MARTIN RITZEN, E. & SAVENDAHL, L. 2003. Localization of estrogen receptors-alpha and -beta and androgen receptor in the human growth plate at different pubertal stages. *J Endocrinol*, 177, 319-26.
- NIU, T. H. & ROSEN, C. J. 2005. The insulin-like growth factor-I gene and osteoporosis: A critical appraisal. *Gene*, 361, 38-56.
- NOGUEIRA, R. C., WEEKS, B. K. & BECK, B. R. 2014. Exercise to improve pediatric bone and fat: a systematic review and meta-analysis. *Med Sci Sports Exerc*, 46, 610-21.
- NORMAN, J., GOODMAN, A. & POLITZ, D. 2011. Calcium, parathyroid hormone, and vitamin D in patients with primary hyperparathyroidism: normograms developed from 10,000 cases. *Endocr Pract*, 17, 384-94.
- OLMEDILLAS, H., GONZALEZ-AGUERO, A., MORENO, L. A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2011. Bone Related Health Status in Adolescent Cyclists. *Plos One*, 6, e24841.

- OLMEDILLAS, H., GONZALEZ-AGUERO, A., MORENO, L. A., CASAJUS, J. A. & VICENTE-RODRIGUEZ, G. 2012. Cycling and bone health: a systematic review. *BMC Med*, 10, 168.
- ORTEGA, F. B., ARTERO, E. G., RUIZ, J. R., VICENTE-RODRIGUEZ, G., BERGMAN, P., HAGSTROMER, M., OTTEVAERE, C., NAGY, E., KONSTA, O., REY-LOPEZ, J. P., POLITO, A., DIETRICH, S., PLADA, M., BEGHIN, L., MANIOS, Y., SJOSTROM, M., CASTILLO, M. J. & GROUP, H. S. 2008. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. *Int J Obes (Lond)*, 32 Suppl 5, S49-57.
- PAOLA PISANI, M. D. R., FRANCESCO CONVERSANO, ERNESTO CASCIARO, MAURIZIO MURATORE, EUGENIO QUARTA, MARCO DI PAOLA, AND SERGIO CASCIARO 2013. Screening and early diagnosis of osteoporosis through X-ray and ultrasound based techniques. *World J Radiol*, 5, 398-410.
- PARFITT, A. M., TRAVERS, R., RAUCH, F. & GLORIEUX, F. H. 2000. Structural and cellular changes during bone growth in healthy children. *Bone*, 27, 487-94.
- PETIT, M. A., BECK, T. J., LIN, H. M., BENTLEY, C., LEGRO, R. S. & LLOYD, T. 2004. Femoral bone structural geometry adapts to mechanical loading and is influenced by sex steroids: the Penn State Young Women's Health Study. *Bone*, 35, 750-9.
- PETIT, M. A., BECK, T. J., SHULTS, J., ZEMEL, B. S., FOSTER, B. J. & LEONARD, M. B. 2005. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone*, 36, 568-576.
- PETIT, M. A., MCKAY, H. A., MACKELVIE, K. J., HEINONEN, A., KHAN, K. M. & BECK, T. J. 2002. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. *J Bone Miner Res*, 17, 363-72.
- PETTIFOR, J. M. 2005. Rickets and vitamin D deficiency in children and adolescents. *Endocrinol Metab Clin North Am*, 34, 537-53, vii.
- PETTIFOR, J. M. & PRENTICE, A. 2011. The role of vitamin D in paediatric bone health. *Best Pract Res Clin Endocrinol Metab*, 25, 573-84.
- PETTWAY, G. J., MEGANCK, J. A., KOH, A. J., KELLER, E. T., GOLDSTEIN, S. A. & MCCAULEY, L. K. 2008. Parathyroid hormone mediates bone growth through the regulation of osteoblast proliferation and differentiation. *Bone*, 42, 806-18.
- PHILLIPS, L. R., PARFITT, G. & ROWLANDS, A. V. 2013. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. *J Sci Med Sport*, 16, 124-8.
- PLAZA-CARMONA, M., VICENTE-RODRIGUEZ, G., MARTIN-GARCIA, M., BURILLO, P., FELIPE, J. L., MATA, E., CASAJUS, J. A., GALLARDO, L. & ARA, I. 2014. Influence of Hard vs. Soft Ground Surfaces on Bone Accretion in Prepubertal Footballers. *International Journal of Sports Medicine*, 35, 55-61.
- POLLOCK, N. K., LAING, E. M., BAILE, C. A., HAMRICK, M. W., HALL, D. B. & LEWIS, R. D. 2007. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr*, 86, 1530-8.
- POTHUAUD, L., BARTHE, N., KRIEG, M. A., MEHSEN, N., CARCELLER, P. & HANS, D. 2009. Evaluation of the Potential Use of Trabecular Bone Score to Complement Bone Mineral Density in the Diagnosis of Osteoporosis: A Preliminary Spine BMD-Matched, Case-Control Study. *Journal of Clinical Densitometry*, 12, 170-176.
- PRENTICE, A., GINTY, F., STEAR, S. J., JONES, S. C., LASKEY, M. A. & COLE, T. J. 2005. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. *J Clin Endocrinol Metab*, 90, 3153-61.
- PRENTICE, A., PARSONS, T. J. & COLE, T. J. 1994. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *Am J Clin Nutr*, 60, 837-42.
- PREVRHAL, S., SHEPHERD, J. A., FAULKNER, K. G., GAITHER, K. W., BLACK, D. M. & LANG, T. F. 2008. Comparison of DXA hip structural analysis with volumetric QCT. *J Clin Densitom*, 11, 232-6.

- RANDELL, A., SAMBROOK, P. N., NGUYEN, T. V., LAPSLEY, H., JONES, G., KELLY, P. J. & EISMAN, J. A. 1995. Direct clinical and welfare costs of osteoporotic fractures in elderly men and women. *Osteoporosis International*, 5, 427-432.
- RAUCH, F. 2005. Bone growth in length and width: the Yin and Yang of bone stability. *J Musculoskelet Neuronal Interact*, 5, 194-201.
- RAUCH, F., BAILEY, D. A., BAXTER-JONES, A., MIRWALD, R. & FAULKNER, R. 2004. The 'muscle-bone unit' during the pubertal growth spurt. *Bone*, 34, 771-775.
- RAUCH, F. & GLORIEUX, F. H. 2004. Osteogenesis imperfecta. Lancet, 363, 1377-85.
- RECTOR, R. S., ROGERS, R., RUEBEL, M. & HINTON, P. S. 2008. Participation in road cycling vs running is associated with lower bone mineral density in men. *Metabolism*, 57, 226-32.
- REGINSTER, J. Y. & BURLET, N. 2006. Osteoporosis: A still increasing prevalence. Bone, 38, 4-9.
- RENNIE, L., COURT-BROWN, C. M., MOK, J. Y. Q. & BEATTIE, T. F. 2007. The epidemiology of fractures in children. *Injury-International Journal of the Care of the Injured*, 38, 913-922.
- RICO, H., REVILLA, M., HERNANDEZ, E. R., GOMEZ-CASTRESANA, F. & VILLA, L. F. 1993a. Bone mineral content and body composition in postpubertal cyclist boys. *Bone*, 14, 93-5.
- RICO, H., REVILLA, M., VILLA, L. F., GOMEZ-CASTRESANA, F. & ALVAREZ DEL BUERGO, M. 1993b. Body composition in postpubertal boy cyclists. *J Sports Med Phys Fitness*, 33, 278-81.
- RIDDOCH, C. J., MATTOCKS, C., DEERE, K., SAUNDERS, J., KIRKBY, J., TILLING, K., LEARY, S. D., BLAIR, S. N. & NESS, A. R. 2007. Objective measurement of levels and patterns of physical activity. *Arch Dis Child*, 92, 963-9.
- RIGGS, B. L., KHOSLA, S. & MELTON, L. J., 3RD 2002. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev*, 23, 279-302.
- RIZZOLI, R., BIANCHI, M. L., GARABEDIAN, M., MCKAY, H. A. & MORENO, L. A. 2010. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone*, 46, 294-305.
- ROCHEFORT, G. Y., PALLU, S. & BENHAMOU, C. L. 2010. Osteocyte: the unrecognized side of bone tissue. *Osteoporosis International*, 21, 1457-1469.
- ROSS, A. C., MANSON, J. E., ABRAMS, S. A., ALOIA, J. F., BRANNON, P. M., CLINTON, S. K., DURAZO-ARVIZU, R. A., GALLAGHER, J. C., GALLO, R. L., JONES, G., KOVACS, C. S., MAYNE, S. T., ROSEN, C. J. & SHAPSES, S. A. 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*, 96, 53-8.
- RUFFING, J. A., COSMAN, F., ZION, M., TENDY, S., GARRETT, P., LINDSAY, R. & NIEVES, J. W. 2006. Determinants of bone mass and bone size in a large cohort of physically active young adult men. *Nutrition & Metabolism*, 3.
- RUIZ, J. R., SILVA, G., OLIVEIRA, N., RIBEIRO, J. C., OLIVEIRA, J. F. & MOTA, J. 2009. Criterion-related validity of the 20-m shuttle run test in youths aged 13-19 years. *J Sports Sci*, 27, 899-906.
- SAMET, J. M., WIPFLI, H., PLATZ, E. A. & BHAVSAR, N. 2009. A Dictionary of Epidemiology, Fifth EditionEdited by Miquel Porta. *American Journal of Epidemiology*, 170, 1449-1451.
- SANDERCOCK, G., VOSS, C., COHEN, D., TAYLOR, M. & STASINOPOULOS, D. M. 2012. Centile curves and normative values for the twenty metre shuttle-run test in English schoolchildren. *J Sports Sci*, 30, 679-87.
- SAYERS, A., MARCUS, M., RUBIN, C., MCGEEHIN, M. A. & TOBIAS, J. H. 2010. Investigation of sex differences in hip structure in peripubertal children. *J Clin Endocrinol Metab*, 95, 3876-83.
- SAYERS, A. & TOBIAS, J. H. 2010. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab*, 95, 699-706.
- SCHEERDER, J., VANDERMEERSCHEN, H., VAN TUYCKOM, C., HOEKMAN, R., BREEDVELD, K. & VOS, S. 2011. Sport participation in Europe. *KU Leuven / Research Unit of Social Kinesiology & Sport Management*, 10.

- SCHIPILOW, J. D., MACDONALD, H. M., LIPHARDT, A. M., KAN, M. & BOYD, S. K. 2013. Bone micro-architecture, estimated bone strength, and the muscle-bone interaction in elite athletes: an HR-pQCT study. *Bone*, 56, 281-9.
- SCHOENAU, E. 2005. From mechanostat theory to development of the "Functional Muscle-Bone-Unit". *J Musculoskelet Neuronal Interact*, 5, 232-8.
- SCHOENAU, E. & FROST, H. M. 2002. The "muscle-bone unit" in children and adolescents. *Calcified Tissue International,* 70, 405-407.
- SCOFIELD, K. L. & HECHT, S. 2012. Bone health in endurance athletes: runners, cyclists, and swimmers. *Curr Sports Med Rep*, 11, 328-34.
- SCOTT, A., KHAN, K. M., DURONIO, V. & HART, D. A. 2008. Mechanotransduction in human bone in vitro cellular physiology that underpins bone changes with exercise. *Sports Medicine*, 38, 139-160.
- SEABRA, A., MARQUES, E., BRITO, J., KRUSTRUP, P., ABREU, S., OLIVEIRA, J., REGO, C., MOTA, J. & REBELO, A. 2012. Muscle strength and soccer practice as major determinants of bone mineral density in adolescents. *Joint Bone Spine*, 79, 403-8.
- SEEMAN, E. 2004. Estrogen, androgen, and the pathogenesis of bone fragility in women and men. *Curr Osteoporos Rep*, 2, 90-6.
- SEEMAN, E. & DELMAS, P. D. 2006. Bone quality--the material and structural basis of bone strength and fragility. *N Engl J Med*, 354, 2250-61.
- SHAWWA, K., ARABI, A., NABULSI, M., MAALOUF, J., SALAMOUN, M., CHOUCAIR, M., HANS, D. & EL-HAJJ FULEIHAN, G. 2016. Predictors of trabecular bone score in school children. Osteoporos Int, 27, 703-10.
- SHEPHERD, J. A., WANG, L., FAN, B., GILSANZ, V., KALKWARF, H. J., LAPPE, J., LU, Y., HANGARTNER, T., ZEMEL, B. S., FREDRICK, M., OBERFIELD, S. & WINER, K. K. 2011. Optimal Monitoring Time Interval Between DXA Measures in Children. *Journal of Bone and Mineral Research*, 26, 2745-2752.
- SHERK, V. D., BARRY, D. W., VILLALON, K. L., HANSEN, K. C., WOLFE, P. & KOHRT, W. M. 2014. Bone loss over 1 year of training and competition in female cyclists. *Clin J Sport Med*, 24, 331-6.
- SILVA, B. C., LESLIE, W. D., RESCH, H., LAMY, O., LESNYAK, O., BINKLEY, N., MCCLOSKEY, E. V., KANIS, J. A. & BILEZIKIAN, J. P. 2014. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. *Journal of Bone and Mineral Research*, 29, 518-530.
- SILVA, C. C., GOLDBERG, T. B., TEIXEIRA, A. S. & DALMAS, J. C. 2011. The impact of different types of physical activity on total and regional bone mineral density in young Brazilian athletes. *J Sports Sci*, 29, 227-34.
- SIOEN, I., LUST, E., DE HENAUW, S., MORENO, L. A. & JIMENEZ-PAVON, D. 2016. Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. *Calcif Tissue Int*, 99, 557-577.
- SIOEN, I., MOURATIDOU, T., KAUFMAN, J. M., BAMMANN, K., MICHELS, N., PIGEOT, I., VANAELST, B., VYNCKE, K. & DE HENAUW, S. 2012. Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study. *Public Health Nutr*, 15, 1093-9.
- SLEMENDA, C. W., MILLER, J. Z., HUI, S. L., REISTER, T. K. & JOHNSTON, C. C., JR. 1991. Role of physical activity in the development of skeletal mass in children. *J Bone Miner Res*, 6, 1227-33.
- SLOOTMAKER, S. M., SCHUIT, A. J., CHINAPAW, M. J., SEIDELL, J. C. & VAN MECHELEN, W. 2009. Disagreement in physical activity assessed by accelerometer and self-report in subgroups of age, gender, education and weight status. *Int J Behav Nutr Phys Act*, 6, 17.
- SODERMAN, K., BERGSTROM, E., LORENTZON, R. & ALFREDSON, H. 2000. Bone mass and muscle strength in young female soccer players. *Calcif Tissue Int*, 67, 297-303.

- SOPHER, A. B., FENNOY, I. & OBERFIELD, S. E. 2015. An update on childhood bone health: mineral accrual, assessment and treatment. *Curr Opin Endocrinol Diabetes Obes*, 22, 35-40.
- SPECKER B, V. M. 2007. Evidence for an interaction between exercise and nutrition for improved bone health during growth. *Med Sport Sci.*, 51, 50-63.
- SPIRO, A. & BUTTRISS, J. L. 2014. Vitamin D: An overview of vitamin D status and intake in Europe. *Nutr Bull*, 39, 322-350.
- SPORT ENGLAND, U. D. O. H. 2016. Active People Survey 10. Sport England, UK Department of Health.
- STAGI, S., CAVALLI, L., IURATO, C., SEMINARA, S., BRANDI, M. L. & DE MARTINO, M. 2013. Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. *Clin Cases Miner Bone Metab*, 10, 172-9.
- STAGI, S., CAVALLI, L., RICCI, S., MOLA, M., MARCHI, C., SEMINARA, S., BRANDI, M. L. & DE MARTINO, M. 2015. Parathyroid Hormone Levels in Healthy Children and Adolescents. *Hormone Research in Paediatrics*, 84, 124-129.
- STEER, C. D. & TOBIAS, J. H. 2011. Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Am J Clin Nutr,* 94, 1861S-1864S.
- STEWART, T. L. & RALSTON, S. H. 2000. Role of genetic factors in the pathogenesis of osteoporosis. *J Endocrinol*, 166, 235-45.
- SUNDBERG, M., GARDSELL, P., JOHNELL, O., ORNSTEIN, E. & SERNBO, I. 1998. Comparison of quantitative ultrasound measurements in calcaneus with DXA and SXA at other skeletal sites: a population-based study on 280 children aged 11-16 years. *Osteoporos Int*, 8, 410-7.
- SURGEON-REPORT 2004. Bone Health and Osteoporosis: A Report of the Surgeon General. *Office of the Surgeon General (US)*.
- SZULC, P. & DELMAS, P. D. 2008. Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. *Osteoporos Int*, 19, 1683-704.
- TAMAKI, J., IKI, M., FUJITA, Y., KOUDA, K., YURA, A., KADOWAKI, E., SATO, Y., MOON, J. S., TOMIOKA, K., OKAMOTO, N. & KURUMATANI, N. 2011. Impact of smoking on bone mineral density and bone metabolism in elderly men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. *Osteoporos Int*, 22, 133-41.
- TAN, V. P., MACDONALD, H. M., KIM, S., NETTLEFOLD, L., GABEL, L., ASHE, M. C. & MCKAY, H. A. 2014. Influence of Physical Activity on Bone Strength in Children and Adolescents: A Systematic Review and Narrative Synthesis. *J Bone Miner Res*.
- TANNER, J. M. & WHITEHOUSE, R. H. 1976. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*, 51, 170-9.
- TENFORDE, A. S. & FREDERICSON, M. 2011. Influence of sports participation on bone health in the young athlete: a review of the literature. *PM R*, 3, 861-7.
- TENFORDE, A. S., SAINANI, K. L., CARTER SAYRES, L., MILGROM, C. & FREDERICSON, M. 2015. Participation in ball sports may represent a prehabilitation strategy to prevent future stress fractures and promote bone health in young athletes. *PM R*, 7, 222-5.
- TERVO, T., NORDSTROM, P. & NORDSTROM, A. 2010. Effects of badminton and ice hockey on bone mass in young males: a 12-year follow-up. *Bone*, 47, 666-72.
- THEINTZ, G., BUCHS, B., RIZZOLI, R., SLOSMAN, D., CLAVIEN, H., SIZONENKO, P. C. & BONJOUR, J. P. 1992. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab*, 75, 1060-5.
- TOBIAS, J. H., GOULD, V., BRUNTON, L., DEERE, K., RITTWEGER, J., LIPPERTS, M. & GRIMM, B. 2014. Physical activity and bone: may the force be with you. *Frontiers in Endocrinology*, 5.

- TOBIAS, J. H., STEER, C. D., MATTOCKS, C. G., RIDDOCH, C. & NESS, A. R. 2007. Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: Findings from a large population-based cohort. *Journal of Bone and Mineral Research*, 22, 101-109.
- TOURNIS, S., MICHOPOULOU, E., FATOUROS, I. G., PASPATI, I., MICHALOPOULOU, M., RAPTOU, P., LEONTSINI, D., AVLONITI, A., KREKOUKIA, M., ZOUVELOU, V., GALANOS, A., AGGELOUSIS, N., KAMBAS, A., DOUROUDOS, I., LYRITIS, G. P., TAXILDARIS, K. & PAPPAIOANNOU, N. 2010. Effect of Rhythmic Gymnastics on Volumetric Bone Mineral Density and Bone Geometry in Premenarcheal Female Athletes and Controls. *Journal of Clinical Endocrinology & Metabolism*, 95, 2755-2762.
- TURNER, C. H. 1998. Three rules for bone adaptation to mechanical stimuli. *Bone*, 23, 399-407. U.S. DEPARTMENT OF HEALTH 2008. Physical activity guidelines for Americans. *Okla Nurse*, 53, 25.
- UBAGO-GUISADO, E., GOMEZ-CABELLO, A., SANCHEZ-SANCHEZ, J., GARCIA-UNANUE, J. & GALLARDO, L. 2015a. Influence of different sports on bone mass in growing girls. *J Sports Sci*, 33, 1710-8.
- UBAGO-GUISADO, E., MATA, E., SÁNCHEZ-SÁNCHEZ, J., PLAZA-CARMONA, M., MARTÍN-GARCÍA, M. & GALLARDO, L. 2015b. Influence of different sports on fat mass and lean mass in growing girls. *Journal of Sport and Health Science*.
- UBAGO-GUISADO, E., VLACHOPOULOS, D., FERREIRA DE MORAES, A. C., TORRES-COSTOSO, A.,
 WILKINSON, K., METCALF, B., SANCHEZ-SANCHEZ, J., GALLARDO, L. & GRACIA-MARCO,
 L. 2017. Lean mass explains the association between muscular fitness and bone outcomes in 13-year-old boys. *Acta Paediatr*, 106, 1658-1665.
- VAITKEVICIUTE, D., LATT, E., MAESTU, J., JURIMAE, T., SAAR, M., PURGE, P., MAASALU, K. & JURIMAE, J. 2016. Longitudinal associations between bone and adipose tissue biochemical markers with bone mineralization in boys during puberty. *Bmc Pediatrics*, 16
- VALIMAKI, M. J., KARKKAINEN, M., LAMBERG-ALLARDT, C., LAITINEN, K., ALHAVA, E., HEIKKINEN, J., IMPIVAARA, O., MAKELA, P., PALMGREN, J., SEPPANEN, R. & ET AL. 1994. Exercise, smoking, and calcium intake during adolescence and early adulthood as determinants of peak bone mass. Cardiovascular Risk in Young Finns Study Group. *BMJ*, 309, 230-5.
- VALTUENA, J., GRACIA-MARCO, L., HUYBRECHTS, I., BREIDENASSEL, C., FERRARI, M., GOTTRAND, F., DALLONGEVILLE, J., SIOEN, I., GUTIERREZ, A., KERSTING, M., KAFATOS, A., MANIOS, Y., WIDHALM, K., MORENO, L. A., GONZALEZ-GROSS, M. & HELENA STUDY, G. 2013. Cardiorespiratory fitness in males, and upper limbs muscular strength in females, are positively related with 25-hydroxyvitamin D plasma concentrations in European adolescents: the HELENA study. *QJM*, 106, 809-21.
- VALTUENA, J., GRACIA-MARCO, L., VICENTE-RODRIGUEZ, G., GONZALEZ-GROSS, M., HUYBRECHTS, I., REY-LOPEZ, J. P., MOURATIDOU, T., SIOEN, I., MESANA, M. I., MARTINEZ, A. E., WIDHALM, K., MORENO, L. A. & GROUP, H. S. 2012. Vitamin D status and physical activity interact to improve bone mass in adolescents. The HELENA Study. *Osteoporos Int*, 23, 2227-37.
- VAN LANGENDONCK, L., LEFEVRE, J., CLAESSENS, A. L., THOMIS, M., PHILIPPAERTS, R., DELVAUX, K., LYSENS, R., RENSON, R., VANREUSEL, B., VANDEN EYNDE, B., DEQUEKER, J. & BEUNEN, G. 2003. Influence of participation in high-impact sports during adolescence and adulthood on bone mineral density in middle-aged men: a 27-year follow-up study. *Am J Epidemiol*, 158, 525-33.
- VANDERSCHUEREN, D., VANDENPUT, L. & BOONEN, S. 2005. Reversing sex steroid deficiency and optimizing skeletal development in the adolescent with gonadal failure. *Endocr Dev*, 8, 150-65.
- VASIKARAN, S., COOPER, C., EASTELL, R., GRIESMACHER, A., MORRIS, H. A., TRENTI, T. & KANIS, J. A. 2011. International Osteoporosis Foundation and International Federation of

- Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. *Clin Chem Lab Med*, 49, 1271-4.
- VATANPARAST, H., BAILEY, D. A., BAXTER-JONES, A. D. & WHITING, S. J. 2007. The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *J Nutr*, 137, 2674-9.
- VICENTE-RODRIGUEZ, G. 2006. How does exercise affect bone development during growth? *Sports Med*, 36, 561-9.
- VICENTE-RODRIGUEZ, G., ARA, I., PEREZ-GOMEZ, J., DORADO, C. & CALBET, J. A. 2005. Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. *Br J Sports Med*, 39, 611-6.
- VICENTE-RODRIGUEZ, G., ARA, I., PEREZ-GOMEZ, J., SERRANO-SANCHEZ, J. A., DORADO, C. & CALBET, J. A. 2004a. High femoral bone mineral density accretion in prepubertal soccer players. *Med Sci Sports Exerc*, 36, 1789-95.
- VICENTE-RODRIGUEZ, G., DORADO, C., PEREZ-GOMEZ, J., GONZALEZ-HENRIQUEZ, J. J. & CALBET, J. A. L. 2004b. Enhanced bone mass and physical fitness in young female handball players. *Bone*, 35, 1208-1215.
- VICENTE-RODRIGUEZ, G., JIMENEZ-RAMIREZ, J., ARA, I., SERRANO-SANCHEZ, J. A., DORADO, C. & CALBET, J. A. 2003. Enhanced bone mass and physical fitness in prepubescent footballers. *Bone*, 33, 853-9.
- VICENTE-RODRIGUEZ, G., URZANQUI, A., MESANA, M. I., ORTEGA, F. B., RUIZ, J. R., EZQUERRA, J., CASAJUS, J. A., BLAY, G., BLAY, V. A., GONZALEZ-GROSS, M., MORENO, L. A. & GRP, A.-Z. S. 2008. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: a cross-sectional study. *Journal of Bone and Mineral Metabolism*, 26, 288-294.
- VLACHOPOULOS, D., BARKER, A. R., UBAGO-GUISADO, E., FATOUROS, I. G., KNAPP, K. M., WILLIAMS, C. A. & GRACIA-MARCO, L. 2017a. Longitudinal Adaptations of Bone Mass, Geometry, and Metabolism in Adolescent Male Athletes: The PRO-BONE Study. *J Bone Miner Res*, 32, 2269-2277.
- VLACHOPOULOS, D., BARKER, A. R., UBAGO-GUISADO, E., ORTEGA, F. B., KRUSTRUP, P., METCALF, B., CASTRO PINERO, J., RUIZ, J. R., KNAPP, K. M., WILLIAMS, C. A., MORENO, L. A. & GRACIA-MARCO, L. 2017b. The effect of 12-month participation in osteogenic and non-osteogenic sports on bone development in adolescent male athletes. The PRO-BONE study. *J Sci Med Sport*.
- VLACHOPOULOS, D., BARKER, A. R., WILLIAMS, C. A., KNAPP, K. M., METCALF, B. S. & GRACIA-MARCO, L. 2015. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. *BMC Public Health*, 15, 361.
- VLACHOPOULOS, D., BARKER, A. R., WILLIAMS, C. A., SA, A. R., KNAPP, K. M., METCALF, B. S., FATOUROS, I. G., MORENO, L. A. & GRACIA-MARCO, L. 2017c. The Impact of Sport Participation on Bone Mass and Geometry in Male Adolescents. *Med Sci Sports Exerc*, 49, 317-326.
- VLACHOPOULOS, D., GRACIA-MARCO, L., BARKER, A. R., HUYBRECHTS, I., MORENO, L. A. & MOURATIDOU, T. 2016. Bone Health: The independent and combined effects of calcium, vitamin D and exercise in children and adolescents. *In:* PREEDY, V. R. (ed.) *Calcium: Chemistry, Analysis, Function and Effects.* London, UK: The Royal Society of Chemistry.
- VLACHOPOULOS, D., UBAGO-GUISADO, E., BARKER, A. R., METCALF, B. S., FATOUROS, I. G., AVLONITI, A., KNAPP, K. M., MORENO, L. A., WILLIAMS, C. A. & GRACIA-MARCO, L. 2017d. Determinants of Bone Outcomes in Adolescent Athletes at Baseline: The PROBONE Study. *Med Sci Sports Exerc*, 49, 1389-1396.
- WANG, M. C., CRAWFORD, P. B., HUDES, M., VAN LOAN, M., SIEMERING, K. & BACHRACH, L. K. 2003. Diet in midpuberty and sedentary activity in prepuberty predict peak bone mass. *Am J Clin Nutr*, 77, 495-503.

- WANG, Q., NICHOLSON, P. H., TIMONEN, J., ALEN, M., MOILANEN, P., SUOMINEN, H. & CHENG, S. 2008. Monitoring bone growth using quantitative ultrasound in comparison with DXA and pQCT. *J Clin Densitom*, 11, 295-301.
- WANG, Q. & SEEMAN, E. 2008. Skeletal growth and peak bone strength. *Best Pract Res Clin Endocrinol Metab*, 22, 687-700.
- WANG, Q. J., SUOMINEN, H., NICHOLSON, P. H., ZOU, L. C., ALEN, M., KOISTINEN, A. & CHENG, S. 2005. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. *Scand J Med Sci Sports*, 15, 100-6.
- WANG, T. J., ZHANG, F., RICHARDS, J. B., KESTENBAUM, B., VAN MEURS, J. B., BERRY, D., KIEL, D. P., STREETEN, E. A., OHLSSON, C., KOLLER, D. L., PELTONEN, L., COOPER, J. D., O'REILLY, P. F., HOUSTON, D. K., GLAZER, N. L., VANDENPUT, L., PEACOCK, M., SHI, J., RIVADENEIRA, F., MCCARTHY, M. I., ANNELI, P., DE BOER, I. H., MANGINO, M., KATO, B., SMYTH, D. J., BOOTH, S. L., JACQUES, P. F., BURKE, G. L., GOODARZI, M., CHEUNG, C. L., WOLF, M., RICE, K., GOLTZMAN, D., HIDIROGLOU, N., LADOUCEUR, M., WAREHAM, N. J., HOCKING, L. J., HART, D., ARDEN, N. K., COOPER, C., MALIK, S., FRASER, W. D., HARTIKAINEN, A. L., ZHAI, G., MACDONALD, H. M., FOROUHI, N. G., LOOS, R. J., REID, D. M., HAKIM, A., DENNISON, E., LIU, Y., POWER, C., STEVENS, H. E., JAANA, L., VASAN, R. S., SORANZO, N., BOJUNGA, J., PSATY, B. M., LORENTZON, M., FOROUD, T., HARRIS, T. B., HOFMAN, A., JANSSON, J. O., CAULEY, J. A., UITTERLINDEN, A. G., GIBSON, Q., JARVELIN, M. R., KARASIK, D., SISCOVICK, D. S., ECONS, M. J., KRITCHEVSKY, S. B., FLOREZ, J. C., TODD, J. A., DUPUIS, J., HYPPONEN, E. & SPECTOR, T. D. 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*, 376, 180-8.
- WARD, K. A., ADAMS, J. E., ROBERTS, S. L., ASHBY, R. L. & MUGHAL, M. Z. 2003. The effects of physical activity on the axial and appendicular skeleton of pre-pubertal children. *Osteoporosis International*, 14, S14-S14.
- WARD, K. A., ROBERTS, S. A., ADAMS, J. E., LANHAM-NEW, S. & MUGHAL, M. Z. 2007. Calcium supplementation and weight bearing physical activity--do they have a combined effect on the bone density of pre-pubertal children? *Bone*, 41, 496-504.
- WARD, K. A., ROBERTS, S. A., ADAMS, J. E. & MUGHAL, M. Z. 2005. Bone geometry and density in the skeleton of pre-pubertal gymnasts and school children. *Bone*, 36, 1012-8.
- WARD, K. D. & KLESGES, R. C. 2001. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcif Tissue Int*, 68, 259-70.
- WARDEN, S. J. & FUCHS, R. K. 2009. Exercise and bone health: optimising bone structure during growth is key, but all is not in vain during ageing. *Br J Sports Med*, 43, 885-7.
- WARDEN, S. J., FUCHS, R. K., CASTILLO, A. B., NELSON, I. R. & TURNER, C. H. 2007. Exercise when young provides lifelong benefits to bone structure and strength. *Journal of Bone and Mineral Research*, 22, 251-259.
- WARNER, S. E., SHAW, J. M. & DALSKY, G. P. 2002. Bone mineral density of competitive male mountain and road cyclists. *Bone*, 30, 281-6.
- WARRINGTON, N. M., KEMP, J. P., TILLING, K., TOBIAS, J. H. & EVANS, D. M. 2015. Genetic variants in adult bone mineral density and fracture risk genes are associated with the rate of bone mineral density acquisition in adolescence. *Hum Mol Genet*, 24, 4158-66.
- WEAVER, C. M., GORDON, C. M., JANZ, K. F., KALKWARF, H. J., LAPPE, J. M., LEWIS, R., O'KARMA, M., WALLACE, T. C. & ZEMEL, B. S. 2016. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int*, 27, 1281-386.
- WEBBER, C. E., SALA, A. & BARR, R. D. 2009. Accounting for body size deviations when reporting bone mineral density variables in children. *Osteoporosis International*, 20, 113-121.
- WEBER, J. L., LYTLE, L., GITTELSOHN, J., CUNNINGHAM-SABO, L., HELLER, K., ANLIKER, J. A., STEVENS, J., HURLEY, J. & RING, K. 2004. Validity of self-reported dietary intake at

- school meals by American Indian children: the Pathways Study. *J Am Diet Assoc,* 104, 746-52.
- WEEKS, B. K. & BECK, B. R. 2012. Are bone and muscle changes from POWER PE, an 8-month in-school jumping intervention, maintained at three years? *PLoS One*, 7, e39133.
- WEEKS, B. K., YOUNG, C. M. & BECK, B. R. 2008. Eight months of regular in-school jumping improves indices of bone strength in adolescent boys and Girls: the POWER PE study. *J Bone Miner Res*, 23, 1002-11.
- WEY, H. E., BINKLEY, T. L., BEARE, T. M., WEY, C. L. & SPECKER, B. L. 2011. Cross-Sectional versus Longitudinal Associations of Lean and Fat Mass with pQCT Bone Outcomes in Children. *Journal of Clinical Endocrinology & Metabolism*, 96, 106-114.
- WIEBE, P. N., BLIMKIE, C. J., FARPOUR-LAMBERT, N., BRIODY, J., MARSH, D., KEMP, A., COWELL, C. & HOWMAN-GILES, R. 2008. Effects of single-leg drop-landing exercise from different heights on skeletal adaptations in prepubertal girls: a randomized controlled study. *Pediatr Exerc Sci*, 20, 211-28.
- WILKINSON, K., VLACHOPOULOS, D., KLENTROU, P., UBAGO-GUISADO, E., DE MORAES, A. C., BARKER, A. R., WILLIAMS, C. A., MORENO, L. A. & GRACIA-MARCO, L. 2017. Soft tissues, areal bone mineral density and hip geometry estimates in active young boys: the PRO-BONE study. *Eur J Appl Physiol*, 117, 833-842.
- WILLIAMS, G. R., ROBSON, H. & SHALET, S. M. 1998. Thyroid hormone actions on cartilage and bone: interactions with other hormones at the epiphyseal plate and effects on linear growth. *J Endocrinol*, 157, 391-403.
- WINZENBERG, T. & JONES, G. 2013. Vitamin D and bone health in childhood and adolescence. *Calcif Tissue Int*, 92, 140-50.
- WINZENBERG, T., POWELL, S., SHAW, K. A. & JONES, G. 2011. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. *BMJ*, 342, c7254.
- WINZENBERG, T., SHAW, K., FRYER, J. & JONES, G. 2006. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. *BMJ*, 333, 775.
- WITZKE, K. A. & SNOW, C. M. 2000. Effects of plyometric jump training on bone mass in adolescent girls. *Med Sci Sports Exerc*, 32, 1051-7.
- WOLFF, I., VAN CROONENBORG, J. J., KEMPER, H. C., KOSTENSE, P. J. & TWISK, J. W. 1999. The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre- and postmenopausal women. *Osteoporos Int*, 9, 1-12.
- WOLFF, J. 1986. The law of bone remodelling. Springer-Verlag, University of Berlin.
- WORLD HEALTH ORGANIZATION 2010. Global Recommendations on Physical Activity for Health
- Global Recommendations on Physical Activity for Health. Geneva.
- XU, L., NICHOLSON, P., WANG, Q., ALEN, M. & CHENG, S. 2009. Bone and muscle development during puberty in girls: a seven-year longitudinal study. *J Bone Miner Res*, 24, 1693-8.
- XU, Y., GUO, B., GONG, J., XU, H. & BAI, Z. Q. 2014. The correlation between calcaneus stiffness index calculated by QUS and total body BMD assessed by DXA in Chinese children and adolescents. *Journal of Bone and Mineral Metabolism*, 32, 159-166.
- YAKAR, S. & ROSEN, C. J. 2003. From mouse to man: redefining the role of insulin-like growth factor-I in the acquisition of bone mass. *Exp Biol Med (Maywood)*, 228, 245-52.
- YUNG, P. S., LAI, Y. M., TUNG, P. Y., TSUI, H. T., WONG, C. K., HUNG, V. W. & QIN, L. 2005. Effects of weight bearing and non-weight bearing exercises on bone properties using calcaneal quantitative ultrasound. *Br J Sports Med*, 39, 547-51.
- ZEMEL, B. S. 2012. Human biology at the interface of paediatrics: measuring bone mineral accretion during childhood. *Ann Hum Biol*, 39, 402-11.
- ZEMEL, B. S., KALKWARF, H. J., GILSANZ, V., LAPPE, J. M., OBERFIELD, S., SHEPHERD, J. A., FREDERICK, M. M., HUANG, X., LU, M., MAHBOUBI, S., HANGARTNER, T. & WINER, K. K. 2011. Revised reference curves for bone mineral content and areal bone mineral

- density according to age and sex for black and non-black children: results of the bone mineral density in childhood study. *J Clin Endocrinol Metab*, 96, 3160-9.
- ZEMEL, B. S., LEONARD, M. B., KELLY, A., LAPPE, J. M., GILSANZ, V., OBERFIELD, S., MAHBOUBI, S., SHEPHERD, J. A., HANGARTNER, T. N., FREDERICK, M. M., WINER, K. K. & KALKWARF, H. J. 2010. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab*, 95, 1265-73.
- ZETHRAEUS, N., BORGSTROM, F., STROM, O., KANIS, J. A. & JONSSON, B. 2007. Cost-effectiveness of the treatment and prevention of osteoporosis--a review of the literature and a reference model. *Osteoporos Int*, 18, 9-23.
- ZHENG, H. F., FORGETTA, V., HSU, Y. H., ESTRADA, K., ROSELLO-DIEZ, A., LEO, P. J., DAHIA, C. L., PARK-MIN, K. H., TOBIAS, J. H., KOOPERBERG, C., KLEINMAN, A., STYRKARSDOTTIR, U., LIU, C. T., UGGLA, C., EVANS, D. S., NIELSON, C. M., WALTER, K., PETTERSSON-KYMMER, U., MCCARTHY, S., ERIKSSON, J., KWAN, T., JHAMAI, M., TRAJANOSKA, K., MEMARI, Y., MIN, J., HUANG, J., DANECEK, P., WILMOT, B., LI, R., CHOU, W. C., MOKRY, L. E., MOAYYERI, A., CLAUSSNITZER, M., CHENG, C. H., CHEUNG, W., MEDINA-GOMEZ, C., GE, B., CHEN, S. H., CHOI, K., OEI, L., FRASER, J., KRAAIJ, R., HIBBS, M. A., GREGSON, C. L., PAQUETTE, D., HOFMAN, A., WIBOM, C., TRANAH, G. J., MARSHALL, M., GARDINER, B. B., CREMIN, K., AUER, P., HSU, L., RING, S., TUNG, J. Y., THORLEIFSSON, G., ENNEMAN, A. W., VAN SCHOOR, N. M., DE GROOT, L. C. P. G. M., VAN DER VELDE, N., MELIN, B., KEMP, J. P., CHRISTIANSEN, C., SAYERS, A., ZHOU, Y. H., CALDERARI, S., VAN ROOIJ, J., CARLSON, C., PETERS, U., BERLIVET, S., DOSTIE, J., UITTERLINDEN, A. G., WILLIAMS, S. R., FARBER, C., GRINBERG, D., LACROIX, A. Z., HAESSLER, J., CHASMAN, D. I., GIULIANINI, F., ROSE, L. M., RIDKER, P. M., EISMAN, J. A., NGUYEN, T. V., CENTER, J. R., NOGUES, X., GARCIA-GIRALT, N., LAUNER, L. L., GUDNASON, V., MELLSTROM, D., VANDENPUT, L., AMIN, N., VAN DUIJN, C. M., KARLSSON, M. K., LJUNGGREN, O., SVENSSON, O., HALLMANS, G., ROUSSEAU, F., GIROUX, S., BUSSIERE, J., ARP, P. P., et al. 2015. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature*, 526, 112-+.
- ZOUCH, M., JAFFRE, C., THOMAS, T., FRERE, D., COURTEIX, D., VICO, L. & ALEXANDRE, C. 2008. Long-term soccer practice increases bone mineral content gain in prepubescent boys. *Joint Bone Spine*, 75, 41-9.
- ZOUCH, M., VICO, L., FRERE, D., TABKA, Z. & ALEXANDRE, C. 2014. Young male soccer players exhibit additional bone mineral acquisition during the peripubertal period: 1-year longitudinal study. *Eur J Pediatr*, 173, 53-61.
- ZUO, C., HUANG, Y., BAJIS, R., SAHIH, M., LI, Y. P., DAI, K. & ZHANG, X. 2012. Osteoblastogenesis regulation signals in bone remodeling. *Osteoporosis International*, 23, 1653-1663.

12. Appendices

Appendix 1: Ethical approvals



NRES Committee South West - Cornwall & Plymouth

Level 3 Block B Whitefriars Lewins Mead Bristol BS1 2NT

Tel: 01173421390 Fax: 01173420445

13 October 2014

Dr Luis Gracia-Marco
Chief Investigator- Lecturer
University of Exeter
Baring Court
University of Exeter St Luke's Campus
Heavitree Road
EX12LU

Dear Dr Gracia-Marco

Study title: PRO-BONE: Effect of a program of short bouts of exercise

on bone health in adolescents involved in different sports

REC reference: 14/SW/0060 Protocol number: 151833 Amendment number: 1

Amendment date: 24th September 2014

IRAS project ID: 151833

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The sub-committee reviewed the following amendment;

The addition of an advertisement flyer.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Copies of advertisement materials for research participants	1	29 September 2014
Notice of Substantial Amendment (non-CTIMP)	1	

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/SW/0060: Please quote this number on all correspondence

Yours sincerely

pp.

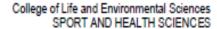
Canon Ian Ainsworth-Smith

Chair

E-mail: nrescommittee.southwest-cornwall-plymouth@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Ms Gail Seymour





St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2014/766 (AM141203-01)

<u>Title:</u> Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports

Applicants: : Dr Luis Gracia-Marco, Prof Craig Williams, Dr Alan Barker, Karen Knapp, Dimitris Vlachopoulos (PhD student), Stuart Cocksedge (UG student), Owen Tomlinson (PhD student), Ricardo Oliveira (PhD student), Emma Cockcroft (PhD student), Bert Bond (PhD student)

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until November 2017

Date: 29/10/2014

Signature:

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Melya Helbolon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.



Ethics Review Report

for Central Review

PROPOSAL PROGRAMME	PROPOSAL ID	ACRONYM
FP7-PEOPLE-2013-CIG	618496	PRO-BONE

REVIEW DATE
16 July 2013

1. Identification of the issues* raised by the project (panel's assessment)

- Healthy children/adolescents
- Human biological samples (peripheral blood samples)
- Data protection and privacy

* (e.g. the involvement	of children, patient	s, vulnerable people,	animals,	non-human	primates,	human
interventions and tissues,	data protection and	privacy, hESC,)				

2. Ethical issues

a) Were the <u>ethical</u>* aspects of the proposed research well described in relation to its objectives?

⊠ Yes	☐ No
-------	------

The objective of PRO-BONE is to compare the osteogenic (healthy bone formation and development) benefits of different forms of physical activity in male adolescents. This will provide a more comprehensive understanding of bone growth and may underpin new approaches in the prevention of osteoporosis. The objectives of the project therefore do not raise any ethical concerns.

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b) Were the <u>ethical</u> * aspects of the proposed research well described in relation to its methodology?
∑ Yes □ No
A number of cohorts of male children/adolescents will be studied. An intervention in the form of plyometric jump training (designed to replicate impact and weight-bearing physical activity) will be evaluated in one group of research participants, and their physical condition and progress measured against a 'less active' control group. Measurement of 25-dihydroxyvitamin D will be performed in blood samples from the cohorts.
In response to the ethics screening report, the applicant has provided comprehensive responses to all of the issues raised, although a number of issues still need to be addressed.
Human samples: No information is provided on whether the samples will be retained or destroyed at the end of the study period.
Consent: Written information leaflets and consent forms will be provided to potential participants and their parents. Copies of these consent forms and information sheets have been attached to the resubmission, although in certain cases, the language as presented may not be understandable for participants and their families e.g. phraseology such as "The percentage of whole body fat mass will be calculated using the BodPod", "Cardiorespiratory fitness, lower leg muscle strength jump height will be measured using standard tests" should be modified.
Indemnity insurance: While evidence of indemnity insurance is provided for the project, it does not cover the entire timeline of the project (Annex 2).
Incidental findings: A policy on incidental findings has not been provided.
Data Protection and Privacy: Data management and security procedures are outlined. The University of Exeter does not have a formal approval process for "the technical data protection procedures" but the applicant indicates that the Ethical Approval and application of the Data Protection Act and related University policies "will help to identify problems". Nevertheless, approvals on the secondary use of data must be provided. No information is provided on whether data will be retained or destroyed at the end of the study period.
c) Were the <u>ethical</u> * aspects of the proposed research well described in relation to the possible implications of its results?
∑ Yes □ No
The benefits associated with this research project include early detection of low bone mineral density and the potential for improved bone mineralization development during growth via selected forms of physical activity. As osteoporosis has now been recognized as a disease that is dramatically increasing in both developed countries and emerging economies, this project may have a strong societal impact.

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d) Do the applicants clearly indicate how the proposal meets the national legal and ethical requirements of the country where the research will be performed?
∑ Yes □ No
Approval from the host institution's research ethics committee is to be sought subsequent to the European Commission's approval of the proposal. However, the national/European ethical/legal frameworks pertaining to the research to be conducted are not presented in sufficient detail.
e) Does the applicant include a timeframe for approval of the proposed study by a relevant authority at national level (local ethics committee and/or competent national authority?)
☐ Yes ⊠ No
The applicant indicates that ethical approvals will be obtained and submitted prior to the commencement of the research, but no precise timeframe has been given.
3. Overall Assessment
a) The proposal adequately identifies and addresses the relevant ethical issues. Specific requirements, if any, are provided in the 'Requirements' section
b) The proposal addresses the ethical issues only in general terms but there are aspects which require substantial clarification. These are highlighted in the 'Requirements' section
c) The proposal fails to identify and to address the relevant ethical issues. A supplementary Ethics Review is recommended.
4. Requirements (Requirements become contractual obligations)
In implementing the following requirements, the analysis and comments made in the sections above must be taken into account. They are considered as an integral part of the requirements.

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- Copies of ethical approvals by the competent local/national Ethics Committees must be submitted to the European Commission prior to the commencement of the research. These must include ethics approval for the secondary use of data.
- All the information forms and consent forms must be written in terms that are understandable to the participants. These forms must indicate if destruction or retention of data/human material collected is proposed following the completion of the study.
- The insurance certificate must be extended to cover the whole study period. A copy of the renewed certificate must be forwarded to the European Commission when available.
- A policy must be set up to handle any incidental findings. A copy of the policy must be provided to the European Commission.

The progress of compliance with the Requirements should be described in the periodic/final Reports under the Section 3.2.2 ('Work progress and achievements during the period' - Guidance Notes on Project Reporting)

5. Would you recommend an Ethics Audit?

(The Ethics Review Sector of DG RTD will undertake an Ethics Audit of selected project(s) in order to ensure compliance with the contractual requirements detailed above)

Yes	No
-----	----

6. Recommendations

(Recommendations are suggestions and advice provided to the applicants. Recommendations do not become contractual obligations)

- Full details of what is entailed in the biological and physiological tests to be conducted
 and samples taken should be provided in the oral presentation during the recruitment
 phase as best practice.
- Given the involvement of children/adolescents, it is recommended that an Ethics Advisor be involved, particularly in the recruitment phase of the project.
- During the life-time of this project the revised Directive 95/46/EC on Data Protection and Privacy may come into force, and the applicant(s) may need to take this into account to ensure continuing compliance.

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Appendix 2: Recruitment forms

A Research Study About: Body Composition In 12-14 Year Old Males.



Researchers at Children's Health and Exercise
Research Centre of University of Exeter are
interested in investigating how your body composition
(especially your bones) influenced by the activities you
practice.

The PRO-BONE study is funded by the **European Commission** and has ethical approval from **NHS**.

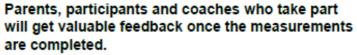
Would the study be a good fit for you if:

- You <u>DO NOT</u> swim, cycle or play football for 3 or more hours per week in the last 3 years.
- You will NOT participate in these sports more than 3 hours/week for the next 3 years.



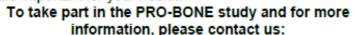
What would happen if you take part in the study?

- You will have to visit our Research Centre only 5 times over the next 3 years.
- We will measure: your body composition (with some completely safe devices shown in the pictures), your physical fitness, your physical activity and we will collect a small amount of blood (only finger drops).



There may be possible benefits if you take part in the study.

 You will obtain results of your body composition that are important for your health.



Email: dv231@exeter.ac.uk Phone number: 07583326273.







CHILDREN'S HEALTH AND EXERCISE RESEARCH CENTRE

SPORT AND HEALTH SCIENCES

College of Life and Environmental Sciences St. Luke's Campus University of Exeter Heavitree Road Exeter EX1 2LU United Kingdom

Telephone +44 (0)1392 724721 Fax +44 (0)1392 724726 Email dv231@exeter.ac.uk

Web: sshs.exeter.ac.uk/research/centres/cherc

Version 3. Date: 01/05/2014

Parent/Guardian Information Sheet

Project Title: Exercise and bone health in adolescents engaged in different sports (PRO-BONE)

We are asking your child if he would like to join in a research project to help answer two questions:

- 1- How your child's bone mass is influenced by the sport he practises?
- 2- How complementary jump training might influence his bones?

Before you decide if you want your child to be involved in this study, it is important to understand why the research is being done and what it will involve. Therefore, please read this information sheet carefully.

1. WHY ARE WE DOING THIS RESEARCH?

We know that some sports can contribute to bone development more than others. This study has two different aims:

- To understand the differences in bone mass of boys playing different sports such as football, cycling and swimming and to compare their values with those from boys not playing these sports regularly.
- To find out if a jump training programme can improve your child's bone strength.

2. WHY HAVE WE INVITED YOUR CHILD TO TAKE PART?

Your child has been invited to join our study because he is regularly engaged in one of these sports: **football, cycling or swimming** (more than 3 hours per week (on average) in the last 3 years). Studies of similar aged boys have been

performed worldwide. This project will involve a total of 105 boys from Exeter and the surrounding area.

3. DOES YOUR CHILD HAVE TO TAKE PART?

No. It is up to you. If he agrees to take part we will ask him to sign an assent form (you will need to sign it too). We will give you a copy of this information sheet, your child's information sheet and the assent form to keep. In addition, you will have to sign the "Parent/Guardian Consent Form". Your child will be free to stop taking part at any time during the research without giving a reason and without any disadvantage to himself.

4. WHAT WILL HAPPEN TO MY CHILD IF HE TAKES PART?

The study will last for 3 years, during which time your child will be asked to attend the University on 5 separate occasions:

1st visit in Autumn-Winter 2014 2nd visit in Autumn-Winter 2015 3rd visit in Summer-Autumn 2016 4th visit in Winter-Spring 2016-2017 5th visit in Summer-Autumn 2017

Each of these visits will last approximately 3-4 hours. In each of these visits we will measure:

Bone mass

We will use a scanner to measure your child's bone health using low dose x-rays. This machine is called "DXA" (see the picture below) and uses a minimal amount of radiation to create a picture of your child's bones, muscle and fat. This amount of radiation is very small and similar to that which your child will receive after a few hours in the sunshine on a hot summer's day. This device is used with adolescents worldwide. To obtain the measurement, your child will need to stay quiet and still on the scanner bed for a maximum of 15 minutes.



Photo 1: DXA

We will use a small device called "ULTRASONOMETER" (see the picture below) to obtain additional information about the strength of your child's heel.

This measurement uses ultrasound, so no radiation is involved. To obtain this measure, you child will have to place his foot in the device and keep it still for 1 minute. This is a non-invasive and pain-free method to measure bone strength.



Photo 2. Ultrasonometer

Body fat

We will use a device called "BodPod" which is shown in the figure below and looks like a small spacecraft. The only thing you have to do is to enter into the device and stay as quiet as you can for around 30 seconds. Your child will wear swim wear and hat. This is a non-invasive method.



Photo 3. BodPod

Body size measurements (anthropometry)

We will measure your child's height, weight, seated height and hip and waist circumference using standard scales and tapes.

Physical fitness:

We will evaluate your child's physical fitness using standard tests that are routinely used for adolescents worldwide. This will include a running test (endurance), a strength test (lower leg muscle strength) and a jumping (jump height) test. All of these tests have been previously used in adolescents without any issue. Physical fitness has been considered a powerful marker of health.

Blood tests:

We will obtain a small amount of blood from your child's finger using a very small lancet. This feels like a small 'pin prick' and is routinely used in research on children and well tolerated. This blood sample will be used to measure the vitamin D status of your child along with bone markers that help form and breakdown bone tissue. It is important that your child does not have any food or drink (except water) from midnight.

Tanner stage:

We will show your child five pictures of physical maturation and he will be asked to choose the one that best represents his current physical development. He will self-assess his physical development in privacy. This is important to know as different physical maturation is associated with different bone levels. It is a routine measure in studies involving children and adolescents.

Diet:

Your child will be asked to give us some information about his diet using a simple questionnaire each day he visits the University.

Physical activity:

We will ask your child to wear a small device on his wrist (it looks like a watch) from the time he wakes up until bedtime for a period of 7 days (see photo below). We will also collect some information about his physical activity patterns using a questionnaire.



5. WHAT ELSE WILL MY CHILD BE ASKED TO DO?

Your child may be asked to perform some jumps per week over a period of 9 months (it will take less than 10 minutes per day and he will not have to do it every day). However, this decision will depend on the group we allocate him. If he is finally in this group, we will explain to him how to do it. There is nothing to worry about, it is very easy and it is not time consuming. By contrast, if your child is not in this group, the only thing he has to do is to continue with his regular training over the 3 years. If your child misses a few sessions this will not be a problem as we will register attendance to the trainings and number of jumps performed, so please do not be concerned about this.

6. WHAT ARE THE POSSIBLE RISKS AND DISADVANTAGES OF TAKING PART?

There are minimal risks of taking part in this study. If we ask your child to do the jumps, then he may feel a bit stiff in his legs. This may also happen after the visit to the University due to the physical fitness tests. However, this is something completely normal and your child may have already had this sensation after doing some sport. We will show him some stretching exercises to remove this sensation. In addition, if we ask your child to do the jumps, it is possible he may twist an ankle, but this is very unlikely and the risk will be even lower if he does it on a hard surface. In fact, no injuries have been reported in previous studies performing similar jumping interventions. However, if your son gets injured for any length of time, you should notify us and we will meet with you to discuss his continued participation in the study.

The study requires a strong commitment from participants as it involves regular attendance to their training sessions over 3 years and it may include (depending on the group we allocate your child) performing jumping exercises over 9 months.

7. WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

You will obtain important information about your child's bones through his adolescence, which is very important and difficult to obtain. We cannot promise the study will help your child but the information we get may help other young people in the future.

8. WHO IS FUNDING THE RESEARCH?

This study has been funded by the European Commission – Marie Curie Career Integration Grants.

9. WHO HAS REVIEWED THE STUDY?

Before any research goes ahead it has to be checked by a Research Ethics Committee. This project has been checked and approved by the Cornwall and Plymouth Research Ethics Committee and the Sport and Health Sciences Ethics Committee (University of Exeter).

Thank you for reading this letter! If you have any questions let us know.



PARENT/GUARDIAN CONSENT FORM

Title of Project: Exercise and bone health in adolescents engaged in different sports

Name of Researchers: Dr. Luis Gracia-Marco, Prof Craig A. Williams, Dr. Alan Barker, Dr. Brad Metcalf, Dr. Karen Knapp, Prof Adrian Taylor, Mr. Dimitris Vlachopoulos

				Please initial bo
1	I have read and understa above study and have ha		neet Version 3, Dated: 01/05/2014 task questions.	for the
2	My child's participation in the project is entirely voluntary (no incentives will be given) and my child is free to withdraw from the project at any time without giving reason or affecting his relationship with the researchers.			
3	I am aware that all data will be stored on computer in coded form and individual results will be confidential to the Children's Health and Exercise Research team. In addition, results and human material collected (i.e. blood samples) will be kept following completion of the study for 5 years and will be used for future publications and research issues.			
4	The results of the propreserved.	ject may be publisl	ned but my child's anonymity w	rill be
5	I give consent for my ch	ild to take part in the	above study.	
Nan	ne of participant	Date	Signature	
Nan	ne of parent/guardian	Date	Signature	
Rese	earcher	Date	Signature	



ASSENT FORM FOR PARTICIPANTS (12-14 years old)

(To be completed by the participant and their parent/guardian)

Date:	01/05/2014
	Version 2.

PRO-BONE Project: Exercise and bone health in adolescents engaged in

<u>different sports</u>	
Adolescent (or if unable, parent on his behalf) to circle all he agrees with:	
Have you read about this project using the Participant Information Sheet*	Yes /
No	
*Participant Information Sheet (Version 3. Date: 01/05/2014)	
Has the research team explained this project to you?	Yes / No
Do you understand what this project is about?	Yes /
No	
Have you asked all the questions you want?	Yes / No
Have you had your questions answered in a way you understand?	Yes /
No	
Do you understand that it is okay to stop taking part at any time?	Yes /
No	
Are you happy to take part?	Yes /
No	

Your name
Sign
Date
Your parent or guardian must write their name here too if they are happy for you to take part in
the project
Print Name
Sign
Date
The person from the research team who explained this project to you needs to sign too:
Print Name
Sign
Date

If you do want to take part, please write your name and today's date in the space below.

Thank you for considering this project!

Please send us by post or email this form and the interest form before your first visit.

Appendix 3: Measurement forms

Maturation Assessment Form

Name
The assessment of your state of puberty is essential for the correct interpretation of your health and exercise performance. All children do not start puberty at the same time and some will progress through puberty more quickly than others. Consequently, two children aged 13 year olds may be the same age, however it is likely that one will be more mature than the other. We therefore require an estimate of your maturation in order to control for the different stages of pubertal development on the outcomes that we will measure. If you have any questions about this assessment, please contact a member of the research team.
Instructions:
On the next page are five drawings showing the changes in pubic hair as a child develops into and adult. Please take your time and read carefully the writing explaining each of the five different stages in pubic hair development. Once you have decided the stage which best describes your present level, please indicate this below by circling the relevant number. Once you have finished, please sign and date this form at the bottom and return it to a member of the research team on your next visit.
ALL INFORMATION WILL BE HANDLED WITH THE STRICTEST OF CONFIDENCE AND ONLY VIEWED BY THE RESEARCH TEAM
Stage: 1 2 3 4 5
Participant signature
Date
PLEASE WEAR THE ACCELEROMETER ON YOUR <u>LEFT WRIST FOR 7</u> <u>DAYS</u>
<u>IF YOU REMOVE THE ACCELEROMETER AT ANY TIME PLEASE</u> COMPLETE THE TABLE BELOW:

PLEASE DO NOT REMOVE IT AT ALL AND SEND IT BACK TO US INCLUDING THIS FORM IN THE PREPAID ENVELOP AND WRITE YOUR

MANY THANKS! ©

ADDRESS ON THE BACK.

THE DRAWINGS ON THIS PAGE SHOW DIFFERENT AMOUNTS OF MALE PUBIC HAIR. A BOY PASSES THROUGH EACH OF THE FIVE STAGES SHOWN BY THESE DRAWINGS. PLEASE LOOK AT EACH DRAWING AND READ THE SENTENCES UNDER THE DRAWING. THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF YOUR BY DEVELOPMENT, MARK A I ON THE LINE ABOVE THAT DRAWING. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST TO YOUR STAGE OF HAIR DEVELOPMENT AND MARK IT A 2, IN CHOOSING THE RIGHT PICTURE, LOOK ONLY AT THE PUBIC HAIR. AND NOT AT THE SIZE OF THE TESTES, SCROTUM, AND PENTS.

S. DRAWING E 4. DRAWING D 3. DRAWING C 2. DRAWING B 1. DRAWING A

THERE IS NO PUBIC HAIR AT ALL,

THERE IS A LITTLE SOFT, LONG, LIGHTLY COLORED HAIR, MOST OF THE MAIR IS AT THE BASE OF THE PENIS. THIS HAIR MAY BE STRAIGHT OR A LITTLE CURLY.

OF AN ADULT MALE. HOW-EVER, THE AREA THAT THE HAIR COVERS IS NOT AS LARGE AS THAT OF AN ADULT MALE, THE HAIR HAS NOT MORE CURLED. IT HAS SPREAD OUT AND THINLY COVERS A SOMEWHAT LARGER AREA. THE HAIR IS DARKER IN THIS STAGE, IT IS COARSER AND

THE HAIR HAS SPREAD OUT TO THE THIGHS. THE HAIR IS NOW LIKE THAT OF AN ADULT MALE. IT COVERS THE SAME AREA AS THAT OF AN ADULT MALE.

THE HAIR IS NOW AS INARK, CURLY, AND COARSE AS THAT

SPREAD OUT TO THE THIGHS

4

Children's Health and Exercise Research Centre (PRO-BONE Study)

	Anthrop	Anthropometry													
<i>©</i>	1 st <u>Measurement</u>	2 nd Measurement	3 rd <u>Measurement</u>												
<u>Seated Height</u> (centimetres)															
Height															
(centimetres)															
Weight															
(kilograms)	(SECA scale)	(BIA)	(BODPOD)												
<u>Waist</u>															
<u>Circumference</u>															
(centimetres)															
<u>Hip</u>															
<u>Circumference</u>															
(centimetres)															

Body Composition Measurements												
	Body (Compositio	n Meas	uremen	ts							
DXA (TB-FN-	BIA		Achille	es Insia	ht Ultra	sonome	eter					
<u>LS)</u>	Fat%:		Achilles Insight Ultrasonometer									
YES / NO	1 41701											
<u>BOD</u>	©		ness lex	В	JA	sos						
Body Volume (mean):		Attempts	Right	Left	Right	Left	Right	Left				
Fat%:	Fat weight:	1 st										
Lean Weight:	Lung Volume:	2 nd										

	Fitness A	ssessme	ent								
<i>©</i>	1 st Atte	empt	2 nd Attempt	3 ^r Attempt							
Jump Height (2 nd value in the											
<u>screen)</u>											
(centimetres)											
Standing Long Jump Test											
<i>©</i>	1 st Atte	empt	2 nd Attempt	3 rd Attempt							
<u>Standing long jump</u> (centimetres)											
20 meter Shuttle Run Tes	it .	Participant obtained:									
		Matura	tion Assessment Fo	rm: Yes / No							
Number of Shuttles:		Accele	rometer:	Yes / No							
		Questi	onnaires (PA+Diet)	Yes / No							

•	•	idential. It will be used only for should accompany this sheet.
DXA scan file code:		D: Date of
Section 1. IRMER (2000) Reg	gulations	
Practitioner: Justification for scan: Definition of benefit:	O	ose constraint set: perator: haperone:
Section 2. Background Scan I	Information	
Body mass (kg):	Stature (cm):	Gender: M / F
Pregnancy assessment: / NO		Breast feeding: YES
Medical conditions:		
Section 3. Personal details (Addentification)	sk participants for the	ese details verbally to ensure
Name: Address:	Age: GP details:	Date of birth:
Section 4. Scan Radiation Rep	port	
Scans aborted and compl	leted Radiat	ion dose (μGy)
	'	
Total radiation dose:	(μGy)	



MY JUMP DIARY

PARTICIPANT ID:

> Jump training progression plan

Levels	Exercise	Vest weights (kg)	Repetitions	Sets / day (¹ Rest)	Trainings / week	Jumps / week
1-3 months	Jumps (CMJ or SJ)	-	20	3	3	180
Total	level 1 (12 weeks)				180 x 12 =	2160
3-6 months	Jumps (CMJ or SJ)	2	20	4	3	240
Total l	level 2 (12 weeks)				240 x 12 =	2880
6-9 months	Jumps (CMJ or SJ)	5	20	4	4	320
Total l	level 3 (12 weeks)				320 x 12 =	3840
Total intervention (36 weeks)						8880

¹Rest between sets = 30 seconds

PARTICIPANT ID:

"If you are unable to complete one of these jumping sessions do not worry about it, it is not a problem, just carry on with your plan".

> 1-3 months

This is your diary for the first 12 weeks. You can either do the "countermovement jumps, CMJ" or the "squat jumps, SJ" as we have explained to you. It is very easy!

As you can see in the diary you have to do the jumps 3 times per day and 3 days per week.

Have a look to this example:

3 TIMES PER WEEK (any days you wish)

1) In the morning: 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)

2) After school: 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)

3) **Before going to bed:** 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)

	DAY 1]	DAY 2	2	DAY 3				
SET	1	2	3	1	2	3	1	2	3		
WEEK 1											
WEEK 2											
WEEK 3											
WEEK 4											
WEEK 5											
WEEK 6											
WEEK 7											
WEEK 8											
WEEK 9											
WEEK 10											
WEEK 11											
WEEK 12											

Please tick \(\square\) the

specific cell after completing each set of repetitions.

GREAT EFFORT! KEEP UP THE GOOD WORK!

PARTICIPANT ID:

"If you are unable to complete one of these jumping sessions do not worry about it, it is not a problem, just carry on with your plan".

> 3-6 months

This is your diary for weeks 13 to 24. You can either do the "countermovement jumps, CMJ" or the "squat jumps, SJ". **NOW** you have to use the **2 kg at the weight vest** as we have explained to you. It is very easy!

As you can see in the diary **NOW** you have to do the jumps 4 times per day and 3 days per week.

Have a look to this example:

3 TIMES PER WEEK (any days you wish) + 2 kg at the weight vest

- 1) In the morning: 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)
- 2) (2+3) After school: 10 jumps (CMJ) + 30 seconds rest + 10 (CMJ) + 30 seconds rest + 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)
- 3) (4) Before going to bed: 10 jumps + 1 minute rest + 10 jumps

	DAY 1				DAY 2				DAY 3			
SET	1	2	3	4	1	2	3	4	1	2	3	4
WEEK 13												
WEEK 14												
WEEK 15												
WEEK 16												
WEEK 17												
WEEK 18												
WEEK 19												
WEEK 20												
WEEK 21												
WEEK 22												
WEEK 23												
WEEK 24	,											

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the specific cell after completing each set of repetitions

EXCELLENT JOB SO FAR!! YOU HAVE DONE REALLY GOOD WORK!!!

PARTICIPANT ID:

"If you are unable to complete one of these jumping sessions do not worry about it, it is not a problem, just carry on with your plan".

> 6-9 months

This is your diary for weeks 25 to 36. You can either do the "countermovement jumps, CMJ" or the "squat jumps, SJ". **NOW** you have to use the **5 kg at the weight vest** as we have explained to you. It is very easy!

As you can see in the diary **NOW** you have to do the jumps 4 times per day and 4 days per week.

Have a look to this example:

4 TIMES PER WEEK (any days you wish) + 5 kg at the weight vest

- 1) In the morning: 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)
- 2) (2+3) After school: 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)
- 3) **Before going to bed:** 10 jumps (CMJ) + 30 seconds rest + 10 jumps (CMJ)

	DAY 1				DAY 2			DAY 3				DAY 4				
SET	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
WEEK 25																
WEEK 26																
WEEK 27																
WEEK 28																
WEEK 29																
WEEK 30																
WEEK 31																
WEEK 32																
WEEK 33																
WEEK 34																
WEEK 35																
WEEK 36																

Please tick
the specific cell after completing each set of repetitions.

YOU HAVE NOW FINISHED, CONGRATULATIONS!!!

CHAPTER 32

Bone Health: The Independent and Combined Effects of Calcium, Vitamin D and Exercise in Children and Adolescents

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32.1 Introduction

Bone-mass acquisition during growth is a key determinant of osteoporosis risk later in life (Marshall *et al.*, 1996). During the last two decades, increasing evidence suggests that young population groups fail to attain adequate

Food and Nutritional Components in Focus No. 10 Calcium: Chemistry, Analysis, Function and Effects Edited by Victor R. Preedy © The Royal Society of Chemistry 2016 Published by the Royal Society of Chemistry, www.rsc.org bone mass; this is alarming given the raised concerns that inadequate bone mass may result in fragile bones and increased fracture risk both in adolescence and in adulthood (Rizzoli *et al.*, 2010). Childhood and adolescence are considered crucial periods of bone-mass acquisition, with up to 50% of total body (TB) bone mass achieved during these life stages (Perez-Lopez *et al.*, 2010). Peak bone mass (PBM) attainment typically occurs between the second and third decade of life, with 80–90% acquired by late adolescence (second decade of life), although this is skeletal site dependent (Baxter-Jones *et al.*, 2011). While up to 80% of bone mass is determined by genetics (Davies *et al.*, 2005), lifestyle factors, such as diet (*i.e.* calcium, vitamin D) and physical activity (PA) have been shown to contribute to skeletal development.

Dietary calcium is an essential component of bone formation in young people as it influences the development of the skeleton during growth. For this reason, adequate calcium levels are necessary for the optimal attainment of PBM (Huncharek *et al.*, 2008). Increasing dietary calcium to optimal levels through consumption of dairy products (fortified or not) may increase bone mass during growth, but the magnitude and the maintenance of the gain in bone mass remain controversial (Huybrechts *et al.*, 2011). Another substantial nutrient in bone mineralization is vitamin D, which plays an important role in calcium absorption from the skeleton and in the attainment of PBM. In addition, vitamin D deficiency may be linked to rickets in children (Pettifor and Prentice, 2011). Therefore, it is important to fully understand the impact of vitamin D on bone development and its association with enhanced bone mass in children and adolescents.

PA contributes to the development of bone mass in young people due to its association with increases in lean mass as explained by the mechanostat theory "bigger muscles exert higher tensile forces on the bones they attach" (Rauch et al., 2004). The importance of PA during childhood is dependent on the ability of the skeleton to adapt to mechanical loading after exercise and appears to elicit the greatest bone-accrual response in the growing skeleton (Bielemann et al., 2013). The intensity, frequency and the type of PA are important factors to consider in order to understand the impact of PA on the paediatric skeleton; due to the ground reaction forces applied at different sites (Vicente-Rodriguez, 2006). In addition, specific PA levels associated with optimal bone health have been published (Gracia-Marco et al., 2011).

Calcium, vitamin D and PA have been shown to independently influence bone-mineral accrual in young people. However, there is evidence suggesting that exercise interacts with calcium intake (Courteix *et al.*, 2005) and vitamin D levels (Valtuena *et al.*, 2012) to improve bone status. Understanding these interactions during childhood and adolescence is a public health priority. Therefore, this chapter will discuss the independent and interactive effects of calcium, vitamin D and exercise on bone status in children and adolescents (Figure 32.1).

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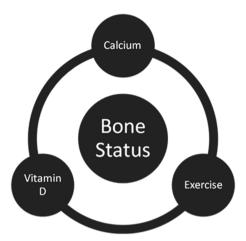


Figure 32.1 Combined effects of calcium, vitamin D and exercise on bone status in children and adolescents. The association of the nutritional factors (calcium and vitamin D) and exercise in bone development.

32.2 Bone Mass Increase during Childhood and Adolescence

Childhood and adolescence is characterized by profound changes in the morphology of the skeleton and bone development determined through the dynamic process of bone formation and resorption. Skeletal growth depends, among other factors, on bone remodeling from osteoblast (formation) and osteoclast (resorption) cells where the ratio of the activity of these cells determines the rate of bone mineral accrual. During the period of bone growth the activity of osteoblasts is higher than the activity of osteoclasts until the time of PBM (Deng *et al.*, 2008). Several other factors are shown to contribute to differences in bone status in children and adolescents such as genetics, sexual maturation, body composition, nutrition and PA.

Pubertal status is associated with bone growth both in size and density, and evidence shows that boys and girls acquire bone mass at similar rates before puberty, whereas after puberty males tend to acquire a higher bone mass than females (Davies *et al.*, 2005). Findings from a longitudinal study monitoring bone mass suggest that the highest rate of bone-mass accrual in females and males occurs at 12–15 years and 14–16 years, respectively. In the same study, bone mass increase rate decreased by the age of 16–18 years in girls and 17–20 years in boys (Bailey *et al.*, 1999). During the years of puberty, girls acquired ~40% of their PBM and ~90% by the age of 18 (Theintz *et al.*, 1992). Maturation onset is another important component of bone-mass acquisition due to the relationship between the pubertal timing and the PBM accrual. The Saskatchewan Paediatric Bone Mineral Accrual Study reported that PBM was achieved 6 months later than peak height

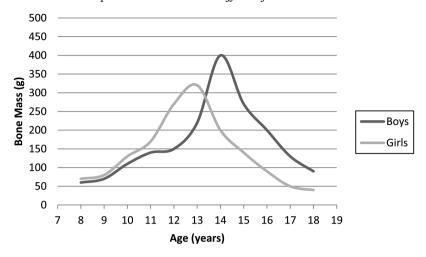


Figure 32.2 Increases in bone mass during childhood and adolescence. Bone mass increases by chronological age are shown for males and females from childhood to adulthood. Peak bone mass increase values achieved ~2 years earlier for girls compared with boys. Adapted from: (Bailey *et al.*, 1999).

velocity (PHV), where boys and girls obtained 90% of total height at PHV but only 57% of total BMC (Bailey *et al.*, 1999). The inverse association of PHV and PBM accrual showed that children who reach PHV earlier tend to have higher BMC than those with a late PHV. In addition, children with a higher stature have greater BMC and BMD z-scores than shorter peers (Zemel, 2013) (Figure 32.2).

32.3 Calcium, Vitamin D and Bone Status in Children and Adolescents

The development of the human skeleton requires an adequate supply of many nutrients, including calcium. Ninety-nine per cent of calcium is concentrated in bone and explains 1–5% of the variance in childhood bone mass and later in PBM attainment (Levine, 2012). Adequate calcium levels are defined as 800 mg per day for children aged 4–8 years old and 1300 mg per day for adolescents (Baker *et al.*, 1999). Adequate calcium levels are crucial during the pubertal growth spurt due to their relationship with peak bone acquisition. Peak calcium accretion occurs during puberty at ~12.5 years in females and ~14 years in males (Bailey *et al.*, 2000).

Increasing dietary calcium to optimal levels through diet rich in calcium and dairy products promotes bone growth in the skeleton. A double-blind placebo-controlled trial in 149 girls aged 7.9 years has shown that 850 mg per day of calcium coming from fortified foods, taken for one year, significantly increased BMD at the femoral and radial sites by 7–12 mg cm⁻² per year and

by 2 mg cm⁻² per year in the lumbar spine (LS) compared to a placebo group. Furthermore, a 7 year longitudinal study in adolescent girls showed that calcium supplementation and milk products were correlated with greater BMC on the hip and forearm sites. However, only dairy products were associated with an increase of BMD in the spine (Matkovic *et al.*, 2004).

In contrast to the above, a systematic review on the effects of dairy products and dietary calcium on bone mass revealed that only 9 out of 27 RCTs reported modest benefits in children's bone mass ranging from 1% to 6%. This suggests that there is no conclusive evidence of the positive effects of dairy products and dietary calcium on bone mass in children and adolescents (Lanou *et al.*, 2005). A more recent meta-analysis included 21 RCTs (study duration 1–2 years) with 3821 participants (83% girls) aged between 4 and 17 years. The findings revealed that increasing the consumption of milk products and calcium intake improved TB BMC by 2 g. In addition, children with calcium intakes below 800 mg per day had a greater increase in BMC (49 g), and vitamin D added to the milk supplement improved BMC at the LS by 35 g. Calcium/milk supplement groups alone showed a nonsignificant improvement of 2.1 g in TB BMC compared to control groups (Huncharek *et al.*, 2008).

Vitamin D plays an important role in calcium homeostasis and is an essential nutrient for the bone mineralization process. This is done via its participation in the absorption of calcium and phosphorous in the skeleton through calcification of the growth plate and mineralization of osteoid on trabecular and cortical bone surfaces. The natural source of vitamin D in the body is sunlight accounting for 95%, with the remaining 5% being dependent on dietary intake. Vitamin D appears in the body in the form of vitamin D_2 and D_3 and is then converted in the liver into 25-hydroxyvitamin D (25(OH)D); the latter is the most appropriate biochemical marker to assess vitamin D availability by measuring serum 25(OH)D concentration in the blood (Perez-Lopez *et al.*, 2010). Table 32.1 shows the recommended dietary intakes of vitamin D for children and adolescents aged 4–18 years (15 µg per day).

Table 32.1 Dietary reference intakes for vitamin D in children and adolescents across the world. Childhood is defined as the age between 4 and 8 years and adolescence as the age between 9 and 18 years. Adapted from: (Ross, 2011).

	Vitamin D (mg per day)						
Age group	Estimated average requirement	Recommended dietary allowance	Upper level intake				
Childhood (4–8 years)							
	10	15	75				
Adolescence (8–18 years)							
Boys	10	15	100				
Girls	10	15	100				

Clinical vitamin D (in)sufficiency in young people includes the following categories: severe deficiency of vitamin D when 25(OH)D levels are below 27.5 nmol L⁻¹, deficiency between 27.5 nmol L⁻¹ and 50 nmol L⁻¹, insufficiency between 50 nmol L⁻¹ and 75 nmol L⁻¹ and sufficiency above 75 nmol L⁻¹ (Gonzalez-Gross et al., 2012). It should be noted that the definition of sufficient 25(OH)D levels in adolescents is controversial and therefore predefined cut-offs are used to define optimal levels \geq 75 mmol L⁻¹ and insufficiency <75 mmol L⁻¹ (Valtuena et al., 2012). Depending on the magnitude and the skeletal site, deficiency in vitamin D may lead to rickets and increase fragility by reducing the absorption of calcium in the skeleton (Pettifor, 2005). Insufficient vitamin D status has been observed in 1006 European adolescents from 9 different countries, aged 12.5 to 17.5 participating in the Healthy Lifestyle in the Europe by Nutrition in Adolescence study. After adjusting by age, gender and weight status, it was revealed that 15% of adolescents were severely deficient, 27% were deficient and 39% insufficient for their 25(OH)D levels (Gonzalez-Gross et al., 2012). The daily dose of vitamin D in children and adolescence is a matter of debate, indicating that optimal vitamin D levels should be maintained throughout the year.

32.4 Impact of Physical Activity on Bone Status during Growth

32.4.1 The Role of Physical Activity on Bone Mass

PA is also considered an important determinant of the accrual and maintenance of bone mass. Weight-bearing PA can increase bone mass due to the ability of the skeleton to adapt to the loads under which it is placed. According to Wolf's law, when the loading on a particular bone increases, the bone remodels itself over time to become stronger to resist that loading (Frost, 1994). Based on the previous theory, studies in animals and humans have shown that the growing skeleton adapts to the mechanical stimuli to a greater extent than mature bone due to a higher osteoblast activity and bone formation (Robling *et al.*, 2002). Thus, engaging in PA is particularly important during childhood and adolescence.

A review of 19 cohort studies from childhood to adulthood highlighted the importance of PA during the growth period and its link with bone mass later in life. The positive association was higher in males than in females at the femoral neck (FN) and LS sites (Bielemann *et al.*, 2013). The results of the six-year Saskatchewan Pediatric Bone Mineral Accrual study in adolescent boys and girls showed that both girls and boys classified in the highest PA quartile acquired 17% and 9% higher TB BMC, respectively compared with their inactive peers one year after peak BMC velocity. At the LS site, active adolescents displayed 18% higher BMC (adjusted for weight and height) one year after peak BMC velocity. In addition, there was a difference of 7% and 11% in active and inactive boys and girls, respectively, at the FN (Bailey *et al.*, 1999).

Table 32.2 Physical activity and bone health during adolescence. Adjusted bone mineral content by height and weight covariates; significant difference set at p < 0.05. Adapted from: (Baxter-Jones *et al.*, 2008).

Significant differences in bone mineral content (g) at total body, lumbar spine, total	
hip and femoral neck at 3 activity groups	

Gender (age at peak height velocity)	Inactive	Average	Active
Males (14.1)	Compared to active group: total body, lumbar spine and total hip	Compared to active group: total body and total hip	Compared to both groups: total body and total hip
Females (11.6)	Compared to active group: total body and lumbar spine	Compared to active group: lumbar spine	Compared to both groups: lumbar spine

In a 7 year study, including 72 males and 82 females, the authors examined whether the positive effects of PA on BMC acquired in adolescence preserved into the second and third decade of life. It was reported that both active males and females during adolescence remained active during adulthood. Furthermore, active boys showed 8–10% greater adjusted BMC for weight, height, calcium intake, PA and maturation status at TB, FN and total hip (TH) in adulthood. In addition, active females had 9–10% higher adjusted BMC at TH and FN in young adulthood (Baxter-Jones *et al.*, 2008). These results highlight the importance of PA for bone accrual during childhood and adolescence and its relation with bone status in adulthood. Therefore, it is important to understand the type of exercise and the optimal "dose" (*i.e.* frequency, intensity, duration) that is required to maximize increases in BMC and BMD during childhood and adolescence (Table 32.2).

32.4.2 Weight-Bearing Exercise and Bone Mass

Weight-bearing exercise such as jumping, running and weight training can enhance bone mass due to the high ground reaction forces (GRF) applied in the developing skeleton of children and adolescents. Evidence supports the beneficial effects of weight-bearing activities in bone accrual by applying GRF of 3.5 to 5 times body weight, which represents a moderate exercise intensity intervention (McKay *et al.*, 2005). The majority of the evidence so far comes from studies conducted in a school-based environment and includ jumping exercises to improve bone health at different stages of growth.

Findings from a 4 year longitudinal study of 205 prepubertal children aged 10 ± 1 years suggested that a 7 month jumping intervention significantly increased the adjusted BMC for age, sexual maturation and tissue mass by 7.9% at LS, 8.4% at TH, 7.7% at FN and 7.3% at TB. Three years after the end of the intervention the effects decreased but remained significant at the above sites accounting for 2.3%, 3.2%, 4.4% and 2.9% higher BMC,

respectively (Gunter et al., 2008). A review of RCTs showed that weight-bearing activities (i.e. games, dance, resistance training and jumping exercise) eliciting GRF of 2-9 times body weight can effectively improve bone mass by 0.9-4.9% in prepubertal children, 1.1-5.5% in early pubertal and 0.3-1.9% in pubertal children (Hind and Burrows, 2007). These findings are in agreement with a recent meta-analysis of 27 studies addressing the impact of weight-bearing activities (i.e. body weight, resistance training machines or both) on BMC and BMD of 2985 boys and girls aged 10.3 ± 2.7 years. The results revealed trivial effect sizes of 0.17 (0.05–0.29) for BMC and 0.26 (0.02– 0.49) for BMD. Interestingly, calcium intake explained 35% of the variance and the changes in BMC were greater during the prepubertal years (Tanner stage 1) when compared with mid- and late pubertal years (Tanner stages 2-5) (Behringer et al., 2014). A meta-analysis of school-based exercise interventions, such as jumping, aerobic and circuit training in children and adolescents confirmed the small but beneficial effect of exercise on BMC and added the negative effect on fat mass. Effect sizes presented that BMC improved at LS by 0.38 (standardized mean difference), at FN by 0.29, at TB by 0.48 and fat mass decreased by 0.25 (Nogueira et al., 2014).

These studies show the positive impact of weight-bearing activities, mainly jumping interventions, on BMC and BMD but, it should be noted that the optimal type, intensity and duration of exercise is not clear yet (Bailey and Brooke-Wavell, 2008). Most of the studies involved exercise interventions lasting from 3 to 20 min per day with a frequency of 3 times per week. This is in agreement with the findings of a recent meta-analysis in girls after controlling for the important confounding factors of maturation stage, exercise mode, intervention strategy, duration and frequency of exercise, design and duration of the study (Ishikawa *et al.*, 2013).

The intensity of exercise may contribute to the changes of BMC and BMD among children and adolescents, and there is evidence to suggest that greater differences are observed when interventions include participation in different sports (Boreham and McKay, 2011). The positive long-term effects of sport participation on BMD were demonstrated in a 27 year follow-up study of 154 Belgian boys aged 13 at baseline. Engagement in high impact sports (GRF > 4 times body weight) during adolescence and adulthood resulted in significantly higher BMD (1.12 g cm⁻²) at LS compared to participants that stopped high impact sports in adulthood (BMD = 1.01 g cm⁻²) or did not engaged in high-impact sports during adolescence and in adulthood (BMD = 0.99 g cm⁻²) (Van Langendonck *et al.*, 2003).

In contrast, other sports, such as swimming and cycling, may not have an effect or have a negative impact on bone status. Cycling training of 10 h per week for 2 to 7 years in adolescent males (<17 years of age) resulted in 10% lower BMC in legs, adjusted for total lean mass and height, but 8% higher in the hip area compared with a control group (Olmedillas *et al.*, 2011). Furthermore, a comparison between adolescent female football players and swimmers that engaged in their sport for 10 h per week, reported significantly higher BMD at all sites for footballers. Also, swimmers had lower values of

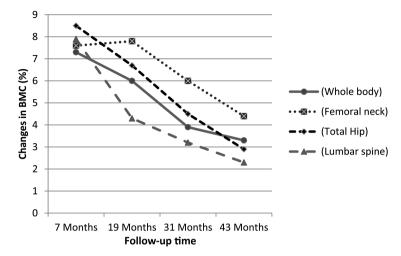


Figure 32.3 Changes (%) in bone-mineral content after a jumping intervention. Percentage of change in bone-mineral content at four sites of the skeleton (total body, femoral neck, total hip, lumbar spine) after a jumping intervention programme. The duration of the school-based intervention was 7 months and the participants were followed up for 3 years. Adapted from: (Gunter et al., 2008).

bone geometry compared with the controls (Ferry *et al.*, 2011). Prolonged participation in weight-bearing activities, such as jumping and osteogenic sports (*e.g.* football, handball, gymnastic), may elicit greater improvements in BMC and BMD compared to nonweight-bearing/osteogenic sports, such as cycling and swimming, which may decrease or not affect the bone development in children and adolescents (Hind and Burrows, 2007) (Figure 32.3).

32.5 Associations of Calcium, Vitamin D and Exercise with Bone Mass of Children and Adolescents

Combined actions of calcium, vitamin D and exercise can determine bone development during growth by taking into account the interactions between hormonal changes and GRF placed on the skeleton. Evidence shows that adequate calcium and vitamin D levels are necessary to acquire bone mass and these nutrients interact with exercise to enhance bone growth (Valtuena *et al.*, 2012). However, the magnitude of the benefits in boys and girls differ at various sites of the skeleton and may depend on the baseline levels of calcium and vitamin D, on the dose of the supplementation and on previous loading experience (Specker and Vukovich, 2007).

The impact of calcium and exercise was investigated in 113 prepubertal females for 12 months. Girls were separated into four groups: two exercise

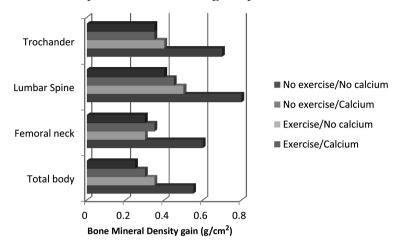


Figure 32.4 Bone-mineral density gain after 12 months of calcium supplementation and exercise intervention. Bone-mineral density gain at total body, femoral neck, lumbar spine and trochanter sites after calcium supplementation and exercise intervention period of 12 months in four groups. Adapted from: (Courteix *et al.*, 2005).

groups (7 \pm 4 h of sport participation per week) that received 800 mg of calcium phosphate (exercise/calcium) or placebo (exercise/placebo) and two sedentary groups (1 \pm 1 h of sport participation per week) that received 800 mg of calcium phosphate (sedentary/calcium) or placebo (sedentary/placebo) (Courteix *et al.*, 2005). The findings showed that in the exercise/calcium group there was a 6.3% greater BMD at the TB, 11% at the LS, 8.2% at FN and 9.3 at Ward's triangle compared to other groups. It should be mentioned that exercise might increase the calcium intake requirements by 422 mg due to calcium excretion through sweat following exercise at intensities. This was observed in adults but there is no evidence for this in young people (Bullen *et al.*, 1999) (Figure 32.4).

The positive combined associations between calcium, vitamin D and bone status have been shown in adolescents at different sites of the skeleton and at different maturation stages (Neville $et\ al.$, 2002). The relationship between PA and 25(OH)D concentration was investigated in 100 adolescents aged 12.5–17.5 years after controlling for calcium and vitamin D intakes, maturation, age, gender, season, PA and fitness level. Findings revealed that the interaction between 25(OH)D levels and PA was significantly related to BMC at TB and legs. Furthermore, it was found that the active group of adolescents had increased total BMC when the vitamin D levels were sufficient (>75 nmol L⁻¹) (Valtuena $et\ al.$, 2012). The beneficial effects of PA have been also demonstrated in participants with vitamin D deficiency. It has been reported that adjusted BMD for age, BMI, parathyroid hormone and bone turnover markers was positively related to PA at TB, FN and LS site in 166 adolescent girls with vitamin D deficiency (Constantini $et\ al.$, 2010).

However, there is evidence that the combined effects of calcium and vitamin D may not have a beneficial effect on BMC and BMD in adolescents. A study of 101 adolescents aged 12.5–17.5 years examined the relationship between calcium, vitamin D, milk intake, 25(OH)D and bone status after adjusting the BMC and BMD for a number of cofounding variables such as height, family affluence scale, maturation status, season and PA (Mouratidou et al., 2013). There was no association between calcium and vitamin D intake and BMC and BMD at any skeletal site. However, a positive relationship was found between milk intake and BMD and BMC in boys. Also, BMD for TB, head and right arm was associated with calcium intakes. In girls there was a significant positive relationship of 25(OH)D and BMC and BMD at TB, subtotal, left and right arm. The exercise contribution to the relationship of bone status and dietary nutrients has been verified by a study that investigated the association of physical fitness and body composition with 25(OH)D levels in 1006 adolescents (Valtuena et al., 2013). Physical fitness was positively related to 25(OH)D levels in boys and upper limp muscular strength was associated with 25(OH)D levels in girls. In addition, boys with higher fitness levels and lower BMI had significantly higher 25(OH)D levels compared to those with lower fitness levels.

32.6 Conclusions

Calcium, vitamin D and exercise are recognized as important factors for bone health in children and adolescences. In this chapter, we discussed the potential independent and combined effects of calcium, vitamin D and exercise on bone health in children and adolescents. The beneficial effects of calcium in relation to vitamin D and exercise were found to be greater during prepubertal years for both boys and girls. Therefore, future interventions should focus on this period of life in order to elicit greater improvements in bone mass that may translate to an increased PBM in adulthood. Current evidence suggests bone development is enhanced to a greater extent when optimal calcium and vitamin D levels combined with weight-bearing exercise. Overall, the findings suggest that regular exercise is considered the most important modifiable factor to enhance bone development during childhood and adolescence. Further longitudinal research is needed to provide valuable information about the continuation of the impact of calcium in combination with vitamin D and exercise from childhood and adolescence into adulthood.

Summary Points

- Peak bone mass increase occurs approximately 2 years earlier in girls compared with boys.
- Boys gain greater amounts of bone mass compared to girls during adolescence.

- Calcium intake between 700 and 1000 mg per day is recommended for normal bone growth in children and 1300 mg per day in adolescents.
- Sufficient vitamin D intake of 15 mg per day or 25-hydroxyvitamin D blood levels above 75 nmol L⁻¹ is recommended for children and adolescents.
- Exercise stimulates bone mineral content and bone-mineral density increase and active children and adolescents have better bone status compared to their nonactive peers.
- Exercise seems to be the most important contributing factor to bone development compared to calcium and vitamin D.

Key Facts of Bone Health during Growth

- 1. Bone-mass acquired during youth is an important determinant of the risk of osteoporotic fracture during later life. The higher the peak bone mass, the lower the risk of osteoporosis.
- 2. From birth to the onset of the physical maturation, the bone mass is the same in girls as in boys.
- 3. During puberty, the speed of bone development in the spine and hip increases by approximately five times.
- 4. By the end of the teen years, approximately 90% of adult bone mass is in place.
- 5. During growth the gain in bone mineral mass is mainly due to an increase in bone size with very little change in bone tissue within the bones.
- 6. Young children who engage in 40 min of normal vigorous activity each day have significantly stronger bones than their less active peers.
- 7. It is estimated that a 10% increase of peak bone mass in children reduces the risk of an osteoporotic fracture during adult life by 50%.

Definitions and Explanations of Key Terms

Bone-mineral content. Amount of bone mineral mass in grams per centimeter.

Bone-mineral density. Amount of mineral mass in grams per square centimeter.

Ground reaction forces. The forces exerted by the ground on a body in contact with it.

Peak bone mineral accrual. The period of most rapid gain in bone mineral. **Peak-height velocity.** Maximum growth in height.

Tanner maturity stages. A five-stage scale system based on breast and pubic hair development.

Peak bone mass. Attainment of maximum bone mass that occurs between the second and third decade of life.

25-Hydroxyvitamin D. Biochemical marker to assess vitamin D availability in the blood.

Osteogenic/nonosteogenic sports. Sports that elicit or not improvements in bone mass.

Weight-bearing exercise. Type of exercise that enhance bone status.

List of Abbreviations

25(OH)D 25-Hydroxyvitamin D BMC Bone-mineral content BMD Bone-mineral density

Ca Calcium FN Femoral neck

GRF Ground reaction forces

LS Lumbar spine
PA Physical activity
PBM Peak bone mass
PHV Peak height velocity

RCT Randomized controlled trial

TB Total body
TH Total hip

References

Bailey, C. A. and Brooke-Wavell, K., 2008. Exercise for optimising peak bone mass in women. *Proceedings of the Nutrition Society*. 67: 9–18.

Bailey, D. A., Martin, A. D., McKay, H. A., Whiting, S. and Mirwald, R., 2000. Calcium accretion in girls and boys during puberty: a longitudinal analysis. *Journal of Bone and Mineral Research*. 15: 2245–2250.

Bailey, D. A., McKay, H. A., Mirwald, R. L., Crocker, P. R. and Faulkner, R. A., 1999. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *Journal of Bone and Mineral Research*. 14: 1672–1679.

Baker, S. S., Cochran, W. J., Flores, C. A., Georgieff, M. K., Jacobson, M. S., Jaksic, T. and Krebs, N. F., 1999. American Academy of Pediatrics. Committee on Nutrition. Calcium requirements of infants, children, and adolescents. *Pediatrics*. 104: 1152–1157.

Baxter-Jones, A. D., Faulkner, R. A., Forwood, M. R., Mirwald, R. L. and Bailey, D. A., 2011. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *Journal of Bone and Mineral Research*. 26: 1729–1739.

Baxter-Jones, A. D., Kontulainen, S. A., Faulkner, R. A. and Bailey, D. A., 2008. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*. 43: 1101–1107.

- Behringer, M., Gruetzner, S., McCourt, M. and Mester, J., 2014. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. *Journal of Bone and Mineral Research*. 29: 467–478.
- Bielemann, R. M., Martinez-Mesa, J. and Gigante, D. P., 2013. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. *BMC Musculoskeletal Disorders*. 14: 77.
- Boreham, C. A. and McKay, H. A., 2011. Physical activity in childhood and bone health. *British Journal of Sports Medicine*. 45: 877–879.
- Bullen, D. B., O'Toole, M. L. and Johnson, K. C., 1999. Calcium losses resulting from an acute bout of moderate-intensity exercise. *International Journal of Sport Nutrition*. 9: 275–284.
- Constantini, N. W., Dubnov-Raz, G., Chodick, G., Rozen, G. S., Giladi, A. and Ish-Shalom, S., 2010. Physical activity and bone mineral density in adolescents with vitamin D deficiency. *Medicine & Science in Sports & Exercise*. 42: 646–650.
- Courteix, D., Jaffre, C., Lespessailles, E. and Benhamou, L., 2005. Cumulative effects of calcium supplementation and physical activity on bone accretion in premenarchal children: a double-blind randomised placebocontrolled trial. *International Journal of Sports Medicine*. 26: 332–338.
- Davies, J. H., Evans, B. A. and Gregory, J. W., 2005. Bone mass acquisition in healthy children. *Archives of Disease in Childhood*. 90: 373–378.
- Deng, Z. L., Sharff, K. A., Tang, N., Song, W. X., Luo, J., Luo, X., Chen, J., Bennett, E., Reid, R., Manning, D., Xue, A., Montag, A. G., Luu, H. H., Haydon, R. C. and He, T. C., 2008. Regulation of osteogenic differentiation during skeletal development. *Frontiers in Bioscience*. 13: 2001–2021.
- Ferry, B., Duclos, M., Burt, L., Therre, P., Le Gall, F., Jaffre, C. and Courteix, D., 2011. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. *Journal of Bone and Mineral Metabolism*. 29: 342–351.
- Frost, H. M., 1994. Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians. *Angle Orthodontist*. 64: 175–188.
- Gonzalez-Gross, M., Valtuena, J., Breidenassel, C., Moreno, L. A., Ferrari, M., Kersting, M., De Henauw, S., Gottrand, F., Azzini, E., Widhalm, K., Kafatos, A., Manios, Y., Stehle, P. and Grp, H. S., 2012. Vitamin D status among adolescents in Europe: the healthy lifestyle in Europe by nutrition in adolescence study. *British Journal of Nutrition*. 107: 755–764.
- Gracia-Marco, L., Moreno, L. A., Ortega, F. B., Leon, F., Sioen, I., Kafatos, A., Martinez-Gomez, D., Widhalm, K., Castillo, M. J. and Vicente-Rodriguez, G., 2011. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. *American Journal of Preventive Medicine*. 40: 599–607.
- Gunter, K., Baxter-Jones, A. D., Mirwald, R. L., Almstedt, H., Fuller, A., Durski, S. and Snow, C., 2008. Jump starting skeletal health: a 4-year longitudinal

study assessing the effects of jumping on skeletal development in pre and circum pubertal children. *Bone*. 42: 710–718.

- Hind, K. and Burrows, M., 2007. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. *Bone*. 40: 14–27.
- Huncharek, M., Muscat, J. and Kupelnick, B., 2008. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. *Bone*. 43: 312–321.
- Huybrechts, I., Lin, Y., De Keyzer, W., Sioen, I., Mouratidou, T., Moreno, L. A., Slimani, N., Jenab, M., Vandevijvere, S., De Backer, G. and De Henauw, S., 2011. Dietary sources and sociodemographic and economic factors affecting vitamin D and calcium intakes in Flemish preschoolers. *European Journal of Clinical Nutrition*. 65: 1039–1047.
- Ishikawa, S., Kim, Y., Kang, M. and Morgan, D. W., 2013. Effects of weight-bearing exercise on bone health in girls: a meta-analysis. *Sports Medicine*. 43: 875–892.
- Lanou, A. J., Berkow, S. E. and Barnard, N. D., 2005. Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. *Pediatrics*. 115: 736–743.
- Levine, M. A., 2012. Assessing bone health in children and adolescents. *Indian Journal of Endocrinology and Metabolism*. 16: S205–S212.
- Marshall, D., Johnell, O. and Wedel, H., 1996. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *British Medical Journal*. 312: 1254–1259.
- Matkovic, V., Landoll, J. D., Badenhop-Stevens, N. E., Ha, E. Y., Crncevic-Orlic, Z., Li, B. and Goel, P., 2004. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. *Journal of Nutrition*. 134: 7018–7058.
- McKay, H., Tsang, G., Heinonen, A., MacKelvie, K., Sanderson, D. and Khan, K. M., 2005. Ground reaction forces associated with an effective elementary school based jumping intervention. *British Journal of Sports Medicine*. 39: 10–14.
- Mouratidou, T., Vicente-Rodriguez, G., Gracia-Marco, L., Huybrechts, I., Sioen, I., Widhalm, K., Valtuena, J., Gonzalez-Gross, M. and Moreno, L. A., 2013. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *Journal of Clinical Densitometry*. 16: 110–117.
- Neville, C. E., Robson, P. J., Murray, L. J., Strain, J. J., Twisk, J., Gallagher, A. M., McGuinness, M., Cran, G. W., Ralston, S. H. and Boreham, C. A., 2002. The effect of nutrient intake on bone mineral status in young adults: the Northern Ireland young hearts project. *Calcified Tissue International*. 70: 89–98.
- Nogueira, R. C., Weeks, B. K. and Beck, B. R., 2014. Exercise to improve pediatric bone and fat: a systematic review and meta-analysis. *Medicine & Science in Sports & Exercise*. 46: 610–621.

- Olmedillas, H., Gonzalez-Aguero, A., Moreno, L. A., Casajus, J. A. and Vicente-Rodriguez, G., 2011. Bone related health status in adolescent cyclists. *PLoS One*. 6: e24841.
- Perez-Lopez, F. R., Chedraui, P. and Cuadros-Lopez, J. L., 2010. Bone mass gain during puberty and adolescence: deconstructing gender characteristics. *Current Medicinal Chemistry*. 17: 453–466.
- Pettifor, J. M., 2005. Rickets and vitamin D deficiency in children and adolescents. *Endocrinology and Metabolism Clinics of North America*. 34: 537–553.
- Pettifor, J. M. and Prentice, A., 2011. The role of vitamin D in paediatric bone health. *Best Practice & Research, Clinical Endocrinology & Metabolism*. 25: 573–584.
- Rauch, F., Bailey, D. A., Baxter-Jones, A., Mirwald, R. and Faulkner, R., 2004. The 'muscle-bone unit' during the pubertal growth spurt. *Bone*. 34: 771–775.
- Rizzoli, R., Bianchi, M. L., Garabedian, M., McKay, H. A. and Moreno, L. A., 2010. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone*. 46: 294–305.
- Robling, A. G., Hinant, F. M., Burr, D. B. and Turner, C. H., 2002. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *Journal of Bone and Mineral Research*. 17: 1545–1554.
- Ross, A. C., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D. *Public Health Nutrition*. 14: 938–939.
- Specker, B. and Vukovich, M., 2007. Evidence for an interaction between exercise and nutrition for improved bone health during growth. *Medicine and Sport Science*. 51: 50–63.
- Theintz, G., Buchs, B., Rizzoli, R., Slosman, D., Clavien, H., Sizonenko, P. C. and Bonjour, J. P., 1992. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *Journal of Clinical Endocrinology and Metabolism.* 75: 1060–1065.
- Valtuena, J., Gracia-Marco, L., Huybrechts, I., Breidenassel, C., Ferrari, M., Gottrand, F., Dallongeville, J., Sioen, I., Gutierrez, A., Kersting, M., Kafatos, A., Manios, Y., Widhalm, K., Moreno, L. A. and Gonzalez-Gross, M., 2013. Cardiorespiratory fitness in males, and upper limbs muscular strength in females, are positively related with 25-hydroxyvitamin D plasma concentrations in European adolescents: the HELENA study. *QJM*. 106: 809–821.
- Valtuena, J., Gracia-Marco, L., Vicente-Rodriguez, G., Gonzalez-Gross, M., Huybrechts, I., Rey-Lopez, J. P., Mouratidou, T., Sioen, I., Mesana, M. I., Martinez, A. E., Widhalm, K. and Moreno, L. A., 2012. Vitamin D status and physical activity interact to improve bone mass in adolescents. The HELENA Study. *Osteoporosis International*. 23: 2227–2237.

Van Langendonck, L., Lefevre, J., Claessens, A. L., Thomis, M., Philippaerts, R., Delvaux, K., Lysens, R., Renson, R., Vanreusel, B., Vanden Eynde, B., Dequeker, J. and Beunen, G., 2003. Influence of participation in high-impact sports during adolescence and adulthood on bone mineral density in middle-aged men: a 27-year follow-up study. *American Journal of Epidemiology*. 158: 525–533.

- Vicente-Rodriguez, G., 2006. How does exercise affect bone development during growth? *Sports Medicine*. 36: 561–569.
- Zemel, B., 2013. Bone mineral accretion and its relationship to growth, sexual maturation and body composition during childhood and adolescence. *World Review of Nutrition and Dietetics*. 106: 39–45.



STUDY PROTOCOL

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Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol

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Abstract

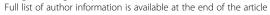
Background: Osteoporosis is a skeletal disease associated with high morbidity, mortality and increased economic costs. Early prevention during adolescence appears to be one of the most beneficial practices. Exercise is an effective approach for developing bone mass during puberty, but some sports may have a positive or negative impact on bone mass accrual. Plyometric jump training has been suggested as a type of exercise that can augment bone, but its effects on adolescent bone mass have not been rigorously assessed. The aims of the PRO-BONE study are to: 1) longitudinally assess bone health and its metabolism in adolescents engaged in osteogenic (football), non-osteogenic (cycling and swimming) sports and in a control group, and 2) examine the effect of a 9 month plyometric jump training programme on bone related outcomes in the sport groups.

Methods/Design: This study will recruit 105 males aged 12–14 years who have participated in sport specific training for at least 3 hours per week during the last 3 years in the following sports groups: football (n = 30), cycling (n = 30) and swimming (n = 30). An age-matched control group (n = 15) that does not engage in these sports more than 3 hours per week will also be recruited. Participants will be measured on 5 occasions: 1) at baseline; 2) after 12 months of sport specific training where each sport group will be randomly allocated into two sub-groups: intervention group (sport + plyometric jump training) and sport group (sport only); 3) exactly after the 9 months of intervention; 4) 6 months following the intervention; 5) 12 months following the intervention. Body composition (dual energy X-ray absorptiometry, air displacement plethysmography and bioelectrical impedance), bone stiffness index (ultrasounds), physical activity (accelerometers), diet (24 h recall questionnaire), pubertal maturation (Tanner stage), physical fitness (cardiorespiratory and muscular), bone turnover markers and vitamin D will be measured at each visit.

Discussion: The PRO-BONE study is designed to investigate the impact of osteogenic and non-osteogenic sports on bone development in adolescent males during puberty, and how a plyometric jump training programme is associated with body composition parameters.

Keywords: Body composition, Longitudinal study, Plyometric jump training intervention, Osteogenic, Non-osteogenic, Sport participation, Weight-bearing exercise

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Background

Osteoporosis is a common skeletal disease associated with high morbidity and mortality [1]. Approximately 2.7 million European men and women suffer an osteoporotic fracture every year [2]. The economic burden of osteoporosis in Europe is higher than most types of cancer (except lung cancer), or chronic cardiorespiratory diseases [2,3] and represents a direct annual cost of ~ €31.7 billion to health care and social services [1]. In order to improve the outcome for osteoporosis, primary prevention remains the most important policy action in public health. Although contested [4], it is generally accepted that acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health [5-7]. Approximately 60% of osteoporotic cases in adult life are related to low bone mineral content (BMC) in adolescence with up to 50% of total body (TB) bone mass achieved during this period of life [8,9]. Peak bone mass attainment typically occurs between the second and third decade of life, with 80-90% acquired by late adolescence, although this is skeletal site dependent [6,10]. Although bone mass is ~ 60-80% genetically determined [11], there are other factors strongly related with bone mass development. Environmental and lifestyle factors such as physical activity (PA) [12] and nutrition, i.e. calcium intake [13] and vitamin D [14], are known to have important osteogenic effects and have been the key focus in several interventions.

Exercise as a tool to improve bone health

Exercise has been proposed as a key factor for developing healthy bones in childhood and adolescence [15,16], mainly when high-impact and weight-bearing PA occurs [15] above a certain intensity and duration [15,17,18]. Longitudinal studies have shown that habitual PA is positively associated with bone health in children and adolescents because of its impact on bone development [19,20]. The long-term positive effects of PA during adolescence remain into young adulthood with active males aged 24.2 years having 8 and 10% higher BMC at TB and femoral neck (FN) respectively compared to nonactive peers, even when adjusted for maturation and size [21]. Research conducted on former professional football players showed that exercise is not only an important factor in the accretion of, but also in the maintenance, of bone mineral density (BMD) [22]. It has been shown that moderate and readily accessible weight-bearing exercise before puberty may increase femoral volumetric BMC, by increasing cortical thickness, and therefore bone strength [23]. In addition, bone development is dependent on the impact of mechanical load and processes that trigger bone modelling and remodelling [24], and possibly on structural adaptations related with trabecular microarchitecture [25].

Sport participation and bone health

It has been shown that sport participation is crucial for healthy bone development, however not all sports have a positive influence on the skeletal mass. According to their characteristics, sports can be described as osteogenic (weight-bearing exercise) and non-osteogenic (non weight-bearing exercise). Apart from numerous health benefits [26], football is considered as an osteogenic sport both in childhood and adolescence as bone mass is augmented [27-30]. In contrast, sports such as cycling [31-40] or swimming [41-46] are associated with no change or a reduction in bone mass when compared to controls. This could be a barrier for obtaining a high peak bone mass which may compromise future bone health [40,41,46,47].

Plyometric exercise intervention to increase bone health

To achieve the benefits of exercise and gain acceptance, PA models must be effective, simple to administer, feasible, inexpensive, short in duration and possible to perform at any location (i.e. at home, at the sports centre). Plyometric jump training (PJT) may be a judicious choice and experimental studies using animal models have repeatedly shown that short, discrete bouts of exercise interspersed with rest periods is more effective than a single longer bout of exercise for improving bone mass and strength [48].

Research in early puberty has shown that a novel and easily implemented 8-month PJT (Bounce at the Bell; ~3 min/ day) enhanced bone mass at the weight bearing proximal femur [49]. Mackelvie et al. showed that a 7-month jumping intervention (10 min, 3 times/week) was associated with more bone at the FN and lumbar spine (LS) in early pubertal girls [50], and these results were maintained after 2 years [51]. In addition, prepubertal Asian and Caucasian boys of average or low body mass index (BMI) augmented bone mineral accrual at several regions after a 7-month jumping intervention (10 min, 3 times/week). However, there are a lack of studies analysing the effect of PJT in the adolescent population, which is crucial as adolescence is the period associated with the greatest increments in BMC and BMD [52]. In addition, this has not been studied in adolescents engaged in different sports (osteogenic vs. non osteogenic), which is important to examine if peak bone mass during adolescence may be maximized and therefore reduce the risk for developing osteoporosis in adulthood.

Bone turnover markers and vitamin D

Bone development depends on its metabolic activity, which includes bone formation, resorption and, as a consequence bone turnover [53]. The relationship of PA and sport participation with bone metabolism markers has been shown previously in adolescents [54,55]. An

increase in the concentrations of bone formation and resorption markers can be observed in non-osteogenic sports, such as swimming, but a comparison between osteogenic and non-osteogenic sports has not been investigated previously [56].

The role of vitamin D in bone metabolism is important due to contribution of vitamin D in calcium homeostasis and bone mineralization processes during growth. Evidence shows that adequate vitamin D levels are necessary to acquire bone mass and interact with exercise to enhance bone growth [57,58]. The magnitude of the benefits in boys and girls differ at sites of the skeleton and may depend on the baseline levels of vitamin D and on previous loading experience [59]. A positive interaction between PA and vitamin D on BMD in adolescents has been described [60,61] however the association between vitamin D with osteogenic and non-osteogenic sports has not been justified.

Objectives

The objectives of the PRO-BONE study are: 1) to longitudinally assess, over 3 years, bone health and its metabolism in adolescents engaged in osteogenic (football) and non-osteogenic (cycling and swimming) sports, and 2) after 12 months of sport participation to examine whether a short and inexpensive 9 months PJT intervention programme is positively associated with bone-related variables and its metabolism in adolescent footballers, cyclists and swimmers.

The secondary aim of the study is to examine whether the PJT programme stimulus is enough to counteract the expected negative consequences of these non-osteogenic sports in bone health and to follow-up the bone-related variables and its metabolism over 12 months after the PJT programme.

Methods/Design

Study design

PRO-BONE is a longitudinal design and involves four cohorts of males aged 12–14 years at the beginning of the study. These four cohorts consist of footballers, cyclists, swimmers and controls that will be followed over a period of 33 months. The timeline of the PRO-BONE study can be seen in Figure 1.

Sample size

The sample size has been calculated according to the primary interest variable, TB BMD (of cyclists (aged 15.5 years) [39] in order to achieve 90% of statistical power to detect differences in the contrast of the null hypothesis H0: μ 1 = μ 2 through bilateral student t, difference between two dependent means (matched pairs). Taking into account a significance level of 5% and assuming that the mean of the reference group 1.133 units (SD = 0.127) and the mean of the experimental group is

1.002 units (SD = 0.093), it will be necessary to include 9 participants in the reference group and 9 participants in the experimental group, totalling 18 participants. It is known that the number of participants to recruit depends also on potential withdrawals [or could use dropouts]: n' = n/(1-p), so that if the withdrawals were 40% the number of participants to be recruited would be n' = 9/(1-0.4) = 15 in each group (e.g. 15 INT cyclists + 15 CON cyclists = 30 cyclists). Therefore, cyclists (n = 30), footballers (n = 30), swimmers (n = 30) and controls (n = 15) will be recruited, yielding a total N = 105.

Recruitment of the participants

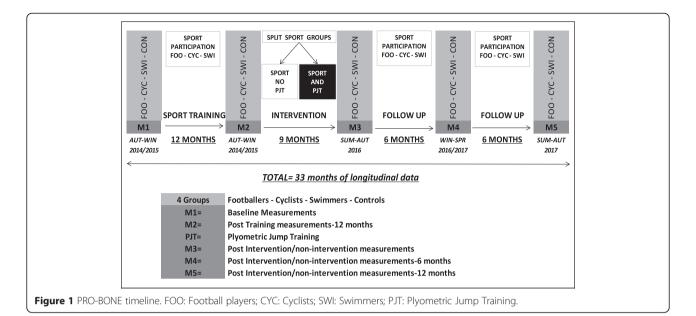
Participants and parents/guardians will be contacted via advert flyers, posters and social media to participate in this study and by contacting sport clubs and schools from the South West of England. Where possible, a meeting will be held to explain the project as well as to answer any questions. At the end of this meeting, consent/assent forms and information sheets will be given out and participants and parents/guardians will have 15 days to return the consent/assent forms. After these 15 days, a reminder (phone call or email) will be provided to those not sending the consent/assent to check if they wish to participate. Seven more days will be given to those that agreed to participate and in the 2nd reminder, they will be asked to send the interest and consent/assent forms signed.

Participants will be screened for eligibility, based on the inclusion/exclusion criteria outlined below, by a member of the research team depending on the information provided in the interest form. If eligible, the baseline assessment will be scheduled for the participant. All participants and parents involved in this project will be carefully informed about the risks and benefits of the study and will be required to sign the approved assent and consent forms before their visit to the laboratory at the Children's Health and Exercise Research Centre (CHERC, University of Exeter).

Inclusion and exclusion criteria

Inclusion criteria include: 1) Males 12-14 years old, engaged (≥ 3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports in the last 3 years or more; 2) Male adolescents 12-14 years old not engaged in any of these sports (≥ 3 h/week) in the last 3 or more years (control group).

Exclusion criteria include: 1) participation in another clinical trial; 2) any acute infection lasting until < 1 week before inclusion; 3) medical history of diseases or medications affecting bone metabolism or the presence of an injury (before inclusion) that may affect participation in their respective sports and/or any variable considered in the present study (i.e. doing the PJT); 4) non-Caucasian



participants. The latter is included since there are differences in body composition (bone, fat and fat-free mass) and biochemical markers (i.e. osteocalcin) between ethnic groups [62].

Ethics approval

The methods and procedures of the PRO-BONE study have been checked and approved by: the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496), the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060). All data and information obtained will be confidential and access to database will be restricted to the researchers of the study. All measurements will be carried out by qualified and experienced researchers that will undergo a Disclosure and Barring Service check for approval to work with young people.

Study protocol and measurements Body composition

Anthropometry Stature (cm), seated height (cm) and body mass (kg) will be measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK; precision 0.1 cm; range 60–210 cm), a sitting height table (Harpenden, Holtain Ltd., Crymych, UK; precision 0.1 cm; range 32–109 cm) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK; precision 100 g; range 2–200 kg) respectively. Body mass index (BMI) will be calculated as body mass (kg) divided by the height (m) squared.

Waist circumference will measured at the midpoint between the lowest rib cage and the top of the iliac crest. Hip circumference will be measured around the widest portion of the buttocks. All measurements will be undertaken by the same trained researcher using the type Seca 201 measuring tape (Seca Ltd, Birmingham, UK; precision 0.1 cm; range 0–205 cm). All anthropometrical measurements will be performed three times and the mean will be calculated. Pubertal maturation will be self-reported by the participants during each visit using adapted drawings of the five stages (Tanner) of pubertal hair development [63].

Dual-energy x-ray absorptiometry Dual-energy x-ray absorptiometry scanner (GE Lunar Healthcare Corp., Madison, WI, USA) will be used to scan participants at four sites due to the evidence of site specific impact of sports participation [64-66]: 1) LS (mean of L1-L4), 2) right hip, 3) left hip, 4) TB. The DXA equipment will be calibrated at the start of each testing day by using a LS phantom as recommended by the manufacturer. The body will be segmented in accordance to standard procedures to evaluate regional bone mass and fat distribution. The scan modes will be automatically selected by the scanner software (standard or thick). All DXA scans and analyses will be performed using the GE enCORE software (2006, version 14.10.022).

Participants will be asked to remain still and they will be scanned in the supine position. The BMC (g) and BMD (g/cm²) with aged-matched Z-scores and age-matched % will be obtained. For LS regions area (cm²), width (cm) and height (cm) will be recorded and for TB regions, fat mass (g), lean mass (kg) and body fat (% and kg) will be obtained. Information about hip strength index, fat mass ratios (trunk/total, legs/total, arms and legs/trunk), android and gynoid regions will also be obtained and have been previously validated in adolescents [67].

This technique uses ionizing radiation that raises ethical issues particularly for child participants. However, this technique uses a minimal radiation dose (similar to spending a day outside in the sunshine), and has been widely used for research purposes with child participants worldwide. The estimated lifetime risks of using GE Lunar Prodigy DXA measurements in the paediatric population was found to be negligible [68].

Air displacement plethysmography Body volume will be measured with BodPod (Body Composition System, Life Measurement Instruments, Concord, California, USA) as it can effectively predict visceral adipose tissue in children [69] and determine the changes of body fat percentage over time [70]. Two measurements will be performed and if there is a difference of more than 150 mL in body volume, a third measurement will be taken. The equipment will be calibrated at the commencement of each testing day following the manufacturer's guidelines and using a cylinder of specific volume (49.887 L). Participants will wear clothing according to the manufacturer's recommendation (a swimsuit and a swim cap) to rule out air trapped in clothes and hair. Participants will be weighed on the BodPod calibrated digital scale and then will enter into the BodPod chamber. During the measurements participants will be asked to remain in a seated position and to breathe normally. A mean value for body volume will be obtained following the manufacturer's recommendations [71] and this value will be integrated into the calculation of lung volume. Percentage of TB fat mass will be calculated using the equation reported by Siri [72,73].

Imaging bone ultrasonometer Qualitative ultrasound measurements will be performed with a Lunar Achilles Insight and the OsteoReport PC software version 5.x + (TM Insight GE Healthcare, Milwaukee, WI, USA). This portable device measures bone stiffness using ultrasound waves and is considered a valid and radiation-free method to assess bone health in children [74,75]. The same device will be used throughout the study and calibration will be carried out prior to each visit. A standard procedure will be followed according to manufacturer's instructions. Participants will be placed on a stable chair in a comfortable position directly in front of the Achilles device. The position of the leg will rest lightly against the calf support so the foot, calf and thigh are aligned with the centre of the calf support and the positioner.

The qualitative ultrasound device provides three outcome variables, the broadband ultrasound attenuation (BUS), the speed of sound (SOS) and the stiffness index (SI). The BUA indicates the absorption of sound waves measured in decibels per megahertz. The SOS shows the stiffness of a material by the ratio of the traversed

distance to the transit time, expressed in meters per second. And the SI is calculated by a linear combination of BUA and SOS: $SI = (0.67 \times BUA) + (0.28 \times SOS) - 420$. The real-time image of the calcaneus and the region of interest ensure that the measurement is precise [74]. Both feet will be measured twice and the mean of the two measurements will be calculated and used for statistical analyses.

Bioelectrical impedance analysis The portable BIA device (Tanita BF-350, Tokyo, Japan; range 2–200 kg; precision 100 g; body fat % range 1-75%; body fat% increments 0.1%) will estimate the percentage of body fat by using the values of resistance and reactance. Participants will be measured in a fasting state and will remove any metal objects and socks prior the measurements. They will be positioned on the posterior surface barefoot according to manufacturer's instructions. Despite the reported prediction measurement error, BIA is considered a practical method to assess body fat in addition to DXA and BodPod in adolescents [76,77].

Biochemical markers and blood collection

The measurement of bone turnover markers, in addition to the measurement of bone mass, is an interesting option to obtain a more dynamic picture of bone tissue, with the advantage that can be repeated at short intervals [78]. Therefore, the combination of both measures (bone mass and bone metabolism) is essential to obtain a better understanding on changes in the skeletal mass. In this regard, the International Osteoporosis Foundation and the International Federation of Clinical Chemistry recommended the use of serum procollagen type 1 aminoterminal propeptide (P1NP) and isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-1) as markers for formation and resorption, respectively [79]. The role of vitamin D in bone metabolism is important due to contribution of vitamin D in calcium homeostasis and bone mineralization processes during growth. Assessment of vitamin D levels can be achieved by measuring the serum 25-hydroxyvitamin D [25(OH)D] in the blood [80]. For the scope of the present study 25(OH)D will be analysed as it has been shown to interact with PA to improve bone mass in adolescents [14].

Blood samples will be collected between 8:00 am and 9:00 am following a 12-hour fast period. A research team experienced in sampling techniques will collect capillary blood samples (\sim 1.2 mL) from a pre-warmed hand into heparin fluoride coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) that will be placed immediately on ice. The microvettes will be centrifuged at 1000 \times G per min for 15 minutes at 4°C and plasma will be separated in Eppendorf tubes of at least 60 μ L, 110 μ L and 60 μ L and stored at -80°C for future analysis of P1NP,

CTX-1 and 25(OH)D respectively. The CTX-1 and 25(OH)D biochemical markers will be analysed by using IDS-iSYS CrossLaps (Immunodiagnostic Systems Ltd, UK) and total P1NP by using ELISA kit (MyBioSource, San Diego, California, USA).

Physical fitness assessment

A battery of tests will be used to assess attributes of physical fitness that may play an important role in the development of skeletal mass and strength during growth and maturation. Cardiorespiratory fitness (aerobic performance) will be estimated using the 20 m shuttle run test [81], which has been shown to be both reliable and valid in youth [82]. The participants will be tested at the end of the day following a standardized warm up. They will be asked to run between two lines set 20 m apart by following the pace of the audio signals produced from a CD player. The starting speed will be 8.5 km·h⁻¹ and will be increased by 0.5 km·h⁻¹ each minute. The participants will be encouraged to continue the test until they reach maximal effort. The test will end when the participant fails to reach the line two consecutive times. The last completed shuttle will indicate the score of the test.

The standing long jump test and the Abalakov jump test will be performed at least half an hour before the 20 m shuttle run test and following a standardized warm up and with 2 minutes rest between the two tests. The starting position of the standing long jump test will be exactly behind a line and with feet at shoulder's width apart. Participants will be allowed to swing their arms during the eccentric contraction phase and they will be advised to jump as far as possible in order to land with both feet on a non-slippery hard surface. The distance (cm) will be measured between the starting line and the participant's heels. Participants will perform Abalakov jump test on a jump mat (Probotics Inc., Huntsville, USA) after having received instructions as to how much can they bend their knees and the position of their arms, they will be asked to jump as high as possible. Then, they will be placed in a standing position with their feet shoulder width apart at the jump mat. For both muscular tests the participants will perform 1 familiarization effort and 2 maximal effort jumps. The mean height and distance (in cm) of the maximal efforts will be used as criterion of measure. The reliability of both tests in adolescents was previously described and is acceptable to be used in this population [83]. The order of all the measurements for each testing day can be seen in Figure 2.

Physical activity measurements

PA will be measured using two different methods: 1) International Physical Activity Questionnaire and 2) a

wrist accelerometer (GENEActiv, GENEA, UK). The validity and reliability of the accelerometer and of the International Physical Activity Questionnaire has been established previously in children and adolescents [84,85]. GENEActiv accelerometers are waterproof so are valid for the swimmers too. Both methods will be used in order to obtain more precise data as, for example, accelerometers do not properly measure PA in cyclists as bouts of activity are not detected [86]. A diary to complement accelerometer data will be administered to the participants to obtain additional information such as calcium and protein intake.

Dietary assessment Assessment of dietary intakes of calcium, vitamin D and milk will be completed by using two non-consecutive 24-h dietary recall questionnaires. CompEat Pro software (Nutrition systems, VIS Visual Information Systems Ltd., UK) will be used for the analysis.

Jumping intervention

Following 12 months of sport specific training, the randomisation process will start in each sport group and participants will be divided into two sub-groups to perform a PJT programme as follows: 1) intervention programme groups, (sport + PJT) and 2) sport groups (sport only). It has been shown that 7 to 9 month PJT programmes can effectively improve BMC and/or BMD at different skeletal sites in children and adolescents and to maintain the benefits for 3 years after the intervention [52,87]. Therefore, a progressive PJT (~10 min/day) will be performed by intervention groups 3 to 4 times/week (depending on progression) as shown in Table 1. Before the intervention, trained staff will ensure that participants fully understand and correctly execute the different jumps and a research assistant will meet with the participants to observe, demonstrate and review the jumps. Participants will be instructed to perform a number of countermovement jumps (CMJ) and squat jumps (SJ) on a hard surface. Jumps will be performed before and after school and before going to bed. The CMJ will be performed by bending the knees prior to the jump. The CMJ activates the stretch-shortening cycle in the muscles, resulting in greater power production in the legs compared to a SJ. For the SJ participants will squat down until the knees are bent at 90 degrees, then they will immediately jump vertically as high as possible, landing back on the ground on both feet simultaneously. For this technique, the participant starts from a stationary semi-squatting position, or pauses at the lower level of the squat before jumping upwards. This removes the factor of the stretch-shortening cycle. The reliability and validity of the CMJ and SJ has been previously reported [88,89].

These jumps are associated with important ground reaction forces, i.e. for a countermovement it is about 5

	Blood Collection	Во	Physical Fitness Tests				
Testing day	Anthropometry	DXA		Ultrasonometer/	Jump Height	Long Jump	20 m shuttle
day		(LS-FN-TB)	BodPod	BIA	Treight	Jump	run test
Time	8:00 am-9:00 am	9:00 am-12:00 pm					12:30 pm

Figure 2 Order of the measurements for each testing day. DXA, dual energy x-ray absorptiometry; LS, lumbar spine; FN, femoral neck; TB, total body; BIA, bioelectrical impedance analysis.

times body weight (BW), compared to 3.5 times BW for jumping jacks. Similarly, the highest rates of change in force are 493 times BW/s for the CMJ, as shown in an independent sample of boys and girls [90]. A diary will be used to record the number of jumps performed each day. Both the intensity and number of jumps will be increased progressively in 3 levels of 12 weeks each. Intensity will be modified using ankle weights (from 1 kg at level 1 to 2.5 kg at level 3). With this an increase in BW between 2 to 5 kg will be achieved. In this regard, it has been shown that adolescents with higher BMI have higher levels of bone mass, because of the higher lean mass that they develop as a consequence of their higher fat mass [91].

Discussion

PRO-BONE will assess the longitudinal impact of osteogenic (football) and non-osteogenic (cycling and swimming) sports on bone development in adolescents aged 12–14 years old. In addition, it will investigate whether a simple, feasible and inexpensive PJT programme can improve bone development and if the effects will be maintained a year after finishing the PJT programme. Several investigations have been conducted in order to improve bone health through exercise, strength, jumping or even

combinations among them [92]. However, to achieve impact and gain acceptance, the intervention must be effective, simple to administer, feasible, inexpensive, short in duration and possible to perform at any place [49]. PRO-BONE has been designed to meet all these requirements and follow-up its effects after the withdrawal of the intervention.

Previous research has shown that exercise is positively associated with bone health [93]. However, there are some sports that due to the impact generated at the skeletal sites may have a minimal or negative effect on BMC and BMD [40,56]. As recent data have shown, jump training is associated with increases in BMC and BMD and may play an important role in the prevention of osteoporosis [94]. It is well known that early prevention is the most effective tool, therefore, it is crucial to analyse the effect of PJT at an early stage (i.e. adolescence). In this sense, it is important to examine if PJT can counteract the potential negative consequences of non-osteogenic sports on bone health and if there is enough stimuli to increase BMC and BMD in adolescents engaged in osteogenic sports.

PRO-BONE will employ different and well known technological devices and methods such as DXA, BodPod, imaging bone ultrasonometer and triaxial accelerometers

Table 1 Plyometric jump training progression

Level	Exercise	Ankle weights (kg)	Repetitions	³ Sets/day (⁴ rest)	⁵ Trainings/week	Jumps/week
1	¹ CMJ	-	10	3	3	180
	² SJ	-	10	3	3	
Total level	1 (12 weeks)				180 x 12 =	2160
2	CMJ	1	10	4	3	240
	SJ	1	10	4	3	
Total level	2 (12 weeks)				240 x 12 =	2880
3	CMJ	2.5	10	4	4	320
	SJ	2.5	10	4	4	
Total level	3 (12 weeks)				320 x 12 =	3840
Total interv	rention (36 weeks)					8880

¹Countermovement jump, ²Squat jump, ³1 set = 10 CMJ + 10 SJ, ⁴Rest between sets = 30 seconds.

Rest between exercises = 1 minute, ⁵When 3 sets/day, jumps will be performed in the morning before going to school (1 set), after school (1 set) and before going to bed (1 set). When 4 series, jumps will be performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1 set).

among others. In addition, the PJT will include a progression in intensity with ankle weights to maximize the potential to augment bone. PRO-BONE is timely as there is a lack of studies analysing the effects of PJT on bone health during the crucial this period of life. It represents a golden opportunity to measure how a simple, feasible and inexpensive PJT is associated with bone health in adolescents engaged in different sports. It will also show if the effect of this intervention differs between sports, expecting a greater effect in cyclists and swimmers than footballers. In addition, PRO-BONE will allow us to compare within each group and investigate changes in body composition in groups doing the PJT plus training vs groups only training. Finally, PRO-BONE will examine whether PIT has any additional effect on footballers. Football is considered one of the most osteogenic sports, but this type of intervention has not yet been studied.

Abbreviations

BMC: Bone mineral content; BMD: Bone mineral density; BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; BIA: Bioelectrical impedance analysis; BodPod: Air displacement plethysmography; BW: Body weight; SOC: Football players; SWI: Swimmers; CYC: Cyclists; CON: Control; DXA: Dual energy x-ray absorptiometry; PJT: Plyometric jump training; CMJ: Counter movement jump; SJ: Squad jump; P1NP: Procollagen type 1 aminoterminal propeptide; CTX-1: Carboxi-terminal telopeptide of type I collagen; LS: Lumbar spine; FN: Femoral neck; TB: Total body.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LGM (principal investigator), ARB and CAW contributed to the draft of the study. DV wrote the initial draft of the manuscript under the supervision of LGM, ARB and CAW. BSM, KMK will contribute to perform the analysis of the data obtained. All authors have read and approved this work.

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References

- Kanis JA, Johnell O. Requirements for DXA for the management of osteoporosis in Europe. Osteoporos Int. 2005;16(3):229–38.
- Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster JY, Borgstrom F, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2008;19(4):399–428.

- Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17(12):1726–33.
- Gafni RI, Baron J. Childhood bone mass acquisition and peak bone mass may not be important determinants of bone mass in late adulthood. Pediatrics. 2007;119 Suppl 2:S131–6.
- Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010;46(2):294–305.
- Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729–39.
- Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. Arch Dis Child. 2005;90(4):373–8.
- Perez-Lopez FR, Chedraui P, Cuadros-Lopez JL. Bone mass gain during puberty and adolescence: deconstructing gender characteristics. Curr Med Chem. 2010;17(5):453–66.
- Baroncelli Gl, Bertelloni S, Sodini F, Saggese G. Osteoporosis in children and adolescents: etiology and management. Paediatr Drugs. 2005;7(5):295–323.
- Henry YM, Fatayerji D, Eastell R. Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: relative contributions of bone size and volumetric bone mineral density.
 Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2004;15(4):263–73.
- Zofkova I. Role of genetics in prediction of osteoporosis risk. Vnitr Lek. 2011;57(1):78–84.
- 12. Branca F, Valtuena S. Calcium, physical activity and bone health-building bones for a stronger future. Public Health Nutr. 2001;4(1A):117–23.
- Vicente-Rodriguez G, Ezquerra J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP, Casajus JA, et al. Independent and combined effect of nutrition and exercise on bone mass development. J Bone Miner Metab. 2008;26(5):416–24.
- Valtuena J, Gracia-Marco L, Vicente-Rodriguez G, Gonzalez-Gross M, Huybrechts I, Rey-Lopez JP, et al. Vitamin D status and physical activity interact to improve bone mass in adolescents. Osteoporos Int: The HELENA Study; 2012.
- Vicente-Rodriguez G. How does exercise affect bone development during growth? Sports Med. 2006;36(7):561–9.
- Boreham CA, McKay HA. Physical activity in childhood and bone health. Br J Sports Med. 2011;45(11):877–9.
- Gracia-Marco L, Moreno LA, Ortega FB, Leon F, Sioen I, Kafatos A, et al. Levels of physical activity that predict optimal bone mass in adolescents the HELENA study. Am J Prev Med. 2011;40(6):599–607.
- Heinonen A: Biomechanics. In., 1st edn. Edited by Khan K; McKay H; Kannus P et al.: Champaign (IL). Physical activity and bone health: Human Kinetics; 2001: 23–34.
- Gustavsson A, Thorsen K, Nordstrom P. A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in healthy adolescent males. Calcified Tissue Int. 2003;73(2):108–14.
- DA Bailey MH, Mirwald RL, Crocker PR, Faulkner RA. A six year longitudinal study of the relationship of PA to BMAccrual in growing children.pdf> J Bone Miner Res. 1999;14(10):1672–9.
- Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. Bone. 2008;43(6):1101–7.
- Uzunca K, Birtane M, Durmus-Altun G, Ustun F. High bone mineral density in loaded skeletal regions of former professional football (soccer) players: what is the effect of time after active career? Br J Sports Med. 2005;39(3):154–7.
- Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, et al. Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study.
 J Bone Miner Res. 1998;13(12):1814–21.
- Wolff I, van Croonenborg JJ, Kemper HC, Kostense PJ, Twisk JW. The effect
 of exercise training programs on bone mass: a meta-analysis of published
 controlled trials in pre- and postmenopausal women. Osteoporos Int.
 1999:9(1):1–12.
- 25. Seeman E. An exercise in geometry. J Bone Miner Res. 2002;17(3):373-80.
- Krustrup P, Dvorak J, Junge A, Bangsbo J. Executive summary: the health and fitness benefits of regular participation in small-sided football games. Scand J Med Sci Sports. 2010;20 Suppl 1:132–5.
- 27. Ara I, Vicente-Rodriguez G, Perez-Gomez J, Jimenez-Ramirez J, Serrano-Sanchez JA, Dorado C, et al. Influence of extracurricular sport activities on

- body composition and physical fitness in boys: a 3-year longitudinal study. Int J Obes (Lond). 2006;30(7):1062–71.
- Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA. High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc. 2004;36(10):1789–95.
- Krustrup P, Hansen PR, Andersen LJ, Jakobsen MD, Sundstrup E, Randers MB, et al. Long-term musculoskeletal and cardiac health effects of recreational football and running for premenopausal women. Scand J Med Sci Sports. 2010;20 Suppl 1:58–71.
- Calbet JA, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez LP. High femoral bone mineral content and density in male football (soccer) players. Med Sci Sports Exerc. 2001;33(10):1682–7.
- Rico H, Revilla M, Villa LF, Gomez-Castresana F, Alvarez del Buergo M. Body composition in postpubertal boy cyclists. J Sports Med Phys Fitness. 1993;33(3):278–81.
- Stewart AD, Hannan J. Total and regional bone density in male runners, cyclists, and controls. Med Sci Sports Exerc. 2000;32(8):1373–7.
- 33. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody JN, Howman-Giles R. Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. Med Sci Sports Exerc. 2002;34(2):286–94.
- Duncan CS, Blimkie CJ, Kemp A, Higgs W, Cowell CT, Woodhead H, et al. Mid-femur geometry and biomechanical properties in 15- to 18-yr-old female athletes. Med Sci Sports Exerc. 2002;34(4):673–81.
- 35. Warner SE, Shaw JM, Dalsky GP. Bone mineral density of competitive male mountain and road cyclists. Bone. 2002;30(1):281–6.
- Nichols JF, Palmer JE, Levy SS. Low bone mineral density in highly trained male master cyclists. Osteoporos Int. 2003;14(8):644–9.
- Barry DW, Kohrt WM. BMD decreases over the course of a year in competitive male cyclists. J Bone Miner Res. 2008;23(4):484–91.
- Rector RS, Rogers R, Ruebel M, Hinton PS. Participation in road cycling vs running is associated with lower bone mineral density in men. Metabolism. 2008;57(2):226–32.
- Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Bone related health status in adolescent cyclists. PLoS One. 2011;6(9):e24841.
- 40. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Cycling and bone health: a systematic review. BMC Med. 2012;10:168.
- Andreoli A, Celi M, Volpe SL, Sorge R, Tarantino U. Long-term effect of exercise on bone mineral density and body composition in post-menopausal ex-elite athletes: a retrospective study. Eur J Clin Nutr. 2012;66(1):69–74.
- 42. Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. Joint Bone Spine. 2013;80(1):57–63.
- 43. Greenway KG, Walkley JW, Rich PA. Does long-term swimming participation have a deleterious effect on the adult female skeleton? Eur J Appl Physiol. 2012;112(9):3217–25.
- 44. Ferry B, Duclos M, Burt L, Therre P, Le Gall F, Jaffre C, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. J Bone Miner Metab. 2011;29(3):342–51.
- Dias Quiterio AL, Carnero EA, Baptista FM, Sardinha LB. Skeletal mass in adolescent male athletes and nonathletes: relationships with high-impact sports. J Strength Cond Res. 2011;25(12):3439–47.
- Tenforde AS, Fredericson M. Influence of sports participation on bone health in the young athlete: a review of the literature. PM R. 2011;3(9):861–7.
- 47. Scofield KL, Hecht S. Bone health in endurance athletes: runners, cyclists, and swimmers. Curr Sports Med Rep. 2012;11(6):328–34.
- Robling AG, Burr DB, Turner CH. Recovery periods restore mechanosensitivity to dynamically loaded bone. J Exp Biol. 2001;204(Pt 19):3389–99.
- McKay HA, MacLean L, Petit M, MacKelvie-O'Brien K, Janssen P, Beck T, et al. "Bounce at the Bell": a novel program of short bouts of exercise improves proximal femur bone mass in early pubertal children. Br J Sports Med. 2005;39(8):521–6.
- Mackelvie KJ, McKay HA, Khan KM, Crocker PR. A school-based exercise intervention augments bone mineral accrual in early pubertal girls. J Pediatr. 2001;139(4):501–8.
- MacKelvie KJ, Khan KM, Petit MA, Janssen PA, McKay HA. A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. Pediatrics. 2003;112(6 Pt 1):e447.
- Witzke KA, Snow CM. Effects of plyometric jump training on bone mass in adolescent girls. Medicine and science in sports and exercise. 2000;32(6):1051–7.

- 53. Jurimae J. Interpretation and application of bone turnover markers in children and adolescents. Curr Opin Pediatr. 2010;22(4):494–500.
- 54. Lima F, De Falco V, Baima J, Carazzato JG, Pereira RM. Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. Med Sci Sports Exerc. 2001;33(8):1318–23.
- Gracia-Marco L, Ortega FB, Jiménez-Pavón D, Rodríguez G, Valtueña J, Díaz-Marténez ÁE, et al. Contribution of bone turnover markers to bone mass in pubertal boys and girls. J Pediatr Endocrinol Metab. 2011;24:11–2.
- Gomez-Bruton A, Gonzalez-Aguero A, Gomez-Cabello A, Casajus JA, Vicente-Rodriguez G. Is bone tissue really affected by swimming? a systematic review. PLoS One. 2013;8(8):e70119.
- Stear SJ, Prentice A, Jones SC, Cole TJ. Effect of a calcium and exercise intervention on the bone mineral status of 16-18-y-old adolescent girls. Am J Clin Nutr. 2003;77(4):985–92.
- 58. Valtuena J, Gracia-Marco L, Vicente-Rodriguez G, Gonzalez-Gross M, Huybrechts I, Rey-Lopez JP, et al. Vitamin D status and physical activity interact to improve bone mass in adolescents The HELENA Study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2012;23(8):2227–37.
- Specker B, Vukovich M. Evidence for an interaction between exercise and nutrition for improved bone health during growth. Med Sport Sci. 2007;51:50–63.
- Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. Best Pract Res Clin Endocrinol Metab. 2011;25(4):573–84.
- Constantini NW, Dubnov-Raz G, Chodick G, Rozen GS, Giladi A, Ish-Shalom S. Physical activity and bone mineral density in adolescents with vitamin D deficiency. Med Sci Sport Exer. 2010;42(4):646–50.
- Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. J ClinEndocrinol Metab. 1999;84(12):4702–12.
- Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976:51(3):170–9.
- Ginty F, Rennie KL, Mills L, Stear S, Jones S, Prentice A. Positive, site-specific associations between bone mineral status, fitness, and time spent at high-impact activities in 16-to 18-year-old boys. Bone. 2005;36(1):101–10.
- Magkos F, Kavouras SA, Yannakoulia M, Karipidou M, Sidossi S, Sidossis LS.
 The bone response to non-weight-bearing exercise is sport-, site-, and sex-specific. Clin J Sport Med. 2007;17(2):123–8.
- 66. Ishikawa S, Kim Y, Kang M, Morgan DW. Effects of weight-bearing exercise on bone health in girls: a meta-analysis. Sports Med. 2013;43(9):875–92.
- Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, El-Hajj Fuleihan G, Kecskemethy HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom. 2014;17(2):225–42.
- 68. Damilakis J, Solomou G, Manios GE, Karantanas A. Pediatric radiation dose and risk from bone density measurements using a GE Lunar Prodigy scanner. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(7):2025–31.
- Winsley RJ, Fulford J, MacLeod KM, Ramos-Ibanez N, Williams CA, Armstrong N. Prediction of visceral adipose tissue using air displacement plethysmography in children. Obes Res. 2005;13(12):2048–51.
- Elberg J, McDuffie JR, Sebring NG, Salaita C, Keil M, Robotham D, et al. Comparison of methods to assess change in children's body composition. Am J Clin Nutr. 2004;80(1):64–9.
- McCrory MA, Gomez TD, Bernauer EM, Mole PA. Evaluation of a new air displacement plethysmograph for measuring human body composition. Med Sci Sports Exerc. 1995;27(12):1686–91.
- Siri WE. Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A, editors. Techniques for measuring body composition. Washington, DC: National Academy of Sciences, National Research Council; 1961. p. 223–34.
- 73. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition. 1993;9(5):480–91. discussion 480, 492.
- Jaworski M, Lebiedowski M, Lorenc RS, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int. 1995;56(5):368–71.
- Baroncelli Gl. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220–8.

- Talma H, Chinapaw MJ, Bakker B, HiraSing RA, Terwee CB, Altenburg TM. Bioelectrical impedance analysis to estimate body composition in children and adolescents: a systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. Obesity reviews: an official journal of the International Association for the Study of Obesity. 2013;14(11):895–905.
- Wu YT, Nielsen DH, Cassady SL, Cook JS, Janz KF, Hansen JR. Cross-validation of bioelectrical impedance analysis of body composition in children and adolescents. Phys Ther. 1993;73(5):320–8.
- Jurimae J, Maestu J, Jurimae T. Bone turnover markers during pubertal development: relationships with growth factors and adipocytokines. Med Sport Sci. 2010;55:114–27.
- Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, et al. International osteoporosis foundation and international federation of clinical chemistry and laboratory medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med. 2011;49(8):1271–4.
- Pérez-López F. Vitamin D and adolescent health. Adolescent Health, Medicine and Therapeutics. 2010;1:1–8.
- 81. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci. 1988;6(2):93–101.
- Castro-Pinero J, Artero EG, Espana-Romero V, Ortega FB, Sjostrom M, Suni J, et al. Criterion-related validity of field-based fitness tests in youth: a systematic review. Br J Sports Med. 2010;44(13):934–43.
- Ortega FB, Artero EG, Ruiz JR, Vicente-Rodriguez G, Bergman P, Hagstromer M, et al. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. Int J Obes (Lond). 2008;32 Suppl 5:S49–57.
- Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. J Sci Med Sport. 2013;16(2):124–8.
- 85. 85. Helmerhorst HJF, Brage S, Warren J, Besson H, Ekelund U: A systematic review of reliability and objective criterion-related validity of physical activity questionnaires. Int J Behav Nutr Phy 2012, 9
- Slootmaker SM, Schuit AJ, Chinapaw MJ, Seidell JC, van Mechelen W. Disagreement in physical activity assessed by accelerometer and self-report in subgroups of age, gender, education and weight status. Int J Behav Nutr Phys Act. 2009;6:17.
- Gunter K, Baxter-Jones AD, Mirwald RL, Almstedt H, Fuller A, Durski S, et al. Jump starting skeletal health: a 4-year longitudinal study assessing the effects of jumping on skeletal development in pre and circum pubertal children. Bone. 2008;42(4):710–8.
- Acero RM, Fernandez-del Olmo M, Sanchez JA, Otero XL, Aguado X, Rodriguez FA. Reliability of squat and countermovement jump tests in children 6 to 8 years of age. Pediatr Exerc Sci. 2011;23(1):151–60.
- Markovic G, Dizdar D, Jukic I, Cardinale M. Reliability and factorial validity of squat and countermovement jump tests. J Strength Cond Res. 2004;18(3):551–5.
- McKay H, Tsang G, Heinonen A, MacKelvie K, Sanderson D, Khan KM. Ground reaction forces associated with an effective elementary school based jumping intervention. Br J Sports Med. 2005;39(1):10–4.
- 91. Gracia-Marco L, Ortega FB, Jimenez Pavon D, Rodriguez G, Castillo MJ, Vicente Rodriguez G, et al. Adiposity and bone health in Spanish adolescents The HELENA. study Osteoporos Int. 2012;23(3):937–47.
- Guadalupe-Grau A, Perez-Gomez J, Olmedillas H, Chavarren J, Dorado C, Santana A, et al. Strength training combined with plyometric jumps in adults: sex differences in fat-bone axis adaptations. J Appl Physiol. 2009;106(4):1100–11.
- Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone. 2007;40(1):14–27.
- MacKelvie KJ, McKay HA, Petit MA, Moran O, Khan KM. Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. J Bone Miner Res. 2002;17(5):834–44.

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The Impact of Sport Participation on Bone Mass and Geometry in Male Adolescents

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ABSTRACT

VLACHOPOULOS, D., A. R. BARKER, C. A. WILLIAMS, S. A. ARNGRIÇMSSON, K. M. KNAPP, B. S. METCALF, I. G. FATOUROS, L. A. MORENO, and L. GRACIA-MARCO. The Impact of Sport Participation on Bone Mass and Geometry in Male Adolescents. Med. Sci. Sports Exerc., Vol. 49, No. 2, pp. 317–326, 2017. Purpose: Exercise is an effective approach for developing bone mass and adolescence is a key period to optimize bone health. However, sports-specific training may have different effects on bone outcomes. This study examined the differences on bone outcomes between osteogenic (football) and nonosteogenic (swimming and cycling) sports and a control group in male adolescents. **Methods**: One hundred twenty one males $(13.1 \pm 0.1 \text{ yr})$ were measured: 41 swimmers, 37 footballers, 29 cyclists, and 14 controls. Dual energy X-ray absorptiometry measured bone mineral density (BMD) and bone mineral content at lumbar spine, right and left hip, and total body. Hip Structural Analysis evaluated bone geometry at the femoral neck. Quantitative ultrasound evaluated bone stiffness at both feet. Results: Footballers had significantly higher BMD at total body less head (7%-9%), total hip (12%-21%), and legs (7%-11%) compared with all groups and significantly higher BMD at the femoral neck than controls (14%). Cyclists had higher BMD at the trochanter (10%) and bone mineral content at the arms (10%) compared with controls. Geometrical analysis showed that footballers had significantly higher cross-sectional area (8%–19%) compared with all groups, cross-sectional moment of inertia (17%) compared with controls and section modulus compared with cyclists (11%) and controls (21%). Footballers had significantly higher bone stiffness compared with all groups (10%-20%) at the dominant foot and (12%-13%) at the nondominant foot compared with swimmers and controls. Conclusions: Adolescent male footballers exhibited higher bone density, geometry, and stiffness compared with swimmers, cyclists and controls. Although swimmers and cyclists had higher bone outcomes compared with controls, these differences were not significant. Key Words: ADOLESCENCE, BONE MASS, BONE GEOMETRY, BONE STIFFNESS, EXERCISE

steoporosis is a disease characterized by reduced bone mass and deterioration of bone microarchitecture, resulting in increased risk of fragility fractures. Bone mass acquisition during adolescence is not only an important

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determinant of skeletal growth but also for reducing the risk of osteoporosis later in life (20). In this regard, a 10% increase in peak bone mass during adolescence might reduce the risk of fracture later in life by 50% and delay the onset of osteoporosis by 13 yr (27). Therefore, early prevention remains one of the most prudent approaches to improve bone health status in later adult life.

It is known that 20% of the variation in peak bone mass can be explained by lifestyle factors, including physical activity (PA) and diet (i.e., calcium and vitamin D intakes) (18,36). In terms of PA, a favorable osteogenic response can be obtained when high-impact, intensive, and weight-bearing exercise is performed, due to the mechanical load imposed on the bone tissue (14). For example, football is considered an "osteogenic" sport and augments bone mineral density (BMD) and bone mineral content (BMC) at the weight-bearing sites in early and late pubertal males (19). In contrast, sports such as swimming and cycling have been considered "nonosteogenic" (33), although the supporting

evidence is unclear. Previous evidence found that adolescent male swimmers to have lower adjusted BMC and BMD compared with controls (10). A recent systematic review concluded that swimmers have similar bone mass with sedentary controls (11). Similarly, although there are reports of cycling showing no effect on bone-related outcomes in adolescents, some studies suggest cycling during adolescence may negatively impact bone health and compromise the acquisition of a high peak bone mass (22). There is limited evidence evaluating the effects of osteogenic and nonosteogenic sports on bone outcomes in male adolescents, and further research is needed to investigate this discrepancy in the literature.

With football, swimming, and cycling among the most popular sports during childhood and adolescence in the United Kingdom, understanding the contribution of these sports to bone health is important. To date, studies evaluating bone-related outcomes in athletic groups have mainly focused in BMD and BMC outcomes provided by dualenergy X-ray absorptiometry (DXA). However, a more comprehensive evaluation of bone structure, as well researched can be obtained using the Hip Structural Analysis (HSA) software from DXA (3). The parameters obtained from HSA software reflect bone strength at the narrow neck site of the clinical important site of the hip. A previous study showed that adolescent female footballers had greater hip strength compared with swimmers and controls, whereas swimmers had lower bone mass at the narrow neck than footballers and controls (9). Another method to assess bone properties is quantitative ultrasound (QUS), which is a nonradiation technique and provides measurements of the bone stiffness changes at the calcaneus site. Currently, there are no studies evaluating bone outcomes in male adolescent athletes using a combination of DXA, HSA, and QUS outcomes. Furthermore, there is a lack of consistency when controlling for the use of confounding variables in the assessment of bone outcomes in youth sports. This is important because uncritical use of confounders can lead to size-related artefacts (25). Previous studies typically use confounders such as age, height, weight, calcium intake, fat mass, fat-free mass and lean mass (17,39). However, the most common inconsistencies observed in many studies are the lack of consideration for size adjustments in adolescent participants and the lack of site-specific adjustment of the skeletal outcomes. Therefore, more studies are needed to assess the bone outcomes by taking into account the relevant confounders according to participant characteristics.

The effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports (PRO-BONE) study was designed to investigate whether the bone properties, assessed by DXA, HSA, and QUS, differ between 12- and 14-yr-old boys who perform osteogenic (football) and nonosteogenic (swimming, cycling) sports in comparison to a control group after controlling for a comprehensive set of confounders. We hypothesized that male adolescent engaged in football will have higher bone outcomes compared to those engaged in cycling and swimming

and compared with a control group, and that male adolescent engaged in cycling and swimming will have similar bone outcomes.

METHODS

Study design and participants. The study represents a cross-sectional analysis of the baseline data derived from the PRO-BONE study, which is a 33-month longitudinal design including a 9-month jump intervention programme. The purpose, methodology, and sample size of the PRO-BONE study have been justified elsewhere (35). Data were collected between autumn and winter 2014/2015 in 121 male adolescents: 41 swimmers, 37 footballers, 29 cyclists, and 14 controls. The inclusion and exclusion criteria were: 1) boys 12–14 yr, engaged ($\geq 3 \text{ h·wk}^{-1}$) in osteogenic (football) and/or nonosteogenic (swimming and cycling) sports for the last 3 yr or more; 2) boys age 12-14 yr not engaged in any of these sports (≥3 h·wk⁻¹) in the last 3 or more years (control group); 3) participants not taking part in another clinical trial; 4) participants not having any acute infection lasting until < 1 wk before inclusion; 5) participants had to be free of any medical history of diseases or medications affecting bone metabolism or the presence of an injury; 6) white ethnicity.

Participants were recruited from athletic clubs and schools across the South West of England. Written informed consent and assent forms were signed from parents and participants accordingly, and all participants completed the first visit at the research centre as part of the study. The methods and procedures of the study have been checked and approved by:

1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496), 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766), and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

Anthropometry and sexual maturity. Stature (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK; precision, 0.1 cm; range, 60–210 cm) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK; precision, 0.1 kg; range, 2–200 kg), respectively. Body mass index was calculated as body mass (kg) divided by the stature squared (m²). Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair (30).

Dual energy X-ray absorptiometry. A DXA scanner (GE Lunar Prodigy Healthcare Corp., Madison, WI) was used to measure BMD (g·cm⁻²), BMC (g), bone area (BA) (cm²), fat mass (g) and lean mass (g). Four scans were performed to obtain data for the lumbar spine (LS, L1–L4), right and left hip (including femoral neck, Ward triangle, trochanter and shaft subregions; the mean of right and left hip scans was used), and the total body scan. The total body scan was then used to obtain data for specific regions, such as arms, legs, pelvis, and total body excluding head. All DXA

scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the GE encore software (2006, version 14.10.022). The positioning of the participants and the analyses of the results were undertaken according to International Society of Clinical Densitometry (4).

HSA. Using the HSA software, analyses were performed at the narrow neck region across the narrowest point of the femoral neck. The HSA program uses the distribution of bone mineral mass in line of pixels across the bone axis to measure the structural dimensions of bone cross sections (3). The geometric properties of the bone were obtained and the following variables used: 1) the cortical width neck (mm), which is the narrowest width of the femoral neck; 2) the diameter of the femoral neck (mm); 3) the cross-sectional area (CSA) (mm³), which is the total bone surface area excluding the soft tissue area and the trabecular; 4) the crosssectional moment of inertia (CSMI) (mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; 5) section modulus (mm³), which is an indicator of maximum bending strength in a cross section; and 6) the hip strength index, which is an advanced feature that has been added to the more recent versions of GE enCore software and indicates the risk of fracture forces generated during a fall on the greater trochanter and the CSA short-term precision percentage coefficient of variation has been reported to be between 2.4% and 7.9% (16).

QUS. QUS measurements were performed with a Lunar Achilles Insight (TM Insight GE Healthcare, Milwaukee, WI) and the OsteoReport PC (software version 5.x+). The stiffness index is then calculated by a linear combination of broadband ultrasound attenuation (BUA) and speed of sound (SOS) as follows: stiffness index = $(0.67 \times BUA) +$ $(0.28 \times SOS) - 420$. Both feet were measured twice, and the mean of the two measures was used for statistical analyses of the dominant and nondominant foot. For the purpose of this study, only stiffness index values were used. QUS is considered a valid and radiation-free method compared with DXA to assess bone health in children (2).

Physical activity and diet. PA was measured for seven consecutive days by using wrist accelerometers (GENEActiv, GENEA, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (24). Participants were instructed to place the accelerometer on their nondominant wrist, and data were collected at 100 Hz. Data were analyzed at 1 s epoch intervals to establish time spent in different intensities. Time spent in moderate PA and vigorous PA (VPA) was calculated using a cutoff point of 1140–3599 counts per minute and ≥3600 counts per minute, respectively (24). Moderate-to-vigorous PA (MVPA) was calculated using a cut-off point of ≥1140 counts per minute. Weekly training hours were obtained by face to face questions during the visit of the participants at the research centre.

Dietary calcium, vitamin D, and energy intake were assessed using a 24-h food recall. The validity and reliability of self-reported dietary intake has been previously reported in children (37). Total energy, calcium, and vitamin D intake were estimated using the CompEat Pro software (Nutrition systems, VIS Visual Information Systems Ltd., UK).

Statistical analysis. Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL), and descriptive data are reported as mean and SD. The distribution of the variables was checked and verified using Shapiro-Wilk test, skewness and kurtosis values, visual check of histograms, Q-Q, and box plots. The analysis of the data was completed in two stages: 1) raw (unadjusted) data using ANOVA with Bonferroni post hoc to detect betweengroup differences on bone-related outcomes (DXA, HSA, and QUS), and 2) adjusted data using one-way ANCOVA with Bonferroni post hoc taking into account the following relevant confounders: age, stature, region-specific lean mass (trunk, total body, arms and legs), calcium intake, and MVPA (12,13,32,37). A preliminary analysis showed maturation to have no effect on bone outcomes after accounting for age and thus was not included in the model. Percentages of difference between groups for all variables were used to quantify the magnitude of the differences. Statistical significance level was set at P < 0.05 and differences of P < 0.001were also indicated.

RESULTS

Descriptive characteristics of the study sample. Table 1 presents the descriptive characteristics of the participants. Swimmers were older, taller, heavier, and had more lean mass than the footballers. Footballers spent more time doing MVPA and VPA than swimmers and controls. Cyclists were older and spent more time doing VPA than controls, and they also spent more time doing MVPA and VPA than the swimmers. In addition, swimmers and footballers trained more hours on average than the cyclists. Finally, controls had more fat mass than all the other groups.

DXA region-specific BMD, BMC, and BA. Table 2 shows the raw differences for the four groups at different sites. Controls had significantly lower BMD and BMC compared with footballers (BMD, ranged from 6.7% to 30.1%; BMC, ranged from 18.1% to 52.4%), swimmers (BMD, 10.9% to 17.9%; BMC, 26.7% to 57.1%), and cyclists (BMD, 8.3% to 17.9%; BMC, 21.0% to 40.9%) for all sites except for the lumbar spine and arms. In addition, controls had significantly lower BA compared with swimmers (BA, 11.6% to 37.8%). Footballers had 7.5%, 10.4%, and 10.1% significantly higher BMD at total hip, trochanter, and Ward triangle sites than the swimmers. In addition, they had 7.8%, 10.4%, and 10.4% significantly higher BMD at total hip, trochanter, and Ward triangle sites than the cyclists. Finally, swimmers had 6.1% significantly higher BMD and 23.1% BMC than footballers at the arms as well as 8.9%, 9.9%, and 17.7% greater BA at the shaft, lumbar spine, and arms, respectively.

Figures 1, 2, and supplementary Table 1 (see Table, Supplemental Digital Content 1, adjusted data for DXA

TABLE 1. Descriptive characteristics of the participants.

Parameters	Swimmers $(n = 41)$	Footballers $(n = 37)$	Cyclists $(n = 29)$	Controls $(n = 14)$
Age (yr)	13.4 (1.0) ^{b,dd}	12.8 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
Stature (cm)	165.5 (9.7) ^{bb,d}	155.2 (9.3)	160.8 (9.9)	154.5 (9.9)
Body mass (kg)	52.4 (9.0) ^{bb}	44.3 (7.9)	49.5 (12.3)	48.3 (13.0)
BMI (kg·m ⁻²)	19.0 (1.7)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
Lean mass (kg)	41.6 (9.1) ^{b,dd}	35.4 (7.2)	37.7 (7.5)	31.7 (5.5)
Fat mass (kg)	8.3 (3.2)	6.6 (2.4)	8.6 (7.2)	14.1 (8.5) ^{a,bb,c}
Percentage of body fat (%)	17.1 (7.1)	15.8 (5.6)	17.8 (8.9)	29.0 (10.5) ^{aa,bb,cc}
Weekly training (h)	9.5 (5.1) ^{cc}	10.0 (2.3) ^{cc}	5.1 (2.1)	`- ´
Pubertal maturation (I/II/III/IV/V) (%)	(15/25/13/45/2)	(24/35/24/16/0)	(14/28/28/27/3)	(29/21/21/29/0)
MVPA (min·d $^{-1}$)	85.9 (30.4)	119.8 (29.7) ^{aa,d}	107.2 (33.3) ^a	83.2 (26.8)
VPA (min·d ⁻¹)	11.9 (7.3)	22.5 (9.0) ^{aa,dd}	18.5 (12.8) ^{a,d}	8.9 (4.0)
Energy intake (kcal·d ⁻¹)	2084.5 (560.6)	2093.7 (755.4)	2219.8 (843.4)	1748.9 (434.6)
Calcium intake (mg·d ⁻¹)	988.8 (429.7)	1017.9 (504.5)	1014.9 (601.9)	881.5 (380.7)
Vitamin D intake (µg·d -1)	1.84 (1.5)	1.82 (1.89)	1.95 (1.69)	1.52 (1.39)

Values presented as mean \pm SD.

Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls), a,b,c,d P < 0.05, aa,bb,cc,d P < 0.001. BMI, body mass index.

region-specific BMD, BMC and BA, http://links.lww.com/MSS/A756) present adjusted differences for the sports groups at different sites compared to the control group. Once the confounders were controlled for, differences remained significant and higher mainly in the football group compared with the other groups. More specifically, footballers had significantly higher BMD (8.8% to 25.1%) and BMC (7.9% to 29.5%) than controls at all sites except for the lumbar spine and arms. In addition, footballers had significantly higher BMD and BMC at all sites except for the lumbar spine and arms than swimmers (BMD, 6.9% to 13.9%; BMC, 8.4% to 20.5%) and cyclists (BMD, 5.2% to 12.7%; BMC, 6.7% to 18.9%). BA of footballers was significantly higher at pelvis

site compared with the other groups (7.1% to 8.9%). Cyclists had 10.3% significantly higher BMD only at the trochanter, 9.8% higher BMC, and 7.3% higher BA only at the arms compared with controls. There was no significant difference in the other skeletal sites between cyclists and controls. However, cyclists had nonsignificant higher bone outcomes (BMD, 3.4% to 11.0%; BMC, 1.1% to 11.8%) in the most sites of the skeleton. At lumbar spine, cyclist had nonsignificant lower BMC (-1.9%) compared with controls. No significant difference was found between swimmers and controls at any skeletal sites. However, swimmers had nonsignificant higher bone outcomes in most skeletal sites (BMD, 0.3% to 9.7%; BMC, 0.8% to 10.8%). At the lumbar spine, swimmers

TABLE 2. Raw data for DXA region-specific BMC (g), BMD (g·cm⁻²), and area (BA, cm²) of all participants.

Parameters		Swimmers $(n = 41)$	Footballers $(n = 37)$	Cyclists $(n = 29)$	Controls $(n = 14)$
TBLH	BMD	0.918 (0.067) ^d	0.931 (0.071) ^{dd}	0.905 (0.086) ^d	0.828 (0.071)
	BMC	1630.66 (333.56) ^d	1473.49 (338.60)	1498.27 (362.08)	1234.38 (347.86)
	BA	1762.63 (250.99) ^{b,d}	1564.89 (248.35)	1636.10 (261.69)	1469.43 (300.17)
Total hip	BMD	0.962 (0.107) ^{dd}	1.034 (0.085) ^{á,c,dd}	0.959 (0.114) ^{dd}	0.830 (0.116)
	BMC	28.87 (5.52) ^{dd}	28.78 (6.18) ^{dd}	27.59 (5.97) ^d	21.12 (5.55)
	BA	29.86 (3.83) ^d	27.60 (4.21)	28.54 (3.86)	25.14 (3.79)
Femoral neck	BMD	0.948 (0.098) ^d	1.001 (0.081) ^{dd}	0.975 (0.192) ^d	0.832 (0.118)
	BMC	4.46 (0.65) ^{dd}	4.53 (0.74) ^{dd}	$4.40 (0.79)^{d}$	3.52 (0.73)
	BA	4.70 (0.43) ^d	4.51 (0.46)	4.61 (0.43) ^d	4.21 (0.45)
Ward's triangle	BMD	0.928 (0.111) ^d	1.022 (0.096) a,c,dd	0.926 (0.127) ^d	0.799 (0.120)
, , , , , , , , , , , , , , , , , , ,	BMC	2.31 (0.49) ^d	2.40 (0.59) ^{dd}	$2.23 (0.55)^{d}$	1.64 (0.45)
	BA	2.48 (0.43) ^d	2.34 (0.44)	2.40 (0.42)	2.04 (0.36)
Trochanter	BMD	0.799 (0.089) ^{dd}	0.882 (0.078) ^{aa,cc,dd}	0.799 (0.108) ^{dd}	0.678 (0.098)
	BMC	9.08 (2.41) ^d	9.31 (2.67) ^{dd}	8.61 (2.39) ^d	6.11 (2.17)
	BA	11.26 (2.30) ^d	10.41 (2.34)	10.66 (2.15)	8.82 (2.12)
Shaft	BMD	1.100 (0.140) ^d	1.170 (0.109) ^{dd}	1.090 (0.132) ^d	0.941 (0.150)
	BMC	15.33 (2.62) ^{dd}	14.94 (2.97) ^{ád}	14.58 (2.98) ^d	11.49 (2.74)
	BA	13.91 (1.36) ^{b,d}	12.67 (1.65)	13.27 (1.51)	12.11 (1.49)
Lumbar spine	BMD	0.892 (0.114) ^d	0.883 (0.095)	0.867 (0.122)	0.791 (0.101)
·	BMC	43.26 (11.21) ^d	38.54 (8.93)	39.50 (11.04)	32.64 (8.67)
	BA	47.94 (7.37) ^{b,d}	43.17 (8.82)	44.88 (6.99)	40.79 (6.99)
Arms	BMD	0.784 (0.071) ^{b,dd}	0.736 (0.047)	0.747 (0.069) ^d	0.690 (0.049)
	BMC	244.93 (64.87) ^{bb,dd}	188.34 (48.05)	212.89 (59.27) ^d	155.89 (40.58)
	BA	308.22 (58.14) ^{bb,dd}	253.62 (51.89)	281.00 (58.00) ^d	223.71 (45.67)
Legs	BMD	1.091 (0.010) ^d	1.124 (0.106) ^{dd}	1.077 (0.116) ^d	0.975 (0.103)
-	BMC	779.05 (141.65) ^d	747.84 (175.02)	745.39 (179.21)	612.28 (179.74)
	BA	709.83 (80.64) ^d	657.46 (96.32)	684.24 (108.91)	617.50 (102.23)
Pelvis	BMD	0.994 (0.087) ^d	1.025 (0.103) ^{dd}	0.989 (0.130) ^d	0.888 (0.087)
	BMC	246.55 (57.43) ^d	238.35 (63.80) ^d	227.93 (63.75) ^d	174.81 (45.97)
	BA	245.85 (41.36) ^{dd}	229.19 (40.89) ^d	226.69 (37.38)	194.07 (34.96)

Values are presented as mean \pm SD

Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d P < 0.05 and aa,bb,cc,dd P < 0.001.

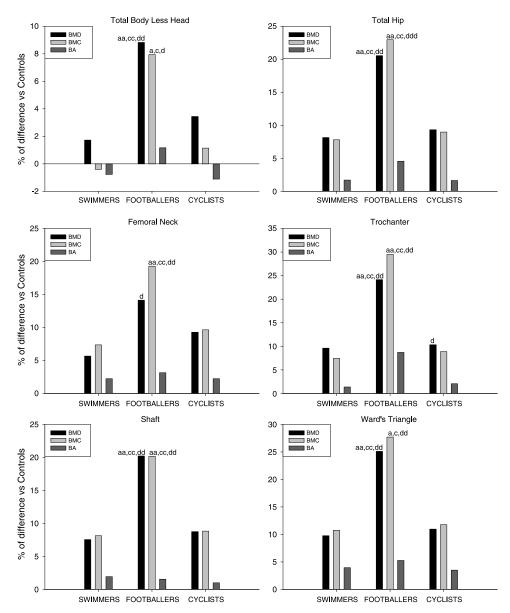


FIGURE 1—Difference (%) in adjusted BMD, BMC, and BA between the sports groups and controls at the total body less head and hip sites. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). $^{a,b,c,d}P < 0.05$ and $^{aa,bb,cc,dd}P < 0.001$.

had nonsignificant lower bone outcomes (BMD, -0.8%; BMC, -4.6%) compared with controls. Cyclists and swimmers had similar BMD, BMC, and BA (-0.9% to 5.0%) with no significant differences at any skeletal site.

Bone geometry—HSA. The adjusted geometrical differences in narrow neck site between the groups are presented in Figure 3, and the raw and adjusted values are presented in supplementary Table 2 (see Table, Supplemental Digital Content 2, raw and adjusted data for HSA and QUS, http://links.lww.com/MSS/A757). Footballers had a significantly higher CSMI than controls (17.4%), greater section modulus than cyclists (10.7%) and controls (21.0%), significantly higher CSA than swimmers (10.8%), cyclists (8.7%), and controls (19.3%) and a significantly greater hip strength index than swimmers (20.7%) and controls (38.9%). Cyclists had only a significantly higher hip strength index compared

with controls (28.6%). Cyclists had nonsignificant higher geometrical outcomes compared to controls (CSMI, 6.4%; section modulus, 9.3%; CSA, 9.8%). Swimmers had nonsignificant higher geometrical outcomes compared with controls (CSMI, 7.8%; section modulus, 10.9%; CSA, 7.6%, hip strength index: 15.1%). Cyclists compared with swimmers had similar geometrical outcomes with minimal differences.

QUS. The adjusted bone stiffness values of the dominant and nondominant foot are presented in Figure 4, and the raw differences are presented at supplementary Table 2 (see Table, Supplemental Digital Content 2, raw and adjusted data for HSA and QUS, http://links.lww.com/MSS/A757). Footballers had significantly higher bone stiffness in the dominant foot than swimmers (13.4%), cyclists (10.3%), and controls (20.1%). In addition, footballers had significantly greater bone stiffness than swimmers (12.2%) and

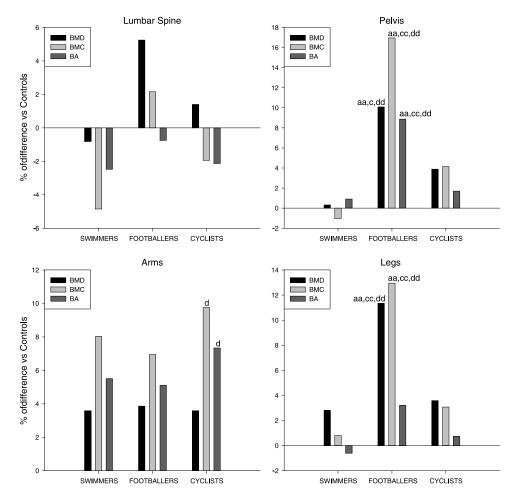


FIGURE 2—Difference (%) in adjusted BMD, BMC, and BA between the sports groups and controls at the lumbar spine, pelvis, arms, and legs. Letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). $^{a,b,c,d}P < 0.05$ and $^{aa,bb,cc,dd}P < 0.001$.

controls (12.9%) at the nondominant foot. No significant differences were found between dominant versus nondominant foot within each group of participants. Cyclists had higher (nonsignificant) stiffness index compared to controls in both dominant (8.9%) and nondominant foot (5.3%). Swimmers had higher (nonsignificant) bone stiffness at the dominant (5.9%) and the nondominant (0.7%) foot. Cyclists compared with swimmers had higher (nonsignificant) bone stiffness at the dominant (2.7%) and the nondominant (4.4%) foot.

DISCUSSION

The key findings from this study are: 1) footballers presented greater adjusted BMD and BMC including clinical relevant sites, an enhanced hip structural geometry at the narrow neck and a greater bone stiffness index compared to swimmers, cyclists, and controls; and 2) swimmers and cyclists had similar bone mass, geometry, and bone stiffness and both groups had higher but not significant bone outcomes compared to controls. The impact of osteogenic (football) and nonosteogenic sports (swimming and cycling)

on bone-related outcomes has not previously been compared in adolescent male athletes, and there is equivocal evidence on the effects of these sports on bone outcomes (10,22,34). To date, there are no studies published using a combination of methods, such as DXA, HSA, and QUS, to assess bone outcomes in this population, and there is a lack of studies taking into consideration the relevant confounders based on the characteristics of the groups studied. The findings of the present study therefore provide a more comprehensive assessment into the effect of sports participation on bone outcomes in male adolescents.

Bone outcomes in footballers versus controls.

Participation in osteogenic sports during adolescence can induce greater adjusted BMD compared with leisure active controls at many sites of the skeleton due to the mechanical loading applied (17). A previous study reported a 10.7% and 10.5% higher adjusted BMC at the total hip and lumbar spine, respectively, in prepubescent male football players (n = 39) compared with active controls (n = 13) (40). The magnitude of the differences might differ among studies due to the use of different confounders and the characteristics of the participants.

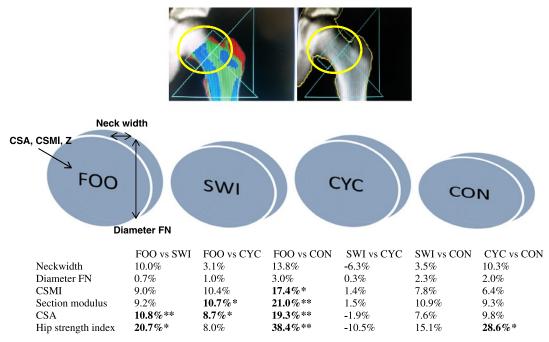


FIGURE 3—Percentage of difference in adjusted geometrical parameters of the narrow neck site between groups. Neck width (mm), *P < 0.05 and **P < 0.001. FN, femoral neck.

In parallel with the findings for BMD and BMC, the geometrical adaptations examined by HSA at the narrow neck of the femoral neck also supported the higher bone geometry in footballers (Fig. 3). One study in oligoamenorrheic female athletes showed that engagement in weight-bearing sports for 4 h·wk⁻¹ resulted in significantly higher CSMI and section modulus compared to nonathletes (1), which is consistent with the improved structural rigidity we found in footballers.

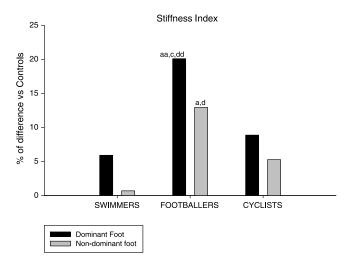


FIGURE 4—Difference (%) in adjusted stiffness index (SI) (mean \pm SE) between the sports groups and the controls at the dominant and nondominant foot. Letters denote a higher significant difference with: a (swimmers), c (cyclists) and d (controls). a,b,c,dP < 0.05 and aa,bb,cc,ddP < 0.001. No differences observed between dominant and non-dominant foot at the groups.

Previous studies using QUS technique observed positive associations between PA and calcaneal bone stiffness index in a sample of Flemish children and adolescents (5). Our results are in agreement with a study reporting that child and adolescent football players have significantly higher QUS parameters at lower extremities compared with age-matched controls (7).

Bone outcomes in swimmers versus controls. A recent meta-analysis of 14 studies summarized that swimming does not induce improvements in BMD during childhood and adolescence, and that swimmers present similar BMD compared with sedentary controls (11). We found that similar BMD and BMC between swimmers and active controls concurs with this meta-analysis, which presents neutral effects of swimming on BMD and BMC at most skeletal sites of the skeleton compared with active controls. The latter could be due to the fact that swimmers and controls have similar bone profile as muscle contraction is not enough to produce bone adaptations (17).

The HSA at the narrow neck site showed that adolescent male swimmers have similar bone geometry parameters compared with active controls. To our knowledge, there is no previous evidence using the HSA technique in adolescent male swimmers. Only one study have used HSA in elite adolescent female swimmers and showed that they had similar bone geometry compared with controls (17). The latter study highlighted the importance of lean body mass because it was highly correlated with CSA and hip strength index.

Regarding QUS, we found similar bone stiffness index in both dominant and nondominant foot of swimmers compared with controls. A previous study in adolescents reported similar QUS parameters between swimmers and controls and indicated that bone adaptations due to swimming might be counterbalanced by other weight-bearing activities (up to 3 h·wk⁻¹) (10); however, this cannot be the case in our study because the QUS parameters were controlled for MVPA.

Bone outcomes in cyclists versus controls. A systematic review revealed that road cyclists did not have any osteogenic benefits due to the nonmechanical loading character of the sport (23). A previous study conducted in adolescent female cyclists showed they had similar BMD compared with nonathletic controls after adjusting for years since menarche, lean mass, and sport specific training (6). According to our study, the skeletal differences between cyclists and active controls are site dependent and, more specifically, we found significantly greater BMC at the arms after controlling for region-specific lean mass and MVPA among other confounding factors.

There is no previous evidence using HSA technique in adolescent cyclists, and only a few studies used volumetric bone parameters. One study conducted in adolescent female cyclists found no significant differences in CSA and CSMI in cyclists compared with controls (6), which is in accordance with our study. However, it should be noted that in our study the HSA revealed cyclists had significantly higher hip strength index than controls (28.6%), something that was not observed with BMD and BMC at any of the hip variables analyzed with DXA. This might be explained by the fact that geometrical bone outcomes may differ from BMD and BMC when using DXA.

The effect of cycling on bone properties using QUS has not been previously evaluated and to the best of our knowledge this study is first that provides evidence for this population. We did not find differences on stiffness index between cyclists and controls, but cyclist had nonsignificant higher stiffness index in both the dominant (8.9%) and nondominant (5.3%) foot compared with controls. Our results support the findings of previous studies that the loading pattern of sports participation may influence the bone stiffness index in adolescents (8).

Comparison of bone outcomes between footballers, swimmers, and cyclists. In the present study, the comparison between osteogenic (football) and nonosteogenic sports (cycling and swimming) showed that adolescent male footballers had significantly greater adjusted BMD and BMC compared with swimmers and cyclists at all sites of the skeleton except for the lumbar spine and the arms. A previous study in athletic female adolescent reported that 3 h·wk⁻¹ of football participation induced greater improvements in height and lean mass adjusted BMD and BMC compared with swimmers at femoral neck and other sites of the skeleton (32). Only one study investigated the effects on bone mass between adolescent male footballers and swimmers and reported greater BMD at the femoral neck site in the footballers (28). To our knowledge, no previous evidence exists on the assessment of bone mass between footballers and cyclists in adolescents. Only one study in children reported positive associations between BMD and football

participation and negative associations found between BMD and cycling participation (29).

In our study, there was no significant difference observed in BMD and BMC between adolescent male swimmers and cyclists at any site of the skeleton. A recent review summarised the impact of sport participation on peak bone mass, and it reported that both swimming and cycling may not be associated with significant improvements in bone health (31). The comparison of BMD and BMC between swimmers and cyclists has been assessed only once in female adolescents, reporting similar values at all skeletal sites after taking into account potential confounders (6). The differences observed in the current study are likely to be explained by the nonweight-bearing environment of both swimming and cycling and by the mechanical loading of the skeleton according to the impact of produced by the sports-specific patterns. In addition, weight training and the plyometric exercises might induce higher bone mass in adolescent athletes (15). A study in adolescent swimmers has shown that participation of adolescent swimmers in other weight-bearing sports or activities involving plyometric exercises can induce higher BMD and BMC (10). In our study, a subsample of our participants has been asked about weight training, and we have shown that almost all footballers were involved in plyometric exercise training. A large number (70.7%) of the swimmers reported participation in plyometric training, but only a few cyclists (37.9%) were doing plyometric exercises. The participation in plyometric training or other weight-bearing activities might explain the difference on bone outcomes between adolescent athletes and needs further investigation to quantify the impact of weight training on bone outcomes.

In parallel with the BMD and BMC findings, the bone geometry evaluated by HSA at the narrow neck of the femoral neck was also higher in footballers compared with swimmers and cyclists. Previous research in adolescent female footballers and swimmers showed that the CSA area and section modulus were significantly higher in footballers compared with swimmers at the narrow neck site which is in agreement with our results (9). There is no evidence comparing football and cycling in children and adolescents and the only evidence exists in young females (21–28 yr) which found that footballers had approximately 10% higher CSA compared with cyclists and after adjusting for age, weight, and height, these results are in accordance with our findings (21).

In relation to QUS parameters, we found improved bone stiffness in footballers compared with cyclists and swimmers at the dominant foot and higher bone stiffness in footballers compared to swimmers at the nondominant foot. Because there is no evidence using QUS in similar age athletic groups, we identified a study in young adults (18–22 yr) that reported higher bone stiffness in footballers compared with swimmers at the dominant and nondominant heels (38) which complies with the findings of the present study.

The strengths of the current study are 1) the investigation of bone outcomes across three male adolescent athletic groups that were not compared before; 2) the combination of DXA, HSA software, and QUS outcomes, which provides a thorough insight of the differences in BMD, BMC, bone geometry, and bone stiffness; 3) the rigorous methodology and strong internal validity to control for specific confounders. It should be noted that the limitation of the crosssectional study precludes any determination of causality in our findings. Nevertheless, our population had strict age inclusion criteria and sport participation characteristics. The limitations of the self-reported maturation assessment should be noted. Most of the participants of our control group met the physical activity guidelines due to inclusion criteria used, but we know that most adolescents of this age do not meet the guidelines (26). However, in this specific case, it seems reasonable to propose that sport participation could have different effects on bone-related outcomes depending on the characteristics of the sport practiced. Therefore, more studies are needed to focus on the determinants affecting bone health for athletic groups during youth.

CONCLUSIONS

This study is the first to investigate the impact of weightbearing (football) and non-weight-bearing sports on bone outcomes in male adolescents. The findings of this study indicate that participation in weight-bearing sports, such as football, can induce greater improvements in bone mass, bone geometry, and stiffness index compared with non-weight-bearing sports, such as swimming and cycling and compared with controls. Swimmers and cyclists had similar bone outcomes, and both groups had higher bone outcomes compared with controls, but these differences were not statistically significant. These findings add to the sport participation recommendations that specific musculoskeletal training may affect the bone development during adolescence. Further longitudinal analyses of the specific sports are needed for this population to identify if these effects will be different after a longer period of sports participation.

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Author's Contribution: D. V. obtained and analysed the data and drafted the manuscript under the supervision of L. G. M. (principal investigator). A. R. B. and C. A. W., B. S. M., K. M. K., S. A. A., I. G. F., and L. A. M. reviewed the draft. All authors have read and approved this work.

Ethics approval and consent to participate: Ethics approval received from the following committees: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West ã Cornwall & Plymouth, ref. number 14/SW/0060).

Conflict of interest: The authors declare that they have no competing interests.

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and the present study do not constitute endorsement by ACSM.

REFERENCES

- 1. Ackerman KE, Pierce L, Guereca G, et al. Hip structural analysis in adolescent and young adult oligoamenorrheic and eumenorrheic athletes and nonathletes. J Clin Endocrinol Metab. 2013;98(4):1742-9.
- 2. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220-8.
- 3. Beck TJ, Ruff CB, Warden KE, Scott WW Jr, Rao GU. Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol. 1990;25(1):6-18.
- 4. Crabtree NJ, Arabi A, Bachrach LK, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom. 2014;17(2):225-42.
- 5. De Smet S, Michels N, Polfliet C, et al. The influence of dairy consumption and physical activity on ultrasound bone measurements in Flemish children. J Bone Miner Metab. 2015;33(2):192-200.
- 6. Duncan CS, Blimkie CJ, Kemp A, et al. Mid-femur geometry and biomechanical properties in 15- to 18-yr-old female athletes. Med Sci Sports Exerc. 2002;34(4):673-81.
- 7. Falk B, Braid S, Moore M, Yao M, Sullivan P, Klentrou N. Bone properties in child and adolescent male hockey and soccer players. J Sci Med Sport. 2010;13(4):387-91.
- 8. Falk B, Bronshtein Z, Zigel L, Constantini NW, Eliakim A. Quantitative ultrasound of the tibia and radius in prepubertal and early-pubertal female athletes. Arch Pediatr Adolesc Med. 2003; 157(2):139-43.

- 9. Ferry B, Duclos M, Burt L, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. J Bone Miner Metab. 2011;29(3):342-51.
- 10. Gómez-Bruton A, González-Agüero A, Gómez-Cabello A, Matute-Llorente A, Casajús JA, Vicente-Rodríguez G. The effects of swimming training on bone tissue in adolescence. Scand J Med Sci Sports. 2015;25(6):e589-602.
- 11. Gomez-Bruton A, Montero-Marín J, González-Agüero A, et al. The effect of swimming during childhood and adolescence on bone mineral density: a systematic review and meta-analysis. Sports Med. 2016;46(3):365-79.
- 12. Gracia-Marco L, Moreno LA, Ortega FB, et al. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. Am J Prev Med. 2011;40(6):599-607.
- 13. Gracia-Marco L, Ortega FB, Jiménez-Pavón D, et al. Adiposity and bone health in Spanish adolescents. The HELENA study. Osteoporos Int. 2012;23(3):937-47.
- 14. Greene DA, Naughton GA. Adaptive skeletal responses to mechanical loading during adolescence. Sports Med. 2006;36(9):723-32.
- 15. Gunter K, Baxter-Jones AD, Mirwald RL, et al. Impact exercise increases BMC during growth: an 8-year longitudinal study. J Bone Miner Res. 2008;23(7):986-93.
- 16. Khoo BC, Beck TJ, Qiao QH, et al. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multi-center clinical trials. Bone. 2005;37(1):112-21.

- Maïmoun L, Coste O, Philibert P, et al. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. *Metabolism*. 2013;62(8):1088–98.
- 18. Michalopoulou M, Kambas A, Leontsini D, et al. Physical activity is associated with bone geometry of premenarcheal girls in a dose-dependent manner. *Metabolism*. 2013;62(12):1811–8.
- Nebigh A, Rebai H, Elloumi M, et al. Bone mineral density of young boy soccer players at different pubertal stages: relationships with hormonal concentration. *Joint Bone Spine*. 2009;76(1):63–9.
- Nikander R, Sievänen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: a systematic review and meta-analysis for optimising bone strength throughout life. BMC Med. 2010;8:47.
- Nikander R, Sievänen H, Heinonen A, Kannus P. Femoral neck structure in adult female athletes subjected to different loading modalities. *J Bone Miner Res.* 2005;20(3):520–8.
- Olmedillas H, González-Agüero A, Moreno LA, Casajús JA, Vicente-Rodríguez G. Bone related health status in adolescent cyclists. *Plos One*. 2011;6(9):e24841.
- Olmedillas H, González-Agüero A, Moreno LA, Casajus JA, Vicente-Rodríguez G. Cycling and bone health: a systematic review. BMC Med. 2012;10:168.
- Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. J Sci Med Sport. 2013;16(2):124–8.
- 25. Prentice A, Parsons TJ, Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *Am J Clin Nutr*. 1994;60(6):837–42.
- Riddoch CJ, Mattocks C, Deere K, et al. Objective measurement of levels and patterns of physical activity. *Arch Dis Child*. 2007; 92(11):963–9.
- Rizzoli R, Bianchi ML, Garabédian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone*. 2010;46(2):294–305.
- Silva CC, Goldberg TB, Teixeira AS, Dalmas JC. The impact of different types of physical activity on total and regional bone mineral density in young Brazilian athletes. J Sports Sci. 2011;29(3):227–34.
- Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CC Jr. Role of physical activity in the development of skeletal mass in children. J Bone Miner Res. 1991;6(11):1227–33.

- Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*. 1976;51(3):170–9.
- 31. Tenforde AS, Sainani KL, Carter Sayres L, Milgrom C, Fredericson M. Participation in ball sports may represent a prehabilitation strategy to prevent future stress fractures and promote bone health in young athletes. *PM R*. 2015;7(2):222–5.
- 32. Ubago-Guisado E, Gómez-Cabello A, Sánchez-Sánchez J, García-Unanue J, Gallardo L. Influence of different sports on bone mass in growing girls. *J Sports Sci.* 2015;33(16):1710–8.
- Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA. High femoral bone mineral density accretion in prepubertal soccer players. *Med Sci Sports Exerc*. 2004; 36(10):1789–95.
- 34. Vicente-Rodriguez G, Dorado C, Perez-Gomez J, Gonzalez-Henriquez JJ, Calbet JA. Enhanced bone mass and physical fitness in young female handball players. *Bone*. 2004;35(5):1208–15.
- 35. Vlachopoulos D, Barker AR, Williams CA, Knapp KM, Metcalf BS, Gracia-Marco L. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. *BMC Public Health*. 2015;15:361.
- Vlachopoulos D, Gracia-Marco L, Barker AR, Huybrechts I, Moreno LA, Mouratidou T. Bone Health: The independent and combined effects of calcium, vitamin D and exercise in children and adolescents. In: Preedy VR, editor. *Calcium: Chemistry, Analysis, Function and Effects*. London, UK: The Royal Society of Chemistry; 2016. pp. 530–46.
- Weber JL, Lytle L, Gittelsohn J, et al. Validity of self-reported dietary intake at school meals by American Indian children: the Pathways Study. *J Am Diet Assoc.* 2004;104(5):746–52.
- Yung PS, Lai YM, Tung PY, et al. Effects of weight bearing and non-weight bearing exercises on bone properties using calcaneal quantitative ultrasound. *Br J Sports Med*. 2005;39(8):547–51.
- 39. Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the bone mineral density in childhood study. *J Clin Endocrinol Metab*. 2011;96(10):3160–9.
- 40. Zouch M, Jaffré C, Thomas T, et al. Long-term soccer practice increases bone mineral content gain in prepubescent boys. *Joint Bone Spine*. 2008;75(1):41–9.

Determinants of Bone Outcomes in Adolescent Athletes at Baseline: The PRO-BONE Study

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ABSTRACT

VLACHOPOULOS D., E. UBAGO-GUISADO, A. R. BARKER, B. S. METCALF, I. G. FATOUROS, A. AVLONITI, K. M. KNAPP, L. A. MORENO, C. A. WILLIAMS, and L. GRACIA-MARCO. Determinants of Bone Outcomes in Adolescent Athletes at Baseline: The PRO-BONE Study. Med. Sci. Sports Exerc., Vol. 49, No. 7, pp. 1389–1396, 2017. Purpose: The determinants of areal bone mineral density (aBMD) and hip geometry estimates in adolescent athletes are poorly understood. This study aimed to identify the determinants of aBMD and hip geometry estimates in adolescent male athletes. **Methods**: One hundred twenty-one men (13.1 ± 0.1 yr) were measured: 41 swimmers, 37 footballers, 29 cyclists, and 14 controls. Dual energy X-ray absorptiometry measured aBMD at lumbar spine, femoral neck and total body. Hip structural analysis evaluated hip geometry estimates at the femoral neck. Multiple linear regression examined the contribution of the sports practised, stature, lean and fat mass, serum calcium and vitamin D, moderate to vigorous physical activity, vertical jump and cardiorespiratory fitness with aBMD and hip geometry estimates. Results: Region-specific lean mass was the strongest positive predictor of aBMD ($\beta = 0.614-0.931$) and football participation was the next strongest predictor $(\beta = 0.304 - 0.579)$. Stature $(\beta = 0.235 - 0.380)$, fat mass $(\beta = 0.189)$, serum calcium $(\beta = 0.103)$, serum vitamin D $(\beta = 0.104 - 0.139)$, and vertical jump ($\beta = 0.146-0.203$) were associated with aBMD across various specific sites. All hip geometry estimates were associated with lean mass ($\beta = 0.370 - 0.568$) and stature ($\beta = 0.338 - 0.430$). Football participation was associated with hip crosssectional area ($\beta = 0.322$) and moderate to vigorous physical activity ($\beta = 0.140-0.142$). Cardiorespiratory fitness ($\beta = 0.183-0.207$) was associated with section modulus and cross-sectional moment of inertia. Conclusions: Region-specific lean mass is the strongest determinant of aBMD and hip geometry estimates in adolescent male athletes. Football participation and stature were important determinants for aBMD and hip geometry estimates, whereas the contribution of the other predictors was site specific. Kev Words: BODY COMPOSITION, BONE MASS, LEAN MASS, PREDICTORS, SPORT PARTICIPATION

uring growth and maturation changes in bone density and geometry occur to withstand the forces applied through external loading of the skeleton (9). Peak bone mass is achieved by early adulthood and is largely determined by nonmodifiable genetic factors (4). However, modifiable factors, such as nutrition (24) and physical activity (11), are also known to alter peak bone mass. Exercise can significantly enhance areal bone mineral density (aBMD) and strength at loaded sites in children but not in adults (25).

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Optimal bone development can be achieved with adequate status of key nutrients, such as calcium and vitamin D, and may attenuate exercise-induced adaptations of aBMD (15). The type of sport practised can affect the skeletal development differently depending on training characteristics (35). Participation in weight-bearing sports, such as football, is associated with greater aBMD than non—weight-bearing sports, such as swimming and cycling (8,38). However, it is poorly understood why the differences exist between different athletic groups and there is limited understanding of the determinants of aBMD and hip geometry in adolescent male athletes.

Total body lean mass has a positive association with aBMD in the growing skeleton (10) but controversy currently exists surrounding the association between fat mass and aBMD (6). There is no evidence distinguishing the site-specific effects of lean mass and fat mass on aBMD in adolescent athletes and there are inconsistencies in the use of confounders to adjust bone parameters in nonathletic groups. Data on nonathletic female prepubescents indicate that leg lean mass is the most important predictor of bone

mineral content at the leg and FN sites (3). Although a positive association between fat mass and aBMD has been reported in nonathletic male and female adolescents (31), this is explained by an increase in lean mass (12). To date, there is no evidence explaining the effects of lean and fat mass on bone outcomes in adolescent athletes, which is of great interest due to the importance of body composition in athletic groups. Cardiorespiratory fitness (CRF) and muscular fitness (vertical jump) have also been found to be positively associated with bone outcomes in nonathletic adolescents (1,13), but their contribution on bone parameters in adolescent athletes is poorly understood.

Geometric properties of the hip, such as cross-sectional area (CSA), obtained by using hip structural analysis (HSA) software, can provide further insight into the determinants of bone hip geometry estimates (19). During growth, bones adapt their geometry due to increases in stature, lean and fat mass (3) and geometric parameters of the femur neck (FN) are closely adapted to lean mass (27). The primary predictor of bone hip geometry in nonathletic boys and girls is muscle CSA, accounting for 10% to 16% of the variance (22), whereas other factors, such as moderate to vigorous physical activity (MVPA), can have a site-specific influence on bone geometry (23).

As highlighted above, numerous factors have been shown to be related to bone outcomes in nonathletic adolescents, but the determinants of aBMD and hip geometry in adolescent male athletes have yet to be comprehensively investigated. Therefore, this study aims to provide novel insight into the contribution of the independent predictors of sports participation (football, swimming and cycling), stature, region-specific lean and fat mass, serum calcium and vitamin D, MVPA, muscular fitness and CRF (all adjusted by each other) on aBMD and hip geometry estimates in adolescent male athletes. It is hypothesized that football participation, lean mass and stature would be the most important determinants of aBMD and hip geometry estimates in adolescent male athletes. It is proposed that other modifiable factors (e.g., nutrition, MVPA and fitness) would have a small but significant contribution on bone outcomes.

METHODS

Study design and participants. Participants comprised 121 male adolescents (41 swimmers, 37 footballers, 29 cyclists and 14 controls) participating in the PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) longitudinal study (39). The data in the current study are taken from the baseline data of the PRO-BONE study and was completed between autumn and winter 2014/2015. The inclusion and exclusion criteria were: 1) boys 12 to 14 yr old, engaged (≥3 h·wk⁻¹) in osteogenic (football) and/or nonosteogenic (swimming and cycling) sports for the last 3 yr or more; 2) active boys 12–14 yr old who were not engaged in football, cycling and swimming (≥3 h·wk⁻¹) in the last 3 or more

years but who were physically active (control group); 3) not taking part in another clinical trial; 4) not having an acute infection lasting until < 1 wk before inclusion; 5) to be free of any medical history of diseases or medications affecting bone metabolism; 6) to be white Caucasian.

Participants were recruited from athletic clubs and schools across the southwest of England. Written informed consent and assent forms were signed from parents and participants accordingly and all participants completed the first visit at the research centre as part of the study. The methods and procedures of the study have been checked and approved by:

1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496);

2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

Dual energy X-ray absorptiometry. A dual energy X-ray absorptiometry (DXA) scanner (GE Lunar Prodigy Healthcare Corp., Madison, WI) was used to measure aBMD (g·cm⁻²), fat mass (g) and lean mass (g) at specific regions of the body. Four scans were performed to obtain data for the lumbar spine (LS, L1-L4), bilateral proximal femora (the mean of both was used for the current analysis) and the total body. The total body scan was then used to obtain data for specific regions such as: arms, legs and total body less head (TBLH). All DXA scans and subsequent insoftware analyses were completed by the same researcher, using the same DXA scanner and the GE encore software (2006, version 14.10.022).

Hip structural analysis. Hip geometry estimates at the FN were determined using HSA software which analyses the distribution of bone mineral mass in a line of pixels across the bone axis. The hip geometry estimates of the bone were obtained and the following variables used: 1) the cross sectional area (CSA, mm²), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia (CSMI, mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; and 3) section modulus (Z, mm³), which is an indicator of maximum bending strength in a cross section. The short term precision percentage coefficient of variation of these variables has been reported to be between 2.4% and 10.1% (19).

Anthropometry, physical activity, and nutritional markers. Stature (cm) and body mass (kg) were measured using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Body mass index was calculated as body mass (kg) divided by the stature (m) squared. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair (34).

Physical activity was measured for seven consecutive days using validated wrist accelerometers (GENEActiv, GENEA, UK) (7). Participants were instructed to place the accelerometer on their nondominant wrist and data was collected at 100 Hz. Data were analyzed using a 1-s epoch to establish time spent in MVPA using a cut-off point of ≥1140 counts per minute previously validated in youth (28).

Total serum levels of calcium and 25 hydroxyvitamin D [25(OH)D] were analyzed. Serum samples were analyzed by using ELISA kits (Abbexa Ltd., Cambridge, UK) for 25(OH)D and had a test range of 3 to 80 μ g·mL⁻¹ and a sensitivity of 1.2 μ g·mL⁻¹ (inter-assay and intra-assay CVs: 5.7% and 9.5%, respectively). Total serum levels of calcium was measured using direct colorimetric assay (Cayman Chemical Company, MI) and had linear assay range of 0.25 to 10 mg·mL⁻¹ (inter-assay and intra-assay CVs: 8.1% and 12.8%, respectively).

Physical fitness. The fitness tests used in the present investigation have been shown to be reliable and valid in youth (26). A counter movement vertical jump test was used to provide an estimate of lower limb muscular power. The jumps were performed on a jump mat (Probotics Inc., Huntsville, AL) which calculates jump height based on flight time. Each participant performed three maximal vertical jumps and the highest jump was used for the analysis.

Cardiorespiratory fitness was evaluated using the 20-m shuttle run test (21). The test ended when the participants failed to reach the line on two consecutive occasions. The last completed shuttle determined the score of the test and the number of shuttles completed was taken as an indicator of CRF.

Statistical analyses. Data were analyzed using SPSS IBM statistics (version 21.0 for Windows, Chicago, IL) and descriptive data are reported as mean and standard deviation (SD). The normal distribution of the raw variables and of the regression model residuals was checked and verified using

Shapiro–Wilk test, skewness and kurtosis values, visual check of histograms, Q-Q and box plots. Collinearity was checked for the variables using the variance inflation factor (VIF) and tolerance levels. One-way ANOVA with Bonferroni *post hoc* comparisons and χ^2 tests were used to detect between-group mean differences for the descriptive variables (Table 1).

Multiple linear regression analyses were used to examine the contribution of sport participation, stature, lean mass, fat mass, total calcium, 25(OH)D, MVPA, vertical jump, and 20-m shuttle run test to bone outcomes. The selection of the predictors was based on their relationship with bone outcomes (22,27,32). To account for the differences between the sports groups a dummy variable was computed (footballers, swimmers, cyclists and controls) and controls were selected as the reference group. In a preliminary analysis we found that Tanner stage was not a significant predictor after adjusting for stature and age and consequently was not included in the model. All remaining predictors were entered into the regression models simultaneously. For the multiple linear regressions, the standardized regression coefficients (β) are reported and significance was set at alpha level of 0.05. The squared semi-partial correlation coefficients (sr²) were used to determine the contribution of each predictor in the overall variance of the model after removing shared contributions with other predictors.

RESULTS

Characteristics of the participants. The raw descriptive characteristics of the participants and the differences

TABLE 1. Descriptive characteristics of the participants.

Characteristics	All (n = 121)	Swimmers $(n = 41)$	Footballers $(n = 37)$	Cyclists $(n = 29)$	Controls $(n = 14)$
Age (yr)	13.1 (1.0)	13.4 (1.0) ^{b,dd}	12.8 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
Stature (cm)	159.9 (10.6)	165.5 (9.7) ^{bb,d}	155.2 (9.3)	160.8 (9.9)	154.5 (9.9)
Pubertal maturation (I/II/III/IV/V) (%)	(18/29/21/30/2)	(13/25/13/46/3)	(24/35/25/16)	(13/28/28/28/3)	(29/21/21/29)
Body composition	,	,	,	,	,
Body mass (kg)	48.7 (10.4)	52.4 (9.0) ^{bb}	44.3 (7.9)	49.5 (12.3)	48.3 (13.0)
BMI (kg·m ⁻²)	18.9 (2.3)	19.0 (1.7)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
Lean mass (kg)	37.6 (8.4)	41.6 (9.1) ^{b,dd}	35.4 (7.2)	37.7 (7.5)	31.7 (5.5)
Fat mass (kg)	8.5 (5.5)	8.3 (3.2)	6.6 (2.4)	8.6 (7.2)	14.1 (8.5) ^{a,bb,c}
Micronutrient status	, ,	• •		* *	, ,
Total calcium (mg·dL ⁻¹)	9.98 (0.41)	10.01 (0.46)	9.97 (0.4)	9.94 (0.41)	10.0 (0.35)
25(0H)D $(\mu g \cdot \hat{L}^{-1})$	14.13 (1.25)	13.75 (1.19)	14.44 (1.63)	14.38 (0.58)	13.92 (0.94)
Physical activity and fitness	, ,	, ,	, ,	, ,	, ,
$MVPA (min \cdot d^{-1})$	101.3 (33.8)	85.9 (30.4)	119.8 (29.7) ^{aa,d}	$107.2 (33.3)^a$	83.2 (26.8)
Vertical jump height (cm)	41.0 (6.7)	$42.3 (6.9)^d$	41.4 (6.0) ^d	41.0 (6.8)	35.9 (5.8)
CRF (no. shuttles)	69.3 (24.2)	69.6 (20.3) ^{dd}	82.9 (17.6) ^{a,c,dd}	69.6 (21.2) ^{dd}	31.8 (16.1)
Weekly training (h)	7.5 (4.8)	9.5 (5.1) ^{cc}	10.0 (2.3) ^{cc}	5.1 (2.1)	<u> </u>
Bone mineral density (DXA)	, ,	• •		* *	
TBLH BMD (g·cm ⁻²)	0.908 (0.079)	0.918 (0.067) ^d	0.931 (0.071) ^{dd}	$0.905 (0.086)^d$	0.828 (0.071)
Lumbar spine BMD (g⋅cm ⁻²)	0.872 (0.112)	0.892 (0.114) ^d	0.883 (0.095)	0.867 (0.122)	0.791 (0.101)
FN BMD (g·cm ⁻²)	0.9516 (0.110)	$0.948 (0.098)^d$	1.001 (0.081) ^{dd}	0.975 (0.192) ^d	0.832 (0.118)
Total hip BMD (g·cm ⁻²)	0.968 (0.119)	0.962 (0.107) ^{dd}	1.034 (0.085) ^{a,c,dd}	0.959 (0.116) ^{dd}	0.830 (0.116)
Legs BMD (g·cm ⁻²)	1.084 (0.113)	1.091 (0.010)	1.124 (0.106)	1.077 (0.116)	0.975 (0.103)
Arms BMD (g·cm ⁻²)	0.750 (0.068)	0.784 (0.071) ^{b,dd}	0.736 (0.047)	$0.747 (0.069)^d$	0.690 (0.049)
Bone geometry (HSA)	, ,	, ,		, ,	, ,
CSA (mm²)	134.9 (22.7)	137.2 (20.2) ^{dd}	140.9 (20.4) ^{dd}	135.9 (22.7) ^d	109.8 (21.0)
$Z (\text{mm}^3)$	530.9 (126.5)	558.3 (121.4) ^{dd}	548.1 (116.7) ^{dd}	530.8 (123.3) ^{dd}	395.0 (123.4)
CSMI (mm ⁴)	8331.5 (2644)	8943.5 (2574) ^d	8471.6 (2607) ^d	8403.1 (2552) ^d	6020.7 (2673)

Values presented as mean \pm SD.

Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls), a.b.c.dP < 0.05, aa.bb.cc.ddP < 0.001. BMI, body mass index; Z, section modulus; 25(0H)D, 25-hydroxyvitamin D.

between sports groups are presented in Table 1. Swimmers were significantly older, taller, heavier, and had more lean mass than the footballers and controls, and cyclists were significantly older than controls. All groups were similar for total serum calcium and 25(OH)D. Swimmers had significantly higher muscular and CRF than the controls. Footballers spent significantly more time in MVPA compared with swimmers and controls and had a significantly higher CRF compared with all the other groups. Cyclists had significantly higher MVPA than swimmers and significantly higher CRF than controls.

The raw unadjusted data showed that swimmers had significantly higher aBMD at the arms compared with footballers and higher aBMD at all sites except for the legs compared with controls. Footballers had significantly higher aBMD at TBLH, FN compared with controls and higher aBMD at TH compared with all groups. Cyclists had significantly higher aBMD at all sites expect LS and legs compared with controls. Swimmers, footballers, and cyclists had significantly enhanced hip geometry estimates compared with controls.

Determinants of bone density and hip geometry estimates. Multivariate regression models significantly explained 49.0%–76.4% (on average, 60.0%) of the variance in the aBMD outcomes (Table 2). Region-specific lean mass and football participation were consistently the strongest significant predictors of aBMD at TBLH, LS, TH, legs and

arms ($\beta = 0.614-0.931$, sr² = 0.031-0.161, P < 0.01). Football participation (compared with the control group) was positively associated with aBMD at TBLH, FN, TH, and legs $(\beta = 0.304 - 0.579, \text{ sr}^2 = 0.031 - 0.068, P < 0.01)$. Stature was positively associated with aBMD at FN and arms (β = 0.235-0.380, sr² = 0.021, P < 0.05). Region-specific fat mass was positively associated with aBMD at TBLH (β = 0.189, sr² = 0.015, P < 0.05). Serum calcium was positively associated with aBMD at the arms ($\beta = 0.103$, sr² = 0.009, P < 0.05). In addition, serum 25(OH)D was positively associated with aBMD at the arms and LS ($\beta = 0.104-0.139$, $sr^2 = 0.009$, P < 0.05). Muscular fitness was positively associated with aBMD at TBLH and LS ($\beta = 0.146-0.203$, sr² = 0.010-0.019, P < 0.05). CRF was not associated with aBMD outcomes at any skeletal site after accounting for the other predictors.

In the multivariate regression analysis of the hip geometry estimates (Table 3), the predictors explained 71.7%–77.8% (on average, 75.7%) of the variance. Region-specific lean mass was the strongest significant predictor and was positively associated with CSA, CSMI and section modulus Z ($\beta = 0.370$ –0.568, sr² = 0.017–0.039, P < 0.05). Football participation (compared with the control group) was positively associated with CSA ($\beta = 0.322$, sr² = 0.023, P < 0.01). Stature was positively associated CSA, CSMI, and Z ($\beta = 0.338$ –0.430, sr² = 0.017–0.025, P < 0.001). MVPA was positively associated with CSMI and Z ($\beta = 0.140$ –0.142,

TABLE 2. Multiple regression models for aBMD variables in adolescent male athletes.

	Predictors	$oldsymbol{eta}$ STD	sr² Values	P Values		Predictors	$oldsymbol{eta}$ STD	sr² values	P Values
TBLH aBMD ($R^2 = 0.75$)	Footballers	0.374	0.031	<0.001	Total hip aBMD ($R^2 = 0.53$)	Footballers	0.549	0.068	<0.001
,	Swimmers	0.077	0.002	0.404	, , ,	Swimmers	0.161	0.007	0.211
	Cyclists	0.139	0.006	0.114		Cyclists	0.212	0.014	0.080
	Stature	0.056	0.000	0.662		Stature	0.216	0.007	0.215
	Lean mass	0.617	0.045	< 0.001		Lean mass	0.226	0.006	0.238
	Fat mass	0.189	0.015	0.013		Fat mass	-0.020	0.000	0.857
	Calcium	0.082	0.006	0.125		Calcium	0.109	0.010	0.137
	25(OH)D	0.083	0.006	0.120		25(OH)D	0.035	0.001	0.628
	MVPA	-0.003	0.000	0.955		MVPA	0.026	0.000	0.741
	Vertical jump	0.146	0.010	0.043		Vertical jump	0.133	0.008	0.192
	CRF	0.136	0.006	0.115		CRF	0.102	0.003	0.382
Lumbar spine aBMD ($R^2 = 0.59$)	Footballers	0.094	0.002	0.475	Legs aBMD ($R^2 = 0.75$)	Footballers	0.304	0.034	<0.001
	Swimmers	-0.061	0.001	0.603		Swimmers	0.024	0.002	0.689
	Cyclists	-0.008	0.000	0.945		Cyclists	-0.061	0.003	0.347
	Stature	-0.090	0.001	0.582		Stature	0.068	0.001	0.596
	Lean mass	0.703	0.058	< 0.001		Lean mass	0.614	0.046	< 0.001
	Fat mass	-0.014	0.000	0.885		Fat mass	0.147	0.008	0.067
	Calcium	0.084	0.006	0.222		Calcium	0.055	0.003	0.305
	25(OH)D	0.139	0.016	0.043		25(OH)D	0.067	0.004	0.212
	MVPA	0.007	0.000	0.927		MVPA	-0.021	0.000	0.711
	Vertical jump	0.203	0.019	0.028		Vertical jump	0.107	0.005	0.153
	CRF	0.000	0.000	1.000		CRF	0.153	0.008	0.076
Femur neck aBMD ($R^2 = 0.49$)	Footballers	0.486	0.053	0.001	Arms aBMD ($R^2 = 0.76$)	Footballers	0.140	0.004	0.161
	Swimmers	0.131	0.005	0.326		Swimmers	0.170	0.008	0.058
	Cyclists	0.208	0.013	0.099		Cyclists	0.158	0.008	0.066
	Stature	0.380	0.021	0.038		Stature	0.235	0.013	0.016
	Lean mass	0.052	0.000	0.792		Lean mass	0.931	0.161	< 0.001
	Fat mass	0.074	0.002	0.515		Fat mass	0.132	0.007	0.069
	Calcium	0.077	0.005	0.311		Calcium	0.103	0.009	0.049
	25(OH)D	0.015	0.000	0.847		25(OH)D	0.104	0.009	0.045
	MVPA	0.039	0.001	0.635		MVPA	-0.037	0.001	0.503
	Vertical jump	0.184	0.015	0.083		Vertical jump	0.058	0.002	0.404
	CRF	0.154	0.008	0.208		CRF	0.066	0.002	0.406

Boldface indicates P < 0.050.

 β STD, standardized regression coefficient.

TABLE 3. Multiple regression models for bone geometry estimates in adolescent male athletes.

	Predictors	βSTD	sr ²	P		Predictors	βSTD	sr ²	P
CSA $(R^2 = 0.72)$	Footballers	0.322	0.023	0.004	$Z(R^2 = 0.78)$	Footballers	0.157	0.005	0.109
	Swimmers	0.068	0.001	0.495		Swimmers	0.019	0.000	0.831
	Cyclists	0.123	0.005	0.190		Cyclists	0.005	0.000	0.951
	Stature	0.394	0.023	0.004		Stature	0.417	0.025	0.001
	Lean mass	0.370	0.017	0.014		Lean mass	0.430	0.023	0.001
	Fat mass	-0.010	0.000	0.905		Fat mass	-0.040	0.001	0.592
	Calcium	0.025	0.001	0.657		Calcium	0.016	0.000	0.755
	25(OH)D	-0.038	0.001	0.498		25(OH)D	-0.054	0.003	0.281
	MVPA	0.103	0.008	0.093		MVPA	0.142	0.014	0.010
	Vertical jump	0.029	0.000	0.713		Vertical jump	-0.024	0.000	0.734
	CRF	0.178	0.010	0.051		CRF	0.207	0.014	0.012
CSMI ($R^2 = 0.78$)	Footballers	0.064	0.001	0.506					
	Swimmers	-0.030	0.000	0.732					
	Cyclists	-0.038	0.000	0.645					
	Stature	0.338	0.017	0.005					
	Lean mass	0.568	0.039	<0.001					
	Fat mass	-0.087	0.003	0.245					
	Calcium	-0.011	0.000	0.820					
	25(OH)D	-0.048	0.002	0.334					
	MVPA	0.140	0.014	0.011					
	Vertical jump	-0.090	0.003	0.199					
	CRF	0.183	0.011	0.024					

Boldface indicates P < 0.050.

 β STD, standardized regression coefficient.

 $sr^2 = 0.014$, P < 0.05). CRF was positively associated with CSMI and $Z(\beta = 0.183-0.207, sr^2 = 0.011-0.014, P < 0.05)$.

DISCUSSION

The present study aimed to identify, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes involved in football, swimming, and cycling. It has recently been shown that football has a beneficial impact on bone outcomes in comparison to cycling and swimming in male adolescents (38). However the determinants responsible for these differences are not known for this population. In support with our hypothesis, regionspecific lean mass was the primary explanatory variable on aBMD and hip geometry estimates at most sites of the skeleton. In addition, we found that only participation in football was a significant predictor of aBMD and hip geometry estimates when contrasted to the control group. Finally, it was observed that modifiable factors such as nutrition status (calcium and vitamin D), MVPA and physical fitness (vertical jump and CRF) had a small but significant contribution to bone density and hip geometry estimates across specific sites of the skeleton.

Determinants of bone mineral density. The determinants explained an important significant variance of aBMD at different skeletal sites (49.0%–76.4%; average, 64.5%) with previous findings in nonathletic population reporting that a similar model of determinants explained 40% to 83% of the variance in bone mineral content (BMC) in prepubertal girls (3). Region-specific lean mass was consistently the strongest determinant of aBMD at TBLH, LS, legs, and arms. A previous study in nonathletic boys and girls reported that total lean mass was the best predictor of total body and lumbar spine aBMD, but they did not report the relationship with other sites of the skeleton (6). Another study in nonathletic children found that total lean mass was

the strongest predictor of the aBMD at total body and LS (16). However the study did not distinguished the sitespecific relationship of lean and bone mass which was considered in the present study. It is of great interest to understand the region-specific relationship of lean mass and aBMD due to the site-specific adaptation of the skeleton during external loading, specifically in athletic populations (2). It is still not clear to what extent fat mass is associated with aBMD in adolescents and especially after adjusting for confounding factors. In our study, we found that regionspecific fat mass only had a positive association with TBLH aBMD, suggesting that after accounting for other covariates its influence on bone development in athletic male adolescents is negligible, perhaps due to the strong effect of region-specific lean mass. In addition, an increase in fat mass in adolescent athletes can have a negative effect on their performance (36).

Football participation was positively associated with aBMD at TBLH, FN, TH, and legs. There was no significant contribution of swimming and cycling in contrast to the control group on aBMD at any sites of the skeleton. The contribution of football on aBMD was independent of lean mass and is likely to be explained by the intermittent and high-intensity characteristics of football that can produce high strains on the skeleton and stimulate bone mineral acquisition (35). The concentric contractions during football generate greater forces compared with cycling and swimming and this might explain the increased skeletal loading in this group (33). In addition, our findings show that stature was positively significant associated with aBMD at the arms and FN. Similarly with our results, previously it was found that stature had a weak and site-specific relationship with BMC at different skeletal sites in nonathletic children (16). The movement characteristics of the sports practiced seems to be important for bone acquisition and the present study found that football participation is one of the most important determinants,

possibly because it includes high intensity concentric contractions that can enhance aBMD in adolescents.

Both MVPA and nutrition (calcium and vitamin D) are considered to be essential for optimal bone growth, but their contribution was diminished once other factors (e.g., stature, lean mass, sports participation) were considered. In the present analysis, we found that blood serum calcium and 25(OH)D had a small contribution on aBMD at the arms and only 25(OH)D contributed to aBMD at LS. Previous findings indicated that dietary calcium and 25(OH)D can have a weak, but significant contribution on specific sites of the skeleton in adolescents (22). The sites of the skeleton, such as arms, are less loaded through sport, and nutritional factors may have a potential influence. The site-specific relationship between nutrition and bone outcomes can be attributed to the interactions of nutrients in relation to bone health (17). In relation to the contribution of MVPA and fitness on aBMD, we found that vertical jump height was the only significant predictor of aBMD at TBLH and LS. These findings show that overall MVPA does not appear to be important once participation in a particular sport is considered in the regression model. This suggests the characteristics of the sport practised and the contribution of lean mass mediates the relationship between fitness and bone outcomes (37).

Determinants of hip geometry estimates. Using the HSA method we showed that the multiple regression model can explain a large proportion of the variance (71.7%-77.8%; average, 75.7%) in geometrical parameters (CSA, CSMI, Z) at the narrow neck site of the hip. To the best of our knowledge, this is the first study examining the determinants of HSA outcomes in adolescent athletes. The strongest predictor for the geometrical parameters was the region-specific lean mass followed by stature. The contribution of region-specific lean mass was consistent for all the geometrical parameters. The findings of the present study highlight the influence of region-specific lean mass on hip geometry estimates during adolescence which is linked with bone outcomes in young adulthood (2). Despite the lack of significant association between region-specific lean mass and aBMD at the FN and TH, all the geometrical parameters of the narrow neck of the femur were significantly associated with region-specific lean mass. This may reflect previous work in children and adolescents showing that HSA can provide more in depth geometrical evaluation at the hip site compared with BMD outcomes (27). In addition, studies using peripheral quantitative computed tomography found that muscle cross sectional area was the strongest predictor of bone strength parameters in early pubertal boys and girls (22). The latter study highlighted the importance of using site-specific lean mass to understand its contribution to hip geometry estimates. On the other hand, region-specific fat mass was not associated with any geometrical parameters, and this is in agreement with findings in nonathletic female adolescent indicating that fat mass was not associated with CSA (29).

Stature was associated with all hip geometry estimates showing that the size of an adolescent athlete plays an important role in modifying hip geometry estimates. A previous study reported that femoral length is one of the most important predictors of CSA and Z in female adolescents (3), highlighting the importance of bone length at the hip. In addition, football participation was associated with CSA in hip geometry estimates of female footballers (5). There was no contribution of swimming and cycling on geometrical parameters which is similar with the findings on aBMD outcomes. The different contribution of stature and football in geometrical parameters compared with aBMD parameters might be due to fact that we used stature and not femoral length to control for the size in geometrical parameters. Also, the estimated geometrical parameters might not be affected from the external loading that football applies at the narrow neck site and higher forces might be needed (20).

All groups of the present study had similar serum levels of calcium and vitamin D, and there was no association found between serum levels of calcium and vitamin D and geometrical bone outcomes, which is consistent with no contribution of hip related aBMD outcomes in the present study. In contrast, MVPA was a significant predictor of CSMI and Z independent of the sport participation suggesting that MVPA might induce changes in geometrical parameters and not aBMD due to mechanical stimuli applied at the hip site (23). The association between MVPA and bone outcomes was evident for the geometrical parameters but not for the aBMD parameters. This may be explained by previous findings showing that geometrical adaptations can occur before the adaptation of aBMD outcomes due to the initial respond inside the bone to the change in external strains (14,40). CRF was a significant predictor of CSMI and Z after accounting for all the other predicting determinants, but there was no association with vertical jump. The different associations between fitness parameters and MVPA with aBMD and the geometrical parameters might be attributed to the sensitivity of the geometrical parameters of the hip to detect changes (19). The bone structure at the hip and specifically CSA site might be associated with CRF due to the use lower leg muscle units during the sport-specific movements. The training characteristics are dominant in the present study and our population was at the 75th percentile for CRF compared with same age and ethnicity matched population (30).

Limitations. To our knowledge, this is the first study conducted in adolescent male athletes to examine the determinants of aBMD and hip geometry estimates. A large list of predictors has been included and their effects have been adjusted by each other. In addition, the present study uses region-specific lean mass as predictor of aBMD and hip geometry estimates due to the site-specific adaptations of the skeleton during exercise and growth (18). The cross-sectional analysis of the present study is a limitation and cannot prove cause and effect between the determinants and bone outcomes studies. In spite of using DXA as a surrogate

estimate of lean mass due to the two-component model, DXA-derived lean mass has been found to be highly correlated (r = 0.82) with muscle cross sectional area measured by peripheral quantitative computed tomography (27).

CONCLUSIONS

The present study has shown, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes. Region-specific lean mass was consistently the most important determinant of aBMD and hip geometry estimates parameters in adolescent male athletes. Football participation and stature were found to be important determinants for the aBMD and HSA parameters, respectively. Calcium and 25(OH)D had a small site-specific contribution only on aBMD. MVPA and CRF positively influenced only the geometrical parameters and vertical jump was associated with aBMD parameters. Studies focusing on bone outcomes of young athletes should account for the region-specific lean mass differences due to the site-specific adaptations of the skeleton to external loading. Future practical approaches of sports clubs should include weight-bearing

REFERENCES

- Baptista F, Mil-Homens P, Carita AI, Janz K, Sardinha LB. Peak vertical jump power as a marker of bone health in children. *Int J Sports Med*. 2016;37(8):653–8.
- Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*. 2008;43(6):1101–7.
- 3. Daly RM, Stenevi-Lundgren S, Linden C, Karlsson MK. Muscle determinants of bone mass, geometry and strength in prepubertal girls. *Med Sci Sports Exerc*. 2008;40(6):1135–41.
- Duren DL, Sherwood RJ, Choh AC, et al. Quantitative genetics of cortical bone mass in healthy 10-year-old children from the Fels Longitudinal Study. *Bone*. 2007;40(2):464–70.
- El Hage R. Geometric indices of hip bone strength in young female football players. *J Musculoskelet Neuronal Interact*. 2013;13(2): 206–12.
- El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffre C. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol*. 2009; 105(5):759–64.
- Esliger DW, Rowlands AV, Hurst TL, Catt M, Murray P, Eston RG. Validation of the GENEA Accelerometer. *Med Sci Sports Exerc*. 2011;43(6):1085–93.
- 8. Ferry B, Duclos M, Burt L, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. *J Bone Miner Metab.* 2011;29(3):342–51.
- Frost HM. Bone's mechanostat: a 2003 update. Anat Rec A Discov Mol Cell Evol Biol. 2003;275(2):1081–101.
- Gracia-Marco L. Physical activity, bone mass and muscle strength in children. Acta Paediatr. 2016;105(10):1127–8.
- Gracia-Marco L, Moreno LA, Ortega FB, et al. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. Am J Prev Med. 2011;40(6):599–607.
- Gracia-Marco L, Ortega FB, Jiménez-Pavón D, et al. Adiposity and bone health in Spanish adolescents. The HELENA study. Osteoporos Int. 2012;23(3):937–47.

and muscle strengthening exercises, such as jumps, which can optimize bone outcomes during the important period of adolescence.

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and the present study do not constitute endorsement by ACSM.

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- Gracia-Marco L, Vicente-Rodriguez G, Casajus JA, Molnar D, Castillo MJ, Moreno LA. Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study. *Eur J Appl Physiol*. 2011;111(11):2671–80.
- 14. Haapasalo H, Kontulainen S, Sievänen H, Kannus P, Järvinen M, Vuori I. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. *Bone*. 2000;27(3):351–7.
- 15. Harvey NC, Cole ZA, Crozier SR, et al. Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. *Osteoporos Int.* 2012;23(1):121–30.
- Hrafnkelsson H, Sigrudsson G, Magnusson KT, Johannsson E, Sigurdsson EL. Factors associated with bone mineral density and content in 7-year-old children. *Bone*. 2010;46(4):1058–62.
- 17. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. *J Am Coll Nutr*. 2000;19(6):715–37.
- Ireland A, Maden-Wilkinson T, McPhee J, et al. Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players. *Med Sci Sports Exerc*. 2013;45(9):1749–58.
- Khoo BC, Beck TJ, Qiao QH, et al. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multi-center clinical trials. *Bone*. 2005;37(1):112–21.
- 20. Korhonen MT, Heinonen A, Siekkinen J, et al. Bone density, structure and strength, and their determinants in aging sprint athletes. *Med Sci Sports Exerc*. 2012;44(12):2340–9.
- Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci.* 1988;6(2): 93–101.
- Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H. Bone strength and its determinants in pre- and early pubertal boys and girls. *Bone*. 2006;39(3):598–608.
- Michalopoulou M, Kambas A, Leontsini D, et al. Physical activity is associated with bone geometry of premenarcheal girls in a dosedependent manner. *Metabolism-Clinical and Experimental*. 2013; 62(12):1811–8.

- 24. Mouratidou T, Vicente-Rodriguez G, Gracia-Marco L, et al. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin Densitom*. 2013;16(1):110–7.
- Nikander R, Sievänen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: a systematic review and meta-analysis for optimising bone strength throughout life. BMC Med. 2010;8:47.
- Ortega FB, Artero EG, Ruiz JR, et al. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. Int J Obes (Lond). 2008;32(5 Suppl):S49–57.
- Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone*. 2005;36(3):568–76.
- Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. J Sci Med Sport. 2013;16(2):124–8.
- Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. Am J Clin Nutr. 2007;86(5):1530–8.
- Sandercock G, Voss C, Cohen D, Taylor M, Stasinopoulos DM. Centile curves and normative values for the twenty metre shuttle-run test in English schoolchildren. *J Sports Sci.* 2012;30(7):679–87.
- Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab*. 2010; 95(2):699–706.
- 32. Sioen I, Mouratidou T, Kaufman JM, et al. Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and

- Lifestyle-Induced Health Effects in Children and Infants) Study. *Public Health Nutr.* 2012;15(6):1093–9.
- 33. Soderman K, Bergstrom E, Lorentzon R, Alfredson H. Bone mass and muscle strength in young female soccer players. *Calcif Tissue Int.* 2000;67(4):297–303.
- Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51(3):170–9.
- Ubago-Guisado E, Gomez-Cabello A, Sanchez-Sanchez J, Garcia-Unanue J, Gallardo L. Influence of different sports on bone mass in growing girls. *J Sports Sci.* 2015;33(16):1710–8.
- Ubago-Guisado E, Mata E, Sánchez-Sánchez J, Plaza-Carmona M, Martín-García M, Gallardo L. Influence of different sports on fat mass and lean mass in growing girls. *J Sport Health Sci.* 2015; doi: https://doi.org/10.1016/j.jshs.2015.06.001.
- Vicente-Rodriguez G, Urzanqui A, Mesana MI, et al. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: a cross-sectional study. *J Bone Miner Metab*. 2008;26(3): 288–94
- 38. Vlachopoulos D, Barker AR, Williams CA, et al. The impact of sport participation on bone mass and geometry in male adolescents. *Med Sci Sports Exerc*. 2017;49(2):317–26.
- 39. Vlachopoulos D, Barker AR, Williams CA, Knapp KM, Metcalf BS, Gracia-Marco L. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. *BMC Public Health*. 2015;15(1):361.
- 40. Wang QJ, Suominen H, Nicholson PH, et al. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. *Scand J Med Sci Sports*. 2005;15(2):100–6.



Longitudinal Adaptations of Bone Mass, Geometry, and **Metabolism in Adolescent Male Athletes: The PRO-BONE** Study

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ABSTRACT

Adolescence is a crucial period for bone development, and exercise can enhance bone acquisition during this period of life. However, it is not known how the different loading sports practiced can affect bone acquisition in adolescent male athletes. Therefore, the purpose of the present study was to determine the 1-year longitudinal bone acquisition among adolescent males involved in osteogenic (football) and non-osteogenic (swimming and cycling) sports and to compare with active controls. A total of 116 adolescent males aged 12 to 14 years at baseline were followed for 1 year: 37 swimmers, 37 footballers, 28 cyclists, and 14 active controls. Bone mineral content (BMC) was assessed using dual-energy X-ray absorptiometry (DXA); cross-sectional area (CSA), crosssectional moment of inertia (CSMI), and section modulus (Z) at the femoral neck was assessed using hip structural analysis (HSA); and bone texture of the lumbar spine was assessed using trabecular bone score (TBS). Serum N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium, and 25 hydroxyvitamin D [25(OH)D] were analyzed. Footballers had significantly higher adjusted BMC at the lumbar spine (7.0%) and femoral neck (5.0%) compared with cyclists, and significantly greater BMC at the lumbar spine (6.9%) compared with swimmers. Footballers presented significantly greater TBS (4.3%) compared with swimmers, and greater CSMI (10.2%), CSA (7.1%), Z (8.9%) and TBS (4.2%) compared with cyclists. No differences were noted between cyclists and swimmers, both groups had similar bone acquisition compared with controls. PINP was significantly higher in footballers and controls compared with cyclists and swimmers (3.3% to 6.0%), and 25(OH)D was significantly higher in footballers and cyclists compared with swimmers and controls (9.9% to 13.1%). These findings suggest that bone acquisition is higher in adolescent male footballers compared with swimmers and cyclists at the femoral neck and lumbar spine sites of the skeleton. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: ADOLESCENCE; BONE MINERAL CONTENT; EXERCISE; HIP STRUCTURAL ANALYSIS; TRABECULAR BONE SCORE

Introduction

puberty is a period of life associated with a rapid increase in bone mass. (1,2) Low bone mass during adolescence is associated with increased fracture risk and osteoporosis later in life. (3-5) The increasing prevalence of fractures for boys is expected to increase by 24%, (6) and the economic burden of fractures will cost £5465 (€6723) million per year by 2025. (7) The childhood and adolescent years are critical for bone acquisition with up to 43% of peak bone mass (PBM) acquired during the 5-year period surrounding the peak height velocity. (8,9) The factors affecting PBM during growth include non-modifiable factors, such as genetics, (10) and modifiable factors, such as nutrition (eg, calcium and vitamin D)^(11,12) and physical activity. (13-15) Exercise and sports participation can enhance bone mineral content (BMC) and bone mineral density (BMD) during youth, (16,17) and the benefits can be maintained into adulthood. (18,19) However, not all types of exercise and sports are beneficial for bone development. Participation in football can augment BMC at the loaded sites of the skeleton, (20,21) whereas participation in swimming and cycling may have a negative or no impact on bone outcomes, (22,23) which may predispose to a suboptimal PBM.

With football, cycling, and swimming being among the most popular sports globally, (24) there is limited evidence comparing the impact of these sports on bone development. (25) To date,

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only cross-sectional studies have evaluated the impact of these "osteogenic" and "non-osteogenic" sports on bone outcomes compared with controls in adolescent males, (25-27) showing higher bone outcomes in those participating in osteogenic sports. However, despite controlling for important confounders, the cross-sectional design precludes any determination of casual relationship between the differences observed between the groups. (28) Therefore, longitudinal studies that can identify sport-specific bone development patterns may be appropriate but are not available to date. Previous longitudinal studies comparing bone acquisition between sports focused only on females and include sports other than football, cycling, and swimming. (17,29-31) A 1-year longitudinal study in adolescent female athletes found that the osteogenic effect of rhythmic gymnastics induced greater bone mass gain at loaded sites of the skeleton compared with swimming participation. (17) The investigation of females and specific weight-bearing sports cannot allow generalization of the bone acquisition in adolescent males or in other sports because of the fact that bone mass gain depends on specific mechanical stimuli that differs between the type of sports practiced during adolescence (32) and because of the hormonal and body composition differences between sexes. (33) In addition, there are no longitudinal studies using a comprehensive analysis of important confounders, such as lean mass and objectively measured moderate-to-vigorous physical activity (MVPA), (34) comparing the effect of swimming, football, and cycling participation on bone outcomes in adolescent male athletes.

Most studies have used dual-energy X-ray absorptiometry (DXA) to evaluate BMC, areal BMD, and bone area (16,20) because of its low cost, radiation, and availability. (35) However, there are few studies using techniques such as hip structural analysis (HSA) to assess bone geometry estimates at the clinically relevant site of the femoral neck in adolescents. (21,36) Previously, 8 months of football participation induced greater acquisition in cross-sectional area (CSA), cross-sectional moment of inertia (CSMI), section modulus, and subperiosteal width compared with swimming participation in adolescent female athletes. (37) However, there are no longitudinal studies available in adolescent males. Moreover, there are no studies in adolescent athletes using the recently developed trabecular bone score (TBS), which can predict fracture risk⁽³⁸⁾ and fragility of the lumbar spine. (39,40) Furthermore, there are no longitudinal studies on the effects of sport participation on bone turnover and nutrition markers, such as N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium, and 25 hydroxyvitamin D [25(OH)D], which can further explain bone formation and resorption in relation to the sports practiced. (41-43)

Therefore, the scope of the present investigation was to determine the longitudinal (1-year) differences on clinically relevant DXA-measured BMC sites, hip geometry estimates, TBS, and bone turnover and nutritional markers in adolescent male athletes engaged in football, swimming, and cycling and active controls aged 12 to 14 years at baseline.

Participants and Methods

Cohort and study design

The present study shows a 12-month longitudinal analysis of sport participation as part of the longitudinal PRO-BONE (effect

of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) study, whose purpose, methodology, and inclusion/exclusion criteria have been described elsewhere. (44) For the present study, the measurements completed at baseline (T0) in autumn/winter 2014-15 and 1 year later (T1) in autumn/winter 2015-16 (mean difference of visits = 1 year and 7 days). Five participants were excluded in the present study because they did not complete the second visit (n=3) or they had missing data in any of the variables included (n = 2). Therefore, 116 adolescent males (13.1 years \pm 1.0 at T0 and 14.1 years \pm 1.0 at T1) were included: 37 swimmers, 37 footballers, and 28 cyclists engaged in these sports more than 3 hours per week the last 3 or more years and 14 active controls not engaged in these sports more than 3 hours per week the last 3 or more years.

Outcome measures: DXA, HSA, TBS, and biochemical analyses

A DXA scanner (GE Lunar Prodigy Healthcare Inc, Wisconsin, USA) was used to measure BMC (g), fat mass (g), and lean mass (g). The lumbar spine (LS, L_1 to L_4) and bilateral proximal femora scans were used to assess BMC. All DXA scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the enCORE software version 14.10.022 (GE Healthcare Inc, Wisconsin, USA) following previous guidelines. The coefficient of variation was not determined in the present study. Previous pediatric studies have shown that the DXA percentage coefficient of variation (CV) was between 1.0% and 2.9% depending on the region. (45)

The hip geometry estimates of the femoral neck were obtained and the following variables used: 1) the CSA (mm²), which is the total bone surface area of the hip, excluding the soft tissue area and the trabecular bone; 2) the CSMI (mm⁴), which is an index of structural rigidity and reflects the distribution of mass in the center of a structural element; and 3) section modulus (Z, mm³), which is an indicator of maximum bending strength in a cross section. The CVs of these variables were previously found to be between 7.9% and 11.7%. (46)

TBS is a DXA-based technological tool that provides an indirect textural index of trabecular microarchitecture in the lumbar spine and has been shown to significantly predict fracture risk independently of BMC. (38) TBS assesses DXA images of the lumbar spine scans using a gray-level analysis as the slope at the origin of the log-log representation of the experimental variogram. (47) All TBS analyses were performed by the same trained researcher using the TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The calculation was performed at the lumbar spine region of interest as in the BMC measurement. The CVs of TBS in relation to BMC has been reported to be from 1.1% to 1.9%. (40)

Capillary blood samples were collected at a non-training weekend day in the morning in heparin fluoride–coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) and centrifuged at 3000 rpm for 15 minutes at 4°C. Serum samples were stored at –80°C until analysis in a single session. Total serum levels of PINP, CTX-I, 25(OH)D, and total calcium were analyzed following guidelines. ELISA kits (Abbexa Ltd, Cambridge, UK) for PINP (test range, 6 to 400 pg·mL⁻¹; sensitivity, 1.2 pg·mL⁻¹; inter- and intra-assay CVs, 3.1% and 8.2%, respectively), CTX-I (test range, 0.1 to 7.0 ng·mL⁻¹; sensitivity, 0.03 ng·mL⁻¹; inter- and intra-assay CVs, 4.9% and

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6.8%, respectively), and 25(OH)D (test range, 3 to 80 ng \cdot mL⁻¹; sensitivity, 1.2 ng \cdot mL⁻¹; inter- and intra-assay CVs, 6.1% and 8.6%, respectively) were used. Total calcium serum was measured using direct colorimetric assay (Cayman Chemical Company, Ann Arbor, MI, USA) and had a sensitivity of 0.25 mg \cdot dL⁻¹ and the absorbance was read at 570 to 590 nm (inter- and intra-assay CVs, 5.1% and 7.3%, respectively).

Other measures

Stature (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair development. (49)

Daily habitual physical activity was measured for 7 consecutive days at T0 and T1 using wrist accelerometers (GENEA, Cambridgeshire, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents. $^{(50)}$ Data were collected at 100 Hz and analyzed at 1-second epoch intervals to establish time spent in MVPA using a cut-off point of $\geq\!1140$ counts per minute previously validated in youth. Weekly training hours were obtained by face-to-face interviews at T0 at T1.

Statistical analyses

Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA). Data were checked for normality using Shapiro-Wilk's test, skewness and kurtosis values, and presented as mean and standard deviation. Data analysis was completed in two stages: 1) unadjusted data using one-way analysis of variance (ANOVA) with Bonferroni post hoc comparisons and chi-square tests used to detect betweengroup differences on bone outcomes (DXA and HSA, TBS) and biochemical markers, and 2) adjusted data using one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect the differences between the groups at T1 using age, height, lean mass, MVPA, and the bone outcomes at TO as covariates. (14,34,51) Preliminary analyses showed that bone outcome results did not change when maturation stage was used as a confounder instead of age. Thus maturation was not included in the model. Percentages of differences between groups for all variables were calculated. Significance was set at p < 0.05 and p < 0.01.

Results

Table 1 presents the descriptive characteristics of the participants at T0 and T1. At T1, swimmers were older, taller, heavier, and had more lean mass than the footballers. Swimmers were more mature than footballers and controls. Swimmers trained more hours and years than cyclists. Footballers spent more time doing MVPA than swimmers and controls. In addition, footballers trained more hours and years than cyclists and swimmers. Cyclists were older than controls. Cyclists spent more time doing MVPA compared with swimmers and controls. Controls had more body fat percentage than all the sport groups. From T0 to T1, all the descriptive characteristics for all groups increased significantly except body fat percentage, which reduced significantly in the sport groups, and MVPA, which decreased significantly in all groups. Results of unadjusted bone outcomes are presented in Supplemental Table S1.

Longitudinal adjusted differences in BMC acquisition between groups

BMC-adjusted results for lumbar spine and femoral neck are presented at Fig. 1 and Table 2. At T1, footballers presented significantly greater BMC at the lumbar spine (7.0%) and femoral neck (5.0%) compared with cyclists. Also, footballers had significantly greater BMC at the lumbar spine (6.9%) and a similar BMC at the femoral neck (2.3%) compared with swimmers. Footballers had higher but not significant BMC at the lumbar spine (6.0%) and femoral neck (2.7%) compared with controls at T1. Cyclists had similar BMC at the lumbar spine (–1.0%) and femoral neck (–2.3%) compared with controls. Swimmers had similar BMC at the lumbar spine (–0.8%) and femoral neck (0.4%) compared with controls. In addition, swimmers had similar BMC at the lumbar spine (0.2%) and femoral neck (2.7%) compared with cyclists.

Longitudinal adjusted differences in HSA and TBS acquisition between groups

Results of adjusted bone geometry acquisition are presented at Fig. 2 and Table 2. At T1, footballers presented significantly greater TBS (4.3%) compared with swimmers, greater CSMI (10.2%), CSA (7.1%), Z (8.9%), and TBS (4.2%) compared with cyclists, and similar CSMI, CSA, Z, and TBS compared with controls (2.6% to 7.8%). In addition, swimmers had similar CSMI, CSA, and Z (1.0% to 4.2%) and similar TBS (-0.1%) compared with cyclists. Swimmers had similar CSMI, CSA, and Z (-0.1% to 0.7%) and non-similar TBS (-1.6%) compared with controls. Finally, cyclists had similar CSMI, CSA, Z, and TBS (-0.3% to -4.0%) compared with controls.

Longitudinal bone turnover and nutrition marker between groups

The biochemical markers are shown in Table 3 and Fig. 3. At T0, there were no significant differences between the groups in any of the biochemical markers. After 1 year of sport participation (T1), bone formation (PINP) was significantly higher in footballers than swimmers (3.3%) and cyclists (6.0%). In addition, footballers had significantly higher 25(OH)D compared with swimmers (12.9%) and controls (13.1%). Cyclists had significantly lower PINP than controls (5.1%), but they had significantly higher 25(OH)D compared with swimmers (9.9%) and controls (11.1%).

CTX-I (14.8%) and 25(OH)D (4.7%) significantly increased from T0 to T1 in footballers, whereas PINP did not change compared with the other groups. In swimmers, there was a significant decrease in PINP (5.8%) and a significant increase in CTX-I (9.8%) from T0 to T1. Similarly, in cyclists, PINP significantly decreased (7.2%) and CTX-I non-significantly increased (4.3%) from T0 to T1.

Discussion

To the best of our knowledge, this is the first longitudinal study that has evaluated bone acquisition in adolescent male athletes and used comprehensive methodology to assess bone status by combining DXA, HSA, TBS, and biochemical markers. The main findings of the present study show that: 1) the osteogenic responses of football participation over 1 year induced significantly greater bone acquisition in BMC, hip geometry

Table 1. Descriptive Characteristics of the Participants at Baseline and After 1 Year of Sport Participation

N = 116	Swimmers $(n = 37)$	Footballers $(n=37)$	Cyclists (n = 28)	Controls $(n = 14)$
Age (years)		,		,
TO TO	13.5 (1.0) ^{b,dd}	12.9 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
T1	14.6 (1.0) ^{b,dd,**}	13.9 (0.9)**	14.2 (1.0) ^{d,**}	13.2 (0.5)**
Stature (cm)				
T0	165.1 (9.7) ^{bb,d}	155.2 (9.3)	160.7 (10)	154.5 (9.9)
T1	171.6 (8.9) ^{bb,dd,**}	162.7 (10.3)**	166.6 (10.7)**	160.7 (10.5)**
Body mass (kg)				
TO TO	51.9 (8.7) ^{bb}	44.3 (7.9)	49.3 (12.5)	48.3 (13.0)
T1	58.9 (8.2) ^{b,**}	50.8 (9.7)**	54.7 (12.5)**	55.2 (15.6)**
BMI (kg/m ²)				
T0	18.9 (1.6)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
T1	19.9 (2.0)**	19.0 (1.8)**	21.0 (3.1)**	21.0 (3.7)**
Lean mass (kg)				
T0	41.1 (9.0) ^{b,dd}	35.4 (7.2)	37.5 (7.5)	31.7 (5.5)
T1	47.8 (8.7) ^{b,dd,**}	41.2 (9.2)**	42.9 (8.2)**	36.8 (7.1)**
Body fat (%)				
T0	17.3 (7.3)**	15.7 (5.6)**	18.0 (9.0)**	29.0 (10.5) ^{aa,bb,cc}
T1	14.4 (6.4)	14.5 (6.0)	16.1 (9.2)	27.9 (10.9) ^{aa,bb,cc}
Tanner stages (%)				
T0	(16/25/16/43/0)	(24/35/24/16/0)	(14/28/25/28/4)	(29/21/21/29/0)
T1	(5/11/11/51/22) ^{b,d,**}	(6/16/35/43/0)**	(7/11/14/57/11)**	(0/21/43/36/0)*
Training (h/wk)				
T0	9.4 (5.1) ^{cc}	10.0 (2.3) ^{cc}	5.2 (2.1)	_
T1	8.9 (3.6) ^{cc}	9.4 (1.7) ^{cc}	5.6 (2.0)	_
Years of training				
T0	5.9 (2.5) ^{cc}	7.5 (2.3) ^{a,cc}	3.9 (1.3)	_
T1	6.9 (2.5) ^{cc,**}	8.5 (2.3) ^{a,cc,**}	4.9 (1.3)**	_
MVPA (min/d)				
T0	85.0 (30.9)**	119.8 (29.7) ^{aa,dd,**}	106.5 (33.7) ^{a,**}	83.2 (26.8)*
T1	62.9 (21.8)	92.4 (25.7) ^{aa,dd}	85.6 (21.8) ^{aa,d}	64.3 (18.1)

 $T0 = baseline \ values; T1 = 1-year \ values; BMI = body \ mass \ index; MVPA = moderate \ to \ vigorous \ physical \ activity.$

Superscript letters denote a higher significant difference between sports: aswimmers, bfootballers, cyclists, dcontrols, a,b,c,dp < 0.05, aa,bb,cc,ddp < 0.001, and between T0 and T1 of each sport: p < 0.05, **p < 0.001.

estimates, and TBS compared with that of swimming and cycling after accounting for relevant confounders; 2) participation in cycling and swimming did not induce significant gains in any bone outcomes and cyclists had similar bone acquisition compared with controls; 3) footballers had significantly higher

PINP than both non-osteogenic sports and significantly higher 25(OH)D concentration compared with swimmers and controls at T1, and PINP significantly decreased over time both in cyclists and swimmers, whereas CTX-I significantly increased in swimmers and footballers.

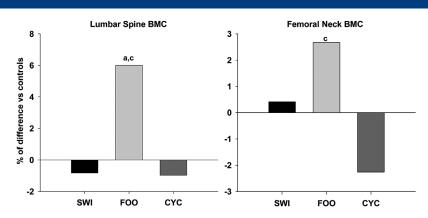


Fig. 1. Difference (%) in adjusted bone mineral content (BMC) between the sports groups and controls after 1 year. The results are adjusted for age, height, lean mass, MVPA, and bone outcomes at baseline. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). *p* < 0.05.

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Values presented as mean $\pm\,\mathrm{SD}$.

Table 2. Adjusted Bone Outcomes Using DXA, HSA, and TBS at Baseline and After 1 Year of Sports Participation in Adolescent Males

N = 116	Swimmers $(n=37)$	Footballers (n = 37)	Cyclists (n = 28)	Controls (n = 14)
Lumbar spine (BMC, g)				
ТО	38.16 (0.89)	40.78 (0.91)	39.67 (0.96)	39.88 (1.47)
T1	46.10 (0.73)	49.27 (0.71) ^{a,c}	46.03 (0.77)	46.48 (1.18)
Femoral neck (BMC, g)				
ТО	4.22 (0.08) ^{dd}	4.68 (0.08) ^{a,c,dd}	4.28 (0.08) ^d	3.92 (0.12)
T1	4.89 (0.05)	5.00 (0.05) ^{cc}	4.76 (0.05)	4.87 (0.09)
CSA (mm²)				
T0	131.4 (2.3)	157.0 (2.4) ^{aa,c,dd}	132.6 (2.4)	121.6 (3.7)
T1	148.0 (2.4)	144.5 (2.3) ^c	146.6 (2.4)	147.0 (4.0)
Section modulus (mm ³)				
T0	521.5 (12.0)	567.0 (12.1) ^{c,d}	511.7 (12.6)	479.4 (19.1)
T1	626.5 (12.8)	655.0 (13.1) ^c	601.4 (13.4)	626.5 (21.2)
CSMI (mm ⁴)				
T0	8113.5 (252.1)	8854.9 (254.0) ^d	7993.8 (264.8)	7527.2 (399.2)
T1	10301.7 (220.2)	11088.7 (219.5) ^c	10063 (229.4)	10284.3 (358.2)
Trabecular bone score				
T0	1.372 (0.011)	1.428 (0.012) ^{a,c}	1.363 (0.013)	1.413 (0.019)
T1	1.365 (0.010)	1.423 (0.010) ^{a,c}	1.366 (0.011)	1.387 (0.016)

DXA = dual-energy X-ray absorptiometry; HSA = hip structural analysis; TBS = trabecular bone score; BMC = bone mineral content; T0 = baseline values; T1 = 1-year values; CSA = cross-sectional area; CSM = cross-sectional moment of inertia.

At T0, bone outcomes were adjusted for age, stature, moderate to vigorous physical activity (MVPA), and lean mass. At T1, bone outcomes were adjusted for age, stature, MVPA, lean mass, and for T0 bone outcomes.

Longitudinal BMC acquisition between groups

In the present study, footballers gained significantly higher BMC at the lumbar spine compared with cyclists and swimmers and also gained significantly higher femoral neck BMC compared with cyclists. Also, footballers gained similar BMC than controls at the lumbar spine and femoral neck. Previous evidence in female footballers and swimmers aged 15.9 to 16.2 years compared BMD gains after 8 months of training and found that BMD at lumbar spine and hip was significantly increased in footballers but not in swimmers, (37) which is in line with the findings of the present study. The differences observed in BMC acquisition among the sports groups in the present longitudinal study might be explained by the loading patterns, such as plyometric exercises, included in the football training that can induce higher impact and bone adaptations in adolescent athletes. (52) A different study in male footballers aged 12.9 years showed that 1 year of football training induced significantly higher gains in BMC at lumbar spine and total hip compared with active controls aged 12.5 years, (20) which is similar with the unadjusted findings of the present investigation. The similar BMC between the non-osteogenic groups (swimmers and cyclists) in the current study might be explained by the low repeated loading applied in the skeleton during participation in these sports, which might not be enough to produce positive bone adaptations. (21)

Longitudinal HSA and TBS acquisition between groups

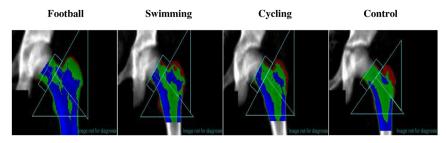
The present findings show that football participation for 1 year induced significantly greater acquisition in bone geometry estimates compared with cyclists and that participation in non-osteogenic sports induced similar acquisition in bone geometry estimates compared with controls. Previous findings in adolescent female footballers and swimmers showed that 8 months of training in their respective sports induced a significant increase in CSA of the narrow neck in footballers compared with swimmers. (37) The geometrical differences between footballers and cyclists in the present study indicate that the continuous unloading environment of cycling may delay the corticalization of the bone structure despite the fewer hours of training in cyclists compared with footballers (5.6 versus 9.4 hours). In swimmers, the continuous unloading environment is present, but the forces applied to the wall in every change of direction during swimming may induce small adaptations to the femoral neck site of the skeleton, (53) which might explain the nonsignificant difference between footballers and swimmers at the hip bone geometry estimates.

The present study also includes novel longitudinal findings using TBS in adolescent athletes, showing that adolescent footballers had significantly higher improvement in TBS score compared with cyclists and swimmers. A recent cross-sectional evaluation in adults showed that participation in repeated moderate-impact loading sports may result in lower TBS score and increased fracture risk compared with high-impact loading sports. (54) The present study indicates that bone texture acquisition at the lumbar spine may be affected from the external loading environment after controlling for potential confounders that have been shown to influence TBS. (39) It can be speculated that the high-intensity loading impact during football participation can result in better trabecular structure at the lumbar spine because of the loading applied both in vertical and horizontal directions.

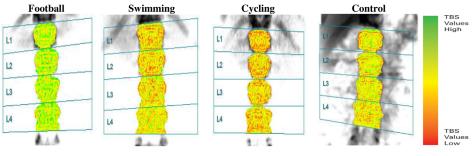
Values are presented as mean \pm SE.

Superscript letters denote a higher significant difference between sports: a swimmers, b footballers, c cyclists, d controls, $^{a,b,c,d}p < 0.05$ and aa,bb,cc, $^{\rm dd}p < 0.001$.

Hip Structural Analysis at the narrow neck of the femur region.



Trabecular bone score at the lumbar spine region.



	FOO vs SWI	FOO vs CYC	FOO vs CON	SWI vs CYC	SWI vs CON	CYC vs CON
CSMI	7.6%	10.2% *	7.8%	2.4%	0.2%	-2.2%
CSA	6.1%	7.1% *	6.8%	1.0%	0.7%	-0.3%
z	4.6%	8.9% *	4.6%	4.2%	-0.1%	-4.0%
TBS	4.3%**	4.2%**	2.6%	-0.1%	-1.6%	-1.5%

Fig. 2. Differences (%) in adjusted bone geometry estimates and trabecular bone score at the femoral neck and lumbar spine regions between the groups after 1 year of sports specific training. The results are adjusted for age, height, region specific lean mass, MVPA and baseline values of bone geometry estimates. CSMI: Cross sectional moment of inertia, CSA: cross-sectional area, TBS: Trabecular Bone score, Z: Section modulus. The figures represent unadjusted results of participants of the same age, height and training hours. Significance at *p < 0.05 and **p < 0.01.

Table 3. Biochemical Markers at Baseline and After 1 Year of Sports Participation in Adolescent Males

N = 116	Swimmers $(n=37)$	Footballers (n = 37)	Cyclists (n = 28)	Controls (n = 14)
Serum calcium (mg/dL)				
T0	10.04 (0.44)	9.97 (0.40)	9.96 (0.41)	10.01 (0.35)
T1	9.91 (0.52)	9.96 (0.39)	9.89 (0.32)	9.74 (0.53)
25(OH)D (ng/mL)				
TO S	13.73 (1.20)	14.44 (1.63)	14.39 (0.59)	13.92 (0.94)
T1	13.42 (0.89)	15.15 (1.17) ^{aa,dd,*}	14.89 (1.13) ^{aa,dd}	13.40 (0.93)
PINP (pg/mL)				
TO	355.15 (10.07)**	352.04 (13.5)	350.83 (12.85)**	350.11 (16.76)
T1	335.67 (18.04)	346.62 (18.16) ^{a,cc}	327.15 (14.15)	344.81 (14.56) ^{cc}
CTX-I (ng/mL)				
TO	1.66 (0.25)	1.61 (0.29)	1.78 (0.19)	1.73 (0.23)
T1	1.84 (0.16)**	1.89 (0.08)**	1.86 (0.09)	1.85 (0.10)

 $T0 = baseline \ values; \ T1 = 1$ -year values; 25(OH)D = 25-hydroxyvitamin D; PINP = N-terminal propertide of procollagen type I; CTX-I = carboxiterminal telopeptide of type 1 collagen.

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Values are presented as mean \pm SD.

Superscript letters denote a higher significant difference between sports: a swimmers, b footballers, c cyclists, d controls, $^{a,b,c,d}p < 0.05$, $^{aa,bb,cc,dd}p < 0.001$, and between T0 and T1 of each sport: $^{*}p < 0.05$, $^{**}p < 0.001$.

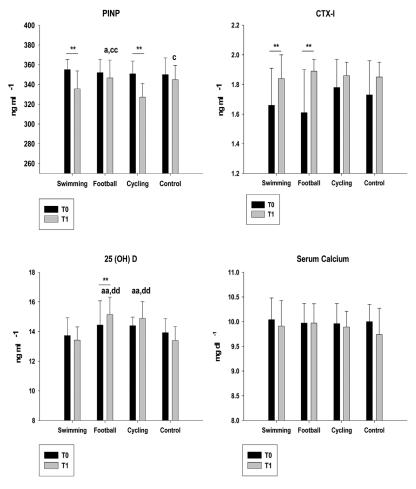


Fig. 3. Differences in bone turnover and nutrition markers between the sports groups and controls after 1 year. 25 (OH) D: 25-hydroxyvitamin D, CTX-I: Carboxi-terminal telopeptide of type 1 collagen (CTX-1), PINP: N-terminal propeptide of procollagen type I. Letters denote a significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls) and * denoted a significant difference between T0 and T1 measurement time. Significance at $^*p < 0.05$ and $^{**}p < 0.01$.

Longitudinal changes in biochemical markers between and within groups

PINP at T1 was significantly higher in footballers compared with swimmers and cyclists, whereas the concentrations of PINP significantly decreased in the non-osteogenic sports from T0 to T1. In addition, CTX-I significantly increased in footballers and swimmers from T0 to T1. The longitudinal changes in PINP and CTX-I suggest an increased bone turnover in footballers and increased bone resorption in swimmers, which reflect the differences in bone acquisition between the groups and indicate a contribution of bone turnover on bone adaptations. (55) A previous longitudinal study in females showed that gymnasts had significantly higher bone formation (osteocalcin) levels than swimmers after 1 year of participation and that the osteocalcin levels decreased in swimmers but not in gymnasts. (17) The bone resorption findings of the present study are in line with a previous longitudinal study in peripubertal girls that did not find significant differences in bone resorption between gymnasts, runners, and controls after 1 year. (28) Also, it has been previously shown that bone remodeling might be increased because of participation in high-intensity weight-bearing activities during puberty, (56,57) which might explain the increased bone remodeling in footballers.

Regarding the serum calcium and 25(OH)D, there were no differences between or within groups in serum calcium, whereas 25 (OH)D levels were significantly higher in footballers and cyclists compared with swimmers and controls at T1. In addition, 25(OH)D significantly increased in footballers from T0 to T1, which may partially contribute to the findings that bone resorption was not affected in this group as it was in the swimmers. All participants had insufficient 25(OH)D levels, which agrees with low 25(OH)D levels previously reported in a European adolescent population. The higher 25(OH)D levels in footballers and cyclists might be explained by the higher exposure to sunlight during training in these sports, although other parameters such as dietary intake and the sampling period have been reported to affect 25(OH)D levels.

Strengths and limitations

The present study includes the 1-year longitudinal evaluation of bone acquisition in adolescent male athletes and a control group that have never been compared before. In addition, the combination of three different methods (DXA, HSA, and TBS) to assess bone development adds novel findings to the literature by showing for the first time that acquisition in HSA and TBS parameters differs between adolescent male athletes in

osteogenic and non-osteogenic sports. Moreover, the serum bone turnover markers used in this study have been previously used in a pediatric population (42,55) and are recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry (IFCC)⁽⁴⁸⁾ in order to provide additional information regarding the bone metabolism changes due to the sports practiced. A limitation of the present study might be the fewer hours of training in cyclists compared with footballers and swimmers. However, the study has strong internal validity because of the comprehensive inclusion criteria and the objective control of potential confounders, such as baseline bone values, lean mass, and MVPA measured by accelerometers. The findings of the present study strongly suggest that the type of exercise practiced during adolescence can induce different bone acquisition on clinical relevant sites of the skeleton, suggesting that weight-bearing activities should be incorporated during adolescence to optimize peak bone mass and reduce low bone status later in life.

Conclusions

This longitudinal study demonstrated for first time that bone acquisition at clinically relevant sites, assessed by BMC, HSA parameters and TBS, is higher in adolescent male footballers compared with swimmers and cyclists. There was no difference between the swimmers and cyclists, and cyclists had similar bone acquisition compared with controls. Bone formation was higher in footballers compared with swimmers and cyclists, and vitamin D levels were higher in footballers and cyclists compared with swimmers and controls. Bone formation decreased over time in cyclists and swimmers but not in footballers or controls, whereas bone resorption increased in swimmers and footballers. These findings suggest that the osteogenic 1 year of football participation is beneficial for bone acquisition and that participation in non-osteogenic sports is not enough to induce positive bone adaptations. Therefore, programs to improve bone outcomes of non-osteogenic athletes during adolescence are required. Further research with longer follow-up needs to address whether the observed changes in this study translate into better bone health in footballers compared with swimmers and cyclists, which could have important training implications for non-osteogenic sports.

Disclosures

All authors state that they have no conflicts of interest.

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Ethics approval was received from the following committees:
1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. no. 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. no. 2014/

766); and 3) the National Research Ethics Service Committee (NRES Committee South West–Cornwall and Plymouth, ref. no. 14/SW/0060).

Authors' roles: DV obtained and analyzed the data and drafted the manuscript under the supervision of LGM (principal investigator), ARB, and CAW. EUG, IGF, and KMK reviewed and improved the manuscript. All authors have read and approved this work.

References

- 1. Gordon CM, Zemel BS, Wren TA, et al. The determinants of peak bone mass. J Pediatr. 2017;180:261–9.
- Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab. 1992;75(4): 1060–5.
- 3. Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. Arch Dis Child. 2005;90(4):373–8.
- Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr. 2001;139(4):509–15.
- Cooper C, Dennison EM, Leufkens HG, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. J Bone Miner Res. 2004;19(12):1976–81.
- Donaldson LJ, Reckless IP, Scholes S, Mindell JS, Shelton NJ. The epidemiology of fractures in England. J Epidemiol Community Health. 2008;62(2):174–80.
- 7. Hernlund E, Svedbom A, Ivergard M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8:136.
- 8. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729–39.
- Boreham CA, McKay HA. Physical activity in childhood and bone health. Br J Sports Med. 2011;45(11):877–9.
- Bonjour JP, Chevalley T, Rizzoli R, Ferrari S. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. Med Sport Sci. 2007;51:64–80.
- Chevalley T, Bonjour JP, Ferrari S, Hans D, Rizzoli R. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebocontrolled trial in prepubertal boys. J Clin Endocrinol Metab. 2005;90:3342–9.
- Moschonis G, Kanellakis S, Papaioannou N, Schaafsma A, Manios Y. Possible site-specific effect of an intervention combining nutrition and lifestyle counselling with consumption of fortified dairy products on bone mass: the Postmenopausal Health Study II. J Bone Miner Metab. 2011;29(4):501–6.
- Janz KF, Letuchy EM, Burns TL, Eichenberger Gilmore JM, Torner JC, Levy SM. Objectively measured physical activity trajectories predict adolescent bone strength: lowa Bone Development Study. Br J Sports Med. 2014;48(13):1032–6.
- Gracia-Marco L, Ortega FB, Jimenez-Pavon D, et al. Adiposity and bone health in Spanish adolescents. The HELENA study. Osteoporos Int. 2012;23:937–47.
- 15. Wilkinson K, Vlachopoulos D, Klentrou P, et al. Soft tissues, areal bone mineral density and hip geometry estimates in active young boys: the PRO-BONE study. Eur J Appl Physiol. 2017; 117(4):833–42.
- Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weightbearing activities on bone mineral content and density in children and adolescents: a meta-analysis. J Bone Miner Res. 2014;29(2):467–78.
- Maimoun L, Coste O, Mura T, et al. Specific bone mass acquisition in elite female athletes. J Clin Endocrinol Metab. 2013;98(7):2844–53.

VLACHOPOULOS ET AL. Journal of Bone and Mineral Research

- Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. Bone. 2008;43(6):1101–7.
- Kato T, Yamashita T, Mizutani S, Honda A, Matumoto M, Umemura Y. Adolescent exercise associated with long-term superior measures of bone geometry: a cross-sectional DXA and MRI study. Br J Sports Med. 2009;43(12):932–5.
- Zouch M, Vico L, Frere D, Tabka Z, Alexandre C. Young male soccer players exhibit additional bone mineral acquisition during the peripubertal period: 1-year longitudinal study. Eur J Pediatr. 2014;173(1):53–61.
- 21. Maimoun L, Coste O, Philibert P, et al. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. Metabolism. 2013;62(8):1088–98.
- Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Cycling and bone health: a systematic review. BMC Med. 2012;10:168.
- Gomez-Bruton A, Gonzalez-Aguero A, Gomez-Cabello A, Casajus JA, Vicente-Rodriguez G. Is bone tissue really affected by swimming? A systematic review. PLoS One. 2013;8(8):e70119.
- 24. Sport England. Active people survey 10. London, UK: UK Department of Health; 2016.
- 25. Vlachopoulos D, Barker AR, Williams CA, et al. The impact of sport participation on bone mass and geometry in male adolescents. Med Sci Sports Exerc. 2017;49(2):317–26.
- 26. Nebigh A, Rebai H, Elloumi M, et al. Bone mineral density of young boy soccer players at different pubertal stages: relationships with hormonal concentration. Joint Bone Spine. 2009;76(1):63–9.
- 27. Ubago-Guisado E, Gomez-Cabello A, Sanchez-Sanchez J, Garcia-Unanue J, Gallardo L. Influence of different sports on bone mass in growing girls. J Sports Sci. 2015;33(16):1710–8.
- Lehtonen-Veromaa M, Mottonen T, Irjala K, Nuotio I, Leino A, Viikari J.
 A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. J Clin Endocr Metab. 2000;85(10):3726–32.
- Ackerman KE, Nazem T, Chapko D, et al. Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. J Clin Endocrinol Metab. 2011;96(10):3123–33.
- 30. Nickols-Richardson SM, O'Connor PJ, Shapses SA, Lewis RD. Longitudinal bone mineral density changes in female child artistic gymnasts. J Bone Miner Res. 1999;14(6):994–1002.
- Maimoun L, Coste O, Galtier F, et al. Bone mineral density acquisition in peripubertal female rhythmic gymnasts is directly associated with plasma IGF1/IGF-binding protein 3 ratio. Eur J Endocrinol. 2010;163(1):157–64.
- 32. Greene DA, Naughton GA. Adaptive skeletal responses to mechanical loading during adolescence. Sports Med. 2006;36(9):723–32.
- 33. Sayers A, Marcus M, Rubin C, McGeehin MA, Tobias JH. Investigation of sex differences in hip structure in peripubertal children. J Clin Endocrinol Metab. 2010;95(8):3876–83.
- Vlachopoulos D, Ubago-Guisado E, Barker AR, et al. Determinants of bone outcomes in adolescent athletes at baseline: the PRO-BONE study. Med Sci Sports Exerc. 2017;49(7):1389–96.
- Crabtree NJ, Arabi A, Bachrach LK, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom. 2014;17(2):225–42.
- 36. Ferry B, Duclos M, Burt L, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. J Bone Miner Metab. 2011;29(3):342–51.
- 37. Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. Joint Bone Spine. 2013:80(1):57–63.
- 38. Hans D, Goertzen AL, Krieg MA, Leslie WD. Bone microarchitecture assessed by TBS predicts osteoporotic fractures independent of

- bone density: the Manitoba study. J Bone Miner Res. 2011; 26(11):2762–9.
- 39. Shawwa K, Arabi A, Nabulsi M, et al. Predictors of trabecular bone score in school children. Osteoporos Int. 2016;27(2):703–10.
- Silva BC, Leslie WD, Resch H, et al. Trabecular bone score: a noninvasive analytical method based upon the DXA image. J Bone Miner Res. 2014;29(3):518–30.
- 41. Banfi G, Lombardi G, Colombini A, Lippi G. Bone metabolism markers in sports medicine. Sports Med. 2010;40(8):697–714.
- 42. Gracia-Marco L, Vicente-Rodriguez G, Valtuena J, et al. Bone mass and bone metabolism markers during adolescence: the HELENA study. Horm Res Paediatr. 2010;74(5):339–50.
- 43. Vaitkeviciute D, Latt E, Maestu J, et al. Longitudinal associations between bone and adipose tissue biochemical markers with bone mineralization in boys during puberty. BMC Pediatr. 2016;16:102.
- Vlachopoulos D, Barker AR, Williams CA, Knapp KM, Metcalf BS, Gracia-Marco L. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. BMC Public Health. 2015;15(1):361.
- Johnson J, Dawson-Hughes B. Precision and stability of dual-energy X-ray absorptiometry measurements. Calcif Tissue Int. 1991;49(3): 174–8.
- 46. Khoo BC, Beck TJ, Qiao QH, et al. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multicenter clinical trials. Bone. 2005;37(1):112–21.
- Pothuaud L, Barthe N, Krieg MA, Mehsen N, Carceller P, Hans D. Evaluation of the potential use of trabecular bone score to complement bone mineral density in the diagnosis of osteoporosis: a preliminary spine BMD-matched, case-control study. J Clin Densitom. 2009;12(2):170–6.
- 48. Vasikaran S, Cooper C, Eastell R, et al. International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med. 2011;49(8):1271–4.
- 49. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51(3):170–9.
- 50. Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. J Sci Med Sport. 2013;16(2):124–8.
- 51. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JA. Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. Br J Sports Med. 2005;39(9):611–6.
- 52. Gunter K, Baxter-Jones AD, Mirwald RL, et al. Impact exercise increases BMC during growth: an 8-year longitudinal study. J Bone Miner Res. 2008;23(7):986–93.
- 53. Gomez-Bruton A, Gonzalez-Aguero A, Gomez-Cabello A, Matute-Llorente A, Casajus JA, Vicente-Rodriguez G. Swimming and bone: is low bone mass due to hypogravity alone or does other physical activity influence it? Osteoporos Int. 2016;27(5):1785–93.
- Heinio L, Nikander R, Sievanen H. Association between long-term exercise loading and lumbar spine trabecular bone score (TBS) in different exercise loading groups. J Musculoskel Neuron. 2015;15(3): 279–85.
- 55. Gracia-Marco L, Ortega FB, Jiménez-Pavón D, et al. Contribution of bone turnover markers to bone mass in pubertal boys and girls. J Pediatr Endocrinol Metab. 2011;24(11–12):971–4.
- Kambas A, Leontsini D, Avloniti A, et al. Physical activity may be a potent regulator of bone turnover biomarkers in healthy girls during preadolescence. J Bone Miner Metab. Epub 2016 Nov 12.
- 57. Jurimae J, Maestu J, Jurimae T. Bone turnover markers during pubertal development: relationships with growth factors and adipocytokines. Med Sport Sci. 2010;55:114–27.
- 58. Gonzalez-Gross M, Valtuena J, Breidenassel C, et al. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. Br J Nutr. 2012;107:755–64.

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Title

The effect of 12-month participation in osteogenic and non-osteogenic sports on bone development in adolescent male athletes. The PRO-BONE study.

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Abstract

Objectives: Research investigating the longitudinal effects of the most popular sports on bone development in adolescent males is scarce. The aim is to investigate the effect of 12-month participation in osteogenic and non-osteogenic sports on bone development.

Design: A 12-month study was conducted in adolescent males involved in football, swimming and cycling and compared with an active control group.

Methods: 116 adolescent males (13.1±0.1 years at baseline): 37 footballers, 37 swimmers, 28 cyclists and 14 active controls were followed for 12 months. Bone mineral content (BMC) was measured by dual-energy x-ray absorptiometry, and bone stiffness was measured by quantitative ultrasound. Bone outcomes at 12 months were adjusted for baseline bone status, age, height, lean mass and moderate to vigorous physical activity.

Results: Footballers had higher improvement in adjusted BMC at the total body, total hip, shaft, Ward's triangle, legs and bone stiffness compared to cyclists (6.3 to 8.0 %). Footballers had significantly higher adjusted BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %). There was no significant difference between swimmers and cyclists for any bone outcomes. Swimming and cycling participation resulted in non-significant lower bone development at most sites of the skeleton compared to controls (-4.3 to -0.6 %).

Conclusions: Football participation induces significantly greater improvements in BMC and bone stiffness over 12 months compared to cycling and swimming.

Abbreviations: BMC: Bone mineral content; BMD: Bone mineral density; DXA: Dual Energy X-Ray Absorptiometry; MVPA: Moderate to vigorous physical activity; PA: Physical activity; QUS: Quantitative ultrasound; PBM: Peak bone mass; T0: baseline measurements; T1: 12-months measurements.

Keywords: Adolescence; Bone mass; Bone stiffness; Cycling; Football; Swimming; Weight-bearing exercise.

1. Introduction

Bone development occurs most rapidly during childhood and adolescence, with 80-90 % of peak bone mass (PBM) acquired by late adolescence depending on the site of the skeleton ¹. PBM is largely determined by genetics ² and by modifiable factors, such as nutrition and physical activity (PA) ^{3,4}. Exercise during this period of life can enhance bone mineral content (BMC) and bone mineral density (BMD) ⁵ and be maintained into adulthood ⁶. Football, cycling and swimming are among the most popular sports performed by adolescents around the world ⁷. However, participation in these sports may have different effects on bone development ⁸. Participation in "osteogenic" sports, such us football, can augment BMC at the loaded sites of the skeleton ^{9,10}. However, participation in "non-osteogenic sports", such as swimming and cycling, may have a negative or no impact on bone outcomes¹¹, which may compromise the achievement of a higher PBM and increase the risk of osteoporotic fractures in adulthood. From a public health perspective, understanding how the most popular sports worldwide among youth affect bone development is of great importance.

Cross-sectional studies have evaluated differences in BMC between adolescents engaged in different sports in comparison to a control group¹¹. Specifically, footballers were found to have higher adjusted-BMC and BMD at most sites of the skeleton compared with age-matched controls ⁹. In contrast, previous evidence found that adolescent male swimmers had lower adjusted-BMC and BMD at several sites compared to controls ¹², but a recent systematic review concluded that swimmers have similar bone mass compared to sedentary controls ¹³. Similarly, in a cross-sectional analysis we found that adolescent male swimmers and cyclists had lower bone outcomes compared to footballers ⁸. However, other studies showed that cycling during adolescence may negatively influence bone health ^{11, 14}. To date, there are only a few longitudinal studies on this topic and it was found that 3 years of

football participation increased femoral neck BMD by 10 % and improved femoral neck and intertrochanteric BMC twice as much compared to age-matched controls in prepubertal males ¹⁵. Previously, 8 months of football training significantly improved bone outcomes at total body, intertrochanteric site, lumbar spine and femoral neck in female adolescent footballers, whereas 8 months of swimming training had no effect on bone outcomes in female adolescent swimmers ¹⁶. Research investigating the longitudinal effects of the most popular sports on bone development in adolescent males is scarce. In a recent study we showed the effect of these sports on clinically relevant sites, including hip geometry estimates and trabecular texture, as well as biochemical markers ¹⁷. It should be noted that a comprehensive analysis of potential confounders, such as lean mass and objectively measured moderate-to-vigorous PA (MVPA) should be used to control for important predictors of bone status in these sports¹⁸.

In addition to Dual energy X-ray Absorptiometry (DXA), Quantitative Ultrasound (QUS) can indicate the risk of osteoporotic fractures at the calcaneus site that is particularly important for adolescent athletes due to their high prevalence of injuries ^{19, 20}. In a cross-sectional study, it was shown that swimming had no effect on bone stiffness compared to age-matched controls in adolescent males and females ¹². Also, in a cross-sectional analysis it was found that footballers had higher bone stiffness than controls but there were no differences in swimmers and cyclists compared to controls ⁸. However, there is lack of longitudinal studies comparing the effects of osteogenic and non-osteogenic sports on QUS bone outcomes in adolescent males athletes ²¹. Therefore, the purpose of this study is to investigate the effect of 12-month participation on BMC and bone stiffness in osteogenic (football) and that non-osteogenic sports (swimming and cycling) compared to an active control group after controlling for baseline bone outcomes, age, height, lean mass and MVPA.

2. Methods

The present study represents a 12-month analysis of sport participation as part of the PRO-BONE study, whose purpose and methodology have been described elsewhere ²². For the present study, data

obtained at baseline (T0) during autumn/winter 2014/15 and at follow-up (T1) during autumn/winter 2015/2016 were used (mean difference of visits = 372 days). Five participants were excluded because they did not complete the second visit (n=3) or they had missing data (n=2). For the present study, 116 adolescent males (37 swimmers, 37 footballers, 28 cyclists and 14 active controls not engaged in these sports more than 3 hour per week) aged 13.1 years \pm 1.0 at T0 and 14.1 years \pm 1.0 at T1 were included. The inclusion criteria at T0 were: 1) males 12–14 years old, engaged (≥3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or more; 2) males 12–14 years old not engaged in any of these sports (≥3 h/week) in the last 3 or more years (control group). The exclusion criteria were at T0 were: 1) participants not taking part in another clinical trial; 2) participants not having any acute infection lasting until < 1 week before inclusion; 3) participants free of any medical history of diseases or medications affecting bone metabolism or injured; 4) white Caucasian ethnicity. Ethics approval received from the following committees: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West -Cornwall & Plymouth, ref. number 14/SW/0060).

A DXA scanner (GE Lunar Prodigy Healthcare Corp., Madison, WI, USA, 2006) was used to measure BMC (g), fat mass (g) and lean mass (g, excluding bone and fat mass). The total body scan was used to obtain BMC at the arms, legs, and total body (excluding head). Dual hip scans were performed to obtain BMC for total hip, femoral neck, Ward's triangle, trochanter and shaft subregions and the mean of right and left hip scans was used. The coefficient of variation (CV) for measurement reliability was not determined in the present study. Previous paediatric studies have shown that the DXA between-day CV was between 1.0 % and 2.9 % depending on the region ²³. In addition, QUS measurements were performed with a Lunar Achilles Insight (TM Insight GE Healthcare, Milwaukee, WI, USA). This portable device measures bone stiffness using ultrasound waves. QUS is a non-ionising radiation technique and evaluates bone stiffness based on broadband ultrasound attenuation (dB/MHz) and speed of sound (m/s) ²⁴. The real-time image of the calcaneus and the region of interest ensures that the measurement is reliable and valid to assess bone health as

demonstrated in paediatric population ²⁵. Daily calibration was completed at all visits and measurements were taken according to the standard procedure provided by the manufacturer. The positioning was standardised between visits by using an adapter for the children's feet in order to get the same position of the calcaneus. Both feet were measured twice and the mean of the two measures was used for statistical analyses.

Stature (cm) and body mass (kg) were measured by using standard procedures and sexual maturity was self-reported using adapted drawings of the five stages of pubic hair development ²⁶. Physical activity was measured for seven consecutive days at T0 and T1 using wrist accelerometers (GENEActiv, GENEA, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents ²⁷. Data were collected at 100 Hz and analysed at 1 s epoch intervals to establish time spent in MVPA using a validated cut-point ²⁷. Weekly training hours were obtained by face to face interviews at T0 at T1. In addition, the coaches indicated participation in weight-training exercises for a subsample of participants.

Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA). Data were normally distibuted and presented as mean and standard deviation. Data were analysed in two stages: 1) raw (unadjusted) data using one-way analysis of variance (ANOVA) with Bonferroni post hoc or Chi-Square tests at T0 and T1 to detect the differences in BMC, and 2) adjusted data using one-way analysis of covariance (ANCOVA) with Bonferroni post hoc to detect the differences between the groups at T1 after controlling for: bone status at T0, age, height, lean mass, MVPA and maturity status ^{18, 28, 29}. Paired t-tests were used to compare differences in values between T0 and T1. Preliminary analyses showed bone outcome results did not change when maturity was used as confounder instead of age. Thus, maturity was not included in the model. Percentages of difference between groups were used to quantify the magnitude of the differences. Significance was set at p < 0.05 and p < 0.01.

3. Results

Table 1 presents the descriptive characteristics of the participants at T0 and T1. From T0 to T1 all the descriptive characteristics significantly increased in all groups except MVPA in all groups and body fat percentage in sports groups that significantly decreased. Between-group differences at T1 showed that swimmers were older, taller, heavier and had more lean mass than the footballers and controls. Swimmers were more mature than footballers and controls. Swimmers trained more hours per week and had more years of training than cyclists. Footballers spent more time doing MVPA than swimmers and controls. In addition, footballers trained more hours per week and had more training years than cyclists and swimmers. Cyclists were older than controls and spent more time doing MVPA compared to swimmers and controls. Controls had a higher body fat percentage than all sports groups.

(Table 1 here)

Table 2 shows the adjusted BMC and bone stiffness at T0 and T1 between the groups and Figure 1 shows the adjusted BMC and bone stiffness differences (%) between the sports groups and controls at T1. At T1 footballers had significantly higher BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %). Also, at T1 footballers had significantly higher BMC at total body, total hip, Ward's triangle, shaft and legs compared to cyclists (6.3 to 8.0 %). At T1 footballers had non-significantly higher bone outcomes than controls (3.3 to 8.4 %). The adjusted bone stiffness was significantly higher in footballers compared to cyclists (7.8 %) at T1. Swimmers and cyclists had similar bone outcomes at T1 (-0.6 to 4.3 %) and both groups had no significant differences at any of the bone outcomes compared to controls (-4.5 to 4.7 %).

(Table 2 and Figure 1here)

Supplementary table 1 shows the unadjusted change in bone outcomes at T0 and T1. At T1 BMC significantly increased at all skeletal sites in swimmers (10.3 to 21.0 %), footballers (13.6 to 23 %), cyclists (9.9 to 19.0 %) and controls (14.8 to 21.0 %) compared to T0. In addition, bone stiffness significantly increased in swimmers (4.5 %), footballers (6.9 %) and controls (5.1 %) from T0 to T1, but the increase was not significantly different in cyclists (0.9 %).

4. Discussion

The main findings of the present study are: 1) after 12 months of sports participation, footballers had significantly higher BMC and bone stiffness gains compared to swimmers and cyclists, and higher but non-significant BMC and bone stiffness compared to active controls; 2) after 12 months swimmers and cyclists had similar BMC and bone stiffness, and both groups had no significant differences in BMC and bone stiffness compared to controls.

The present study shows that after 12 months footballers had higher adjusted BMC compared to cyclists and swimmers at most skeletal sites. The only study comparing these sports was conducted in female adolescent swimmers and footballers and showed that 8 months period of sport-specific training increased total body BMD by 2.9 % in footballers, whereas BMD remained constant in swimmers ¹⁶. The present study found that footballers had 2.4 % higher adjusted BMC compared to swimmers after 12 months. Cross-sectional evidence in adolescent males found that footballers had greater BMD at the femoral neck compared to swimmers ³⁰. The differences observed in BMC gains among the sports groups in the present study might be explained by the plyometric exercises included in the football training that can induce higher bone mass in adolescent athletes despite the reduced lean mass in footballers compared to swimmers ³¹. In this regard, Larsen et al. found that a 10-month programme that included small-sided ball games improved BMD at the legs and total body compared to controls, and BMD at the legs compared to a circuit strength training ³².

In the present study, BMC development over 12 months was similar between adolescent male swimmers and cyclists at any skeletal sites. This is in line with studies showing that swimming and cycling seem to have no additional effect on bone growth ^{11, 12}, which could be due to the low ground reaction forces produced during participation in the non-osteogenic environment. In regards to bone stiffness, the present study showed that footballers significantly increased bone stiffness compared to cyclists. The latter is in accordance with cross-sectional analysis from this cohort showing that footballers had significantly higher bone stiffness compared to swimmers and cyclists ⁸.

Football participation during adolescence may induce higher bone outcomes compared to leisure active controls according to cross-sectional evidence ^{9, 10}. However, evidence from a study in

prepubescent boys found that footballers had non-significant but higher bone outcomes compared to active controls after 10 months of training ³³. These results are in line with our findings showing that footballers had higher (3.3 % to 8.4 %) but not significant bone outcomes compared to active controls after 12 months. It should be noted that the control group was physically active (MVPA= 64 min/day) and some controls engaged in other weight-bearing sports (< 3 hours per week) which might explain the non-significant difference compared to footballers. A previous cross-sectional study showed that footballers had significantly higher bone stiffness at lower extremities compared to active controls ³⁴. The differences in bone outcomes between adolescent footballers and controls might increase in the future due to the previous findings showing that 3 years of football training exhibited significantly greater adjusted BMC in total body, legs and intertrochanteric sites compared to age-matched controls

Swimming is considered a non-osteogenic sport and does not promote positive changes on bone development above that observed due to growth. According to a recent meta-analysis, swimmers and sedentary controls have similar bone outcomes ¹³. In addition, adolescent males that participated only in swimming had lower BMD and BMC at several sites of the skeleton compared to age-matched controls ¹². In the present study swimmers had similar BMC gains with active controls after controlling for relevant covariates (including T0 BMC). Similarly, we found swimmers to have similar bone outcomes with controls at baseline after controlling for the same covariates ⁸. A possible explanation is that swimming has non-gravitational training characteristics and despite swimmers having augmented higher lean mass it was not enough to produce bone adaptations after 12-months of training ³⁵. Regarding bone stiffness, previous cross-sectional findings showed similar values between swimmers and controls ¹².

Cycling is a widely practised sport that applies low mechanical forces to the skeleton during training ³⁶ and the present analysis showed that cyclists had lower but non-significant adjusted BMC and bone stiffness than controls. Previous evidence exist only from cross-sectional studies indicating that adolescent female cyclists had similar or lower bone outcomes compared to non-athletic controls¹⁶. Another cross-sectional study found that males cyclists (< 17 years) had significantly lower BMC at the legs compared to age-matched controls ¹¹. According to the baseline cross-sectional

analysis of this cohort, cyclists had non-significantly higher adjusted BMC at the most skeletal sites ⁸. However, after one year cyclists had non-significant lower bone development in BMC and bone stiffness than controls. The differences observed in the current study might be explained by the non-osteogenic environment of both swimming and cycling and by the mechanical loading produced by the sports-specific patterns. In addition, participation in plyometric training or other weight-bearing activities might explain the difference on bone outcomes between adolescent athletes and needs further investigation to quantify the impact of weight training on bone outcomes.

The strengths of the present study are 1) the investigation of bone outcomes across osteogenic and non-osteogenic male adolescent groups over 12 months; 2) the combination of DXA and QUS, which provides a comprehensive insight into BMC and bone stiffness outcomes and 3) the rigorous methodology that enabled the inclusion of a selection of specific confounders which increases the internal validity of the study. A limitation of the present study is the lack of nutrition-related covariates and the two time points of the longitudinal assessment. However, we have observed that dietary intakes (total energy, protein and calcium) were no different between the groups at T0 and T1 (data not reported). In addition, despite the two measurements completed, this is the first stud to assess the differences in bone development of these sports over 12 months. Also, it should be noted that all sport groups were very active, but cyclists trained less compared to footballers and swimmers.

5. Conclusions

In summary, the findings of this study suggest that 12 months of football participation induces greater BMC and bone stiffness compared to cycling or swimming participation. In addition, footballers had higher BMC although not significant compared to an active control group. Swimmers and cyclists had similar bone outcomes after 12 months, and both groups no significant differences in any of the bone outcomes compared to active controls. These findings suggest that participation in non-osteogenic sports during adolescence should be combined with weight-bearing activities in order to optimise bone development. Studies focusing on females and using specific interventions to improve bone mineralization in non-osteogenic sports during growth are needed.

Practical implications

- Football participation for 12-months induces significantly higher increase in bone mineral content and bone stiffness compared to cycling and swimming in adolescent males.
- Participation in cycling and swimming for 12-months has similar effects on bone development in adolescent males and both groups have non-significant lower bone outcomes compared to active controls.
- Cycling and swimming participation may compromise the optimal bone development during adolescence suggesting intervention studies are needed to improve bone development in adolescents participating in these sports.

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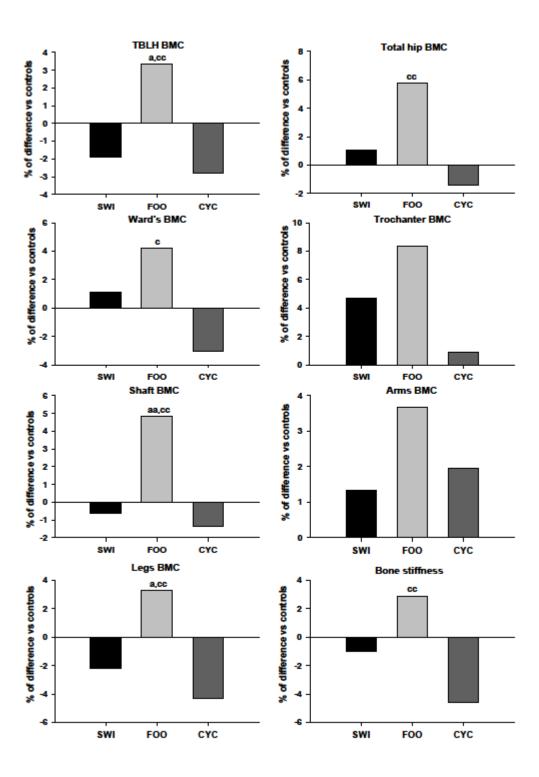
References

- 1. Gordon CM, Zemel BS, Wren TA, et al. The Determinants of Peak Bone Mass. *J Pediatr*. 2016.
- 2. Bonjour JP, Chevalley T, Rizzoli R, Ferrari S. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. *Med Sport Sci.* 2007; 51:64-80.
- 3. Gracia-Marco L, Moreno LA, Ortega FB, et al. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. *Am J Prev Med.* 2011; 40(6):599-607.
- 4. Mouratidou T, Vicente-Rodriguez G, Gracia-Marco L, et al. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin Densitom.* 2013; 16(1):110-117.
- 5. Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. *J Bone Miner Res*. 2014; 29(2):467-478.
- 6. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone.* 2008; 43(6):1101-1107.
- 7. Active People Survey 10: Sport England, UK Department of Health; 2016.
- 8. Vlachopoulos D, Barker AR, Williams CA, et al. The Impact of Sport Participation on Bone Mass and Geometry in Male Adolescents. *Med Sci Sports Exerc.* 2017; 49(2):317-326.
- 9. Zouch M, Vico L, Frere D, Tabka Z, Alexandre C. Young male soccer players exhibit additional bone mineral acquisition during the peripubertal period: 1-year longitudinal study. *Eur J Pediatr.* 2014; 173(1):53-61.
- Ubago-Guisado E, Gomez-Cabello A, Sanchez-Sanchez J, Garcia-Unanue J, Gallardo L. Influence of different sports on bone mass in growing girls. *J Sports Sci.* 2015; 33(16):1710-1718.
- 11. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Bone Related Health Status in Adolescent Cyclists. *Plos One.* 2011; 6(9):e24841.
- 12. Gomez-Bruton A, Gonzalez-Aguero A, Gomez-Cabello A, Matute-Llorente A, Casajus JA, Vicente-Rodriguez G. The effects of swimming training on bone tissue in adolescence. *Scand J Med Sci Sports*. 2015; 25(6):e589-602.
- 13. Gomez-Bruton A, Montero-Marin J, Gonzalez-Aguero A, et al. The Effect of Swimming During Childhood and Adolescence on Bone Mineral Density: A Systematic Review and Meta-Analysis. *Sports Med.* 2016; 46(3):365-379.
- 14. Rico H, Revilla M, Hernandez ER, Gomez-Castresana F, Villa LF. Bone mineral content and body composition in postpubertal cyclist boys. *Bone*. 1993; 14(2):93-95.
- 15. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA. High femoral bone mineral density accretion in prepubertal soccer players. *Med Sci Sports Exerc*. 2004; 36(10):1789-1795.
- 16. Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. *Joint Bone Spine*. 2013; 80(1):57-63.
- 17. Vlachopoulos D, Barker AR, Ubago-Guisado E, et al. Longitudinal Adaptations of Bone Mass, Geometry and Metabolism in Adolescent Male Athletes. The PRO-BONE Study. *J Bone Miner Res.* 2017.
- 18. Vlachopoulos D, Ubago-Guisado E, Barker AR, et al. Determinants of Bone Outcomes in Adolescent Athletes at Baseline: The PRO-BONE Study. *Med Sci Sports Exerc*. 2017; 49(7):1389-1396.
- 19. Krieg MA, Barkmann R, Gonnelli S, et al. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. *J Clin Densitom*. 2008; 11(1):163-187.
- 20. Frush TJ, Lindenfeld TN. Peri-epiphyseal and Overuse Injuries in Adolescent Athletes. *Sports Health.* 2009; 1(3):201-211.
- 21. Boreham CA, McKay HA. Physical activity in childhood and bone health. *Br J Sports Med.* 2011; 45(11):877-879.

- 22. Vlachopoulos D, Barker AR, Williams CA, Knapp KM, Metcalf BS, Gracia-Marco L. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. *BMC Public Health*. 2015; 15(1):361.
- 23. Johnson J, Dawson-Hughes B. Precision and stability of dual-energy X-ray absorptiometry measurements. *Calcif Tissue Int.* 1991; 49(3):174-178.
- 24. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. *Pediatr Res.* 2008; 63(3):220-228.
- 25. Sioen I, Lust E, De Henauw S, Moreno LA, Jimenez-Pavon D. Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. *Calcif Tissue Int.* 2016; 99(6):557-577.
- 26. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child.* 1976; 51(3):170-179.
- 27. Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. *J Sci Med Sport*. 2013; 16(2):124-128.
- 28. Wilkinson K, Vlachopoulos D, Klentrou P, et al. Soft tissues, areal bone mineral density and hip geometry estimates in active young boys: the PRO-BONE study. *Eur J Appl Physiol*. 2017; 117(4):833-842.
- 29. Ubago-Guisado E, Vlachopoulos D, de Moraes ACF, et al. Lean mass explains the association between muscular fitness and bone outcomes in 13-year-old boys. *Acta paediatrica* (*Oslo, Norway : 1992*). 2017.
- 30. Silva CC, Goldberg TB, Teixeira AS, Dalmas JC. The impact of different types of physical activity on total and regional bone mineral density in young Brazilian athletes. *J Sports Sci.* 2011; 29(3):227-234.
- 31. Gunter K, Baxter-Jones AD, Mirwald RL, et al. Impact exercise increases BMC during growth: an 8-year longitudinal study. *J Bone Miner Res.* 2008; 23(7):986-993.
- 32. Larsen MN, Nielsen CM, Helge EW, et al. Positive effects on bone mineralisation and muscular fitness after 10 months of intense school-based physical training for children aged 8-10 years: the FIT FIRST randomised controlled trial. *Br J Sports Med.* 2016.
- 33. Zouch M, Jaffre C, Thomas T, et al. Long-term soccer practice increases bone mineral content gain in prepubescent boys. *Joint Bone Spine*. 2008; 75(1):41-49.
- 34. Falk B, Braid S, Moore M, Yao M, Sullivan P, Klentrou N. Bone properties in child and adolescent male hockey and soccer players. *J Sci Med Sport*. 2010; 13(4):387-391.
- 35. Maimoun L, Coste O, Philibert P, et al. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. *Metabolism.* 2013; 62(8):1088-1098.
- 36. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Cycling and bone health: a systematic review. *BMC Med.* 2012; 10:168.

Figure 1.

Differences (%) in adjusted bone mineral content (BMC) between the sports groups and controls after 12 months. The results adjusted for age, height, lean mass, moderate to vigorous physical activity and bone outcomes at baseline (T0). TBLH: Total body less head. Letters denote a significant difference with: a (Swimmers, SWI), b (Footballers, FOO), c (Cyclists, CYC) and d (Controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.01.



Tables

Table 1.Descriptive characteristics of the participants at baseline (T0) and after 12 months (T1) of sport participation

N = 116	Swimmers	Footballers	Cyclists	Controls
	(N = 37)	(N = 37)	(N=28)	(N = 14)
Age (yrs)	,	·	,	,
TÖ	13.5 (1.0) ^{b,dd}	12.9 (0.9)	13.2 (1.0) ^d	12.3 (0.5)
T1	14.6 (1.0) ^{b,dd,*}	13.9 (0.9)*	$14.2 (1.0)^{d,*}$	13.2 (0.5)*
Stature (cm)	, ,	` ,	, ,	, ,
T0	165.1 (9.7) ^{bb,d}	155.2 (9.3)	160.7 (10)	154.5 (9.9)
T1	171.6 (8.9) ^{bb,dd,*}	162.7 (10.3)*	166.6 (10.7)*	160.7 (10.5)*
Body mass (kg)				
TO	51.9 (8.7) ^{bb}	44.3 (7.9)	49.3 (12.5)	48.3 (13.0)
T1	58.9 (8.2) ^{b,*}	50.8 (9.7)*	54.7 (12.5)*	55.2 (15.6)*
BMI (kg/m^2)				
T0	18.9 (1.6)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
T1	$19.9(2.0)^*$	$19.0 (1.8)^*$	$21.0(3.1)^*$	21.0 (3.7)*
Lean mass (kg)				
T0	$41.1 (9.0)^{b,dd}$	35.4 (7.2)	37.5 (7.5)	31.7 (5.5)
T1	47.8 (8.7) ^{b,dd,*}	41.2 (9.2)*	42.9 (8.2)*	36.8 (7.1)*
Body fat (%)				
T0	17.3 (7.3)*	15.7 (5.6)*	$18.0 (9.0)^*$	29.0 (10.5) ^{aa,bb,cc}
T1	14.4 (6.4)	14.5 (6.0)	16.1 (9.2)	27.9 (10.9) ^{aa,bb,cc}
Tanner stages (1-5;				
%)				
T0	(16/25/16/43/0)	(24/35/24/16/0)	(14/28/25/28/4)	(29/21/21/29/0)
T1	$(5/11/11/51/22)^{b,d,*}$	$(6/16/35/43/0)^*$	$(7/11/14/57/11)^*$	$(0/21/43/36/0)^*$
Training (h/week)				
T0	$9.4(5.1)^{cc}$	$10.0 (2.3)^{cc}$	5.2 (2.1)	-
T1	$8.9 (3.6)^{cc}$	9.4 (1.7) ^{cc}	5.6 (2.0)	-
Years of training				
T0	$5.9(2.5)^{cc}$	$7.5 (2.3)^{a,cc}$	3.9 (1.3)	-
T1	6.9 (2.5) ^{cc,*}	8.5 (2.3) ^{a,cc,*}	4.9 (1.3)	-
MVPA (min/day)				
T0	85.0 (30.9)*	119.8 (29.7) ^{aa,dd,*}	106.5 (33.7) ^{a,*}	83.2 (26.8)*
<u>T1</u>	62.9 (21.8)	92.4 (25.7) ^{aa,dd}	85.6 (21.8) ^{aa,d}	64.3 (18.1)

Values presented as mean (SD). BMI: Body mass index, MVPA: Moderate to vigorous physical activity. TO = baseline values, T1 = 1 year values. Superscript letters denote a higher significant difference between sports: a (swimmers), b (footballers), c (cyclists), d (controls), a,b,c,d p<0.05, aa,bb,cc,dd p<0.001 and within each sports group at T0 and T1: *p<0.05.

Table 2. Adjusted bone mineral content (BMC, g) and bone stiffness at baseline (T0) and after 12 months (T1) of sports participation in adolescent males

N = 116	Swimmers	Footballers	Cyclists	Controls
	(N = 37)	(N = 37)	(N = 28)	(N=14)
TBLH (g)				
T0	1453.9 (21.1)	1574.5 (21.5) ^{a,c,d}	1459.9 (22.7)	1451.8 (34.4)
T1	1752.9 (20.9)	1846.7 (20.9) ^{a,cc}	1737.0 (21.9)	1787.1 (33.6)
Total hip (g)				
T0	26.50 (0.50)	30.24 (0.51) ^{aa,cc,d}	26.62 (0.53)	24.61 (0.79)
T1	32.04 (0.42)	33.53 (0.44) ^c	31.26 (0.45)	31.70 (0.70)
Ward's (g)				
T0	2.15 (0.06)	$2.48 (0.06)^{a,c,dd}$	2.14 (0.06)	1.92 (0.1)
T1	2.66 (0.05)	$2.74 (0.05)^{c}$	2.55 (0.05)	2.63 (0.08)
Trochanter (g)				
T0	8.11 (0.23)	9.85 (0.23) ^{aa,cc,d}	8.22 (0.24)	7.60 (0.36)
T1	10.99 (0.27)	11.38 (0.28)	10.59 (0.28)	10.50 (0.43)
Shaft(g)				
T0	14.16 (0.26)	15.71 (0.26) ^{a,cc,d}	14.12 (0.27)	13.09 (0.41)
T1	16.20 (0.16)	$17.09 (0.17)^{a,cc}$	16.08 (0.17)	16.30 (0.27)
Arms (g)				
T0	209.27 (3.23)	207.24 (3.19)	211.98 (3.48) ^d	193.43 (5.22)
T1	252.85 (2.95)	258.71 (2.85)	254.39 (3.15)	249.52 (4.84)
Legs (g)				
T0	215.69 (4.51)	253.79 (4.59) ^{aa,cc,d}	223.08 (4.89)	216.31 (7.43)
T1	854.67 (9.67)	902.89 (9.82) ^{a,cc}	836.26 (9.85)	873.90 (15.24)
Stiffness index				
T0	89 (2)	$100 (2)^{a,c,d}$	91 (2)	86 (3)
T1	97 (1)	101 (1) ^c	93 (1)	98 (2)

Values are presented as mean (SE). TBLH: Total body less head. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). a,b,c,d p<0.05 and aa,bb,cc,dd p<0.001. At T0 BMC values were adjusted for age, stature, MVPA and lean mass. At T1 BMC values were adjusted for age, stature, MVPA, lean mass and for baseline BMC (T0).

"The roots of education are bitter, but the fruits are sweet"

Aristotle (384 BC, Stagira - 322 BC, Chalcis)