Examining the cross-sectional and longitudinal associations of lifestyle factors, endocrine factors and body composition with bone mass in childhood and adolescence: The Physical Activity and Nutrition in Children (PANIC) Study

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Abstract

Childhood and adolescence are a crucial period for determining peak bone mass, which is a risk factor for the lifetime risk of osteoporosis. However, there is a lack of evidence regarding the associations between lifestyle factors, endocrine factors, and body composition with bone in this demographic. This thesis used data from the Physical Activity and Nutrition in Children (PANIC) Study, a longitudinal study in a population sample of Finnish children, to investigate the cross-sectional and longitudinal associations of physical activity (PA) and other movement behaviours, vitamin D status, lean mass, and fat mass and associated endocrine factors, with bone mass. Chapter 4 showed that moderate PA, moderate-to-vigorous PA (MVPA), and vitamin D status were independently positively associated with bone mass in children aged 6 to 8 years. Chapter 5 applied novel mediation analysis methods and revealed a positive association between fat mass and bone mass in children aged 9 to 11 years, which was suppressed by mediation through free leptin index in both sexes, and moderated by adiponectin and free leptin index in females and males respectively. Chapter 6 used novel methods of summarising PA data in 9 to 11 year olds. PA volume was positively associated with bone mass in females and males, and PA intensity distribution was negatively associated with bone mass in males. Chapter 7 found that MVPA was positively associated with bone mass longitudinally in females, and that time spent in sport and exercise participation was positively associated with bone mass longitudinally in females and males. Sedentary time, screen time and sleep were not associated with bone mass longitudinally. These findings contribute to the literature by providing novel insights into the relationships between PA, summarised with different assessment methods and metrics, body composition, and associated endocrine

factors, and vitamin D status, with bone mass in children and adolescents. This thesis emphasises the importance of utilising different methods for assessing and summarising PA, and supports the consideration of fat and lean mass when looking at relationships between PA and bone mass. Further, the importance of PA promotion and behaviours to encourage improved vitamin D status to improve children's bone health during growth are highlighted.

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Skinner, A. M., Rowlands, A. V., Vlachopoulos, D., Barker, A. R., Janz, K. F. and Moore, S. A. (2023) “The Influence of Accelerometer Epoch Length on Associations of Physical Activity Intensity and Volume with Bone Outcomes”. Journal of Sports Science and Medicine 22: 117 - 132. https://doi.org/10.52082/jssm.2023.117

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Agostinete, R. R., Werneck, A. O., Maillane-Vanegas, S., Gracia-Marco, L., Ubago-Guisado, E., **Constable, A. M.**, Fernandes, R. A., and Vlachopoulos, D. (2021) "The Mediating Role of Lean Soft Tissue in the Relationship between Somatic Maturation and Bone Density in Adolescent Practitioners and Non-Practitioners of Sports." International Journal of Environmental Research and Public Health. 18(6):3008. https://doi.org/10.3390/ijerph18063008

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assessed were TBLH BMC, spine aBMD, total hip aBMD, femoral neck cross-sectional area, femoral neck section modulus.

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Abbreviations and Acronyms

25(OH)D	25-hydroxyvitamin D
aBMD	Areal bone mineral density
ALSPAC	Avon Longitudinal Study of Parents and Children
BA	Bone area
BIA	Bioelectric impedance analysis
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMDCS	Bone Mineral Density in Childhood Study
BMI	Body mass index
BPAQ	Bone-specific physical activity questionnaire
CI	Confidence interval
CT	Computed tomography
CV	Coefficient of variation
DHEAS	Dehydroepiandrosterone sulphate
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
EU27+2	European Union plus the UK and Switzerland
FITT	Frequency, intensity, time, type
GRF	Ground reaction force
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IBDS	Iowa Bone Development Study
IOTF	International Obesity Task Force
IQR	Interquartile range

ISCOLE	International Study of Childhood Obesity, Lifestyle and the Environment
LC-MS	Liquid chromatography-mass spectrometry
LPA	Light physical activity
MET	Metabolic equivalent
MPA	Moderate physical activity
MRI	Magnetic resonance imaging
MVPA	Moderate-to-vigorous physical activity
MX	The intensity above which a person's most active X minutes are accumulated
P-P	Probability-probability plot
PA	Physical activity
PAEE	Physical activity energy expenditure
PANIC	Physical Activity and Nutrition in Children Study
PAQ-A	Physical Activity Questionnaire for Adolescents
PAQ-C	Physical Activity Questionnaire for Children
PBM	Peak bone mass
PBMAS	University of Saskatchewan Pediatric Bone Mineral Accrual Study
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
Q-Q	Quantile-quantile plot
QUS	Quantitative ultrasound
RANKL	Receptor activator of the nuclear factor kappa-B ligand
RCT	Randomised controlled trial
SD	Standard deviation
SDS	Standard deviation score
SWS	Southamption Women's Survey
TBLH	Total-body-less-head
vBMD	Volumetric bone mineral density

VIF	Variance inflation factor
VPA	Vigorous physical activity
WHO	World Health Organisation

Units

cm	Centimetres
dB	Decibel
g	Grams
g	Gravitational acceleration units
g/cm^2	Grams per centimetre squared
g/cm^3	Grams per centimetre cubed
Hz	Hertz
kg	Kilograms
kg/m^2	Kiograms per metre squared
mm	Millimetres
mL	Millilitres
mU/L	Milliunits per litre
m/s^2	Metres per second squared
ng/ml	Nanograms per millilitre
nmol/L	Nanomoles per litre
pmol/L	Picomoles per litre
µg/ml	Micrograms per millilitre
µmol/L	Micromoles per litre
µSv	MicroSievert
µε	Microstrain
µε/s	Microstrain per second
°C	Degrees celsius
Δµε/d	Microstrain per linear distance

Chapter 1 Introduction

Osteoporosis, a disease characterised by low bone mass and increased fracture risk, carries a significant economic, societal, and individual burden, in terms of fracture-related healthcare monetary costs, increased disability and increased mortality rates due to fragility fractures (World Health Organisation, 1994; Hernlund *et al.*, 2013; Borgström *et al.*, 2020; Kanis *et al.*, 2021). Bone mass during adulthood is determined by both peak bone mass (PBM), achieved in young adulthood when bone accrual plateaus, and subsequent age-related bone loss (Eisman *et al.*, 1993; Hernandez, Beaupre and Carter, 2003; Gordon *et al.*, 2017). As bone mass tracks across the lifespan, those with greater bone mass in early adulthood are likely to continue to have greater bone mass in later life (Melton III *et al.*, 2000; Emaus *et al.*, 2006). Further, a 10% increase in PBM has been predicted to delay the onset of osteoporosis by 13 years, whereas a 10% change in bone loss has been predicted to delay the onset of osteoporosis by 2 years (Hernandez, Beaupre and Carter, 2003). This highlights the importance of maximising PBM in reducing the risk of osteoporosis in later life. Bone accrual during growth contributes to PBM, with around 40% of adult total body bone mineral content (BMC) accrued in the 4 years surrounding peak height velocity (PHV) (Baxter-Jones *et al.*, 2011). This has led to osteoporosis being described as a paediatric concern (Faulkner and Bailey, 2007). Therefore, understanding the factors which influence bone accrual during childhood and adolescence is important for promoting skeletal health across the lifespan (Bonjour *et al.*, 1994).

The development of bone mass during childhood and adolescence is determined by various factors, including genetics, sex, physical activity (PA),

lean mass, fat mass, endocrine factors, nutritional components, and exposure to risk factors, such as chronic diseases and medications (Bonjour *et al.*, 1994; Bonjour *et al.*, 2009; Pitukcheewanont *et al.*, 2013). Although genetic predisposition accounts for 60 to 80% of the variance in PBM, the remaining 20 to 40% is influenced by environmental and endocrine factors (Davies, Evans and Gregory, 2005; Weaver *et al.*, 2016). Further understanding of how PA, body composition, endocrine factors and nutrition influence bone accrual during childhood and adolescence is important for improving PBM and reducing the risk of osteoporosis in later life.

PA increases bone mass by increasing mechanical loads on bones, both directly and indirectly via lean mass (Gunter, Almstedt and Janz, 2012; Hart *et al.*, 2017). The skeleton adapts in response to these strains, as explained by the Mechanostat hypothesis (Fuchs, Warden and Turner, 2009; Gunter, Almstedt and Janz, 2012; Hart *et al.*, 2017). Systematic review evidence indicates that more active children have better current and future bone health parameters (Poitras *et al.*, 2016). However, the role of PA intensity remains unclear, particularly as previous literature is founded on energy expenditure-based estimates of PA intensity (Poitras *et al.*, 2016), which may be a suboptimal way to consider PA in relation to bone mass, and is more concerned with cardiometabolic outcomes. Further, the type of PA may be important for bone health, with a rapid evidence review highlighting agreement in the literature that weight-bearing PA, including sports participation, has an osteogenic effect (Public Health England, 2021). However, as much of the evidence on the relationships between osteogenic sports and bone health is based on studies in athletes, little is known about the relationship between bone

strengthening activities and bone mass in the general population. From a public health perspective, it is important to understand these relationships in the general population, to better inform PA guidelines relating to bone health.

Throughout childhood and adolescence, bone, lean and fat mass increase substantially, with the increases closely related to pubertal timing and tempo (Baxter-Jones *et al.*, 2003). There are critical synergies between the trajectories of bone, lean and fat accrual, whereby PHV is observed, followed by peak lean mass velocity, and then by peak fat velocity and peak BMC velocity (Iuliano-Burns, Mirwald and Bailey, 2001). Lean mass is positively related to bone mass in childhood and adolescence, and it has been suggested that lean mass may drive bone mass development, as the largest loads on the skeleton are from muscle contraction, which transfers forces to bones via tendons (Rauch *et al.*, 2004; Sioen *et al.*, 2016). Further, lean mass mediates the relationship between PA and bone (Zymbal *et al.*, 2019). Despite the critical role lean mass plays in the skeletal response to PA, there is limited research considering bone and lean outcomes together. Fat mass may influence bone mass by increasing mechanical loads on bone (Tobias, 2010), and has been positively associated with bone mass in pre- and early-pubertal children (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006; Soininen *et al.*, 2018). However, this association may disappear (Clark, Ness and Tobias, 2006) or invert (Gracia-Marco *et al.*, 2012) in late or post-puberty. In addition to increasing mechanical loads on bones, it has also been hypothesised that adiposity related changes in endocrine factors may mediate the relationship between fat mass and bone mass (Dimitri, 2018), though further research is needed to examine the mediating role of endocrine factors.

Vitamin D influences the development, maintenance and resorption of bone through regulation of calcium and phosphate homeostasis (Institute of Medicine, 2011). Despite the well-established physiological functions of vitamin D in bone metabolism, review evidence has indicated that the associations of serum 25-hydroxyvitamin D (25(OH)D), the clinically-relevant marker of vitamin D status, with bone measures in children and adolescents are unclear (Weaver *et al.*, 2016). Further, vitamin D status may modify the relationship between PA and bone, though studies so far have presented conflicting findings for the moderating role of vitamin D status in the PA and bone relationship (Valtuena *et al.*, 2012; Rønne *et al.*, 2018a). As such, the interaction between vitamin D status and PA with bone in children remains unknown.

The Physical Activity and Nutrition in Children (PANIC) study is ideally suited to provide novel insights into the relationships between PA, body composition and associated endocrine factors, and vitamin D status with bone mass. The PANIC study is an ongoing, longitudinal study with device-measured PA, blood biomarkers, and gold-standard body composition and bone measurements. It has an extended follow-up period in a population sample of 504 children at baseline at age 6 to 9 years, 438 children at 2-year follow-up at age 9 to 11 years, and 277 adolescents at 8-year follow-up at age 15 to 17 years. As such, research using the PANIC dataset can address previously unanswered questions regarding the contributions of PA, body composition and associated endocrine factors, and vitamin D status with bone health in children and adolescents.

The purpose of the present thesis is to present a series of novel investigations into PA, body composition and associated endocrine factors, and vitamin D status as determinants of bone mass during childhood and adolescence. This thesis will contribute to the research area by applying novel statistical analysis methods, the use of different methods to quantify PA, and including both cross-sectional and longitudinal designs. This research will add important insights regarding the contribution and interaction of PA, body composition and associated endocrine factors, and vitamin D status with bone health in childhood and adolescence. This may be useful for guiding future interventional research and informing lifestyle recommendations to improve bone health in childhood and adolescence.

Chapter 2 Literature Review

2.1 Importance of Peak Bone Mass

Osteoporosis is a skeletal disorder characterised by low bone mass, in which areal bone mineral density (aBMD) falls ≥ 2.5 standard deviations (SD) below the young adult mean, matched for sex and race (World Health Organisation, 1994). This low aBMD leads to increased bone fragility, and ultimately an increased risk of fractures (World Health Organisation, 1994). Researchers found that at age 65 years, the risk ratio of hip fracture increased 2.88 in females and 2.94 in males for each SD decrease in hip aBMD, emphasising the importance of aBMD in determining fracture risk (Johnell *et al.*, 2009). The prevalence of osteoporosis in the 27 countries in the European Union (EU), plus Switzerland and the UK (EU27+2), was estimated at 25.5 million women and 6.5 million men in 2019 (Willers *et al.*, 2022). In the same year, incident and prior fragility fracture-related monetary cost was estimated at €57 billion in the EU27+2, with 248,487 causally related deaths (Kanis *et al.*, 2021; Willers *et al.*, 2022). When compared to data from 2010, it is clear that the osteoporosis burden is increasing, as predicted, along with the ageing population (Kanis *et al.*, 2021). Since 2010, direct costs of fragility fractures in the EU27+2 have increased by 64% (Kanis *et al.*, 2021). The incidence for all fracture sites increased, with hip fracture incidence increasing by 33% in 2019 compared to 2010 (Kanis *et al.*, 2021). Further, the annual number of osteoporotic fractures in the EU27+2 is predicted to increase by 25% by 2034 (Willers *et al.*, 2022). In addition to the economic and societal burden, the significant pain, disability, reduced mobility, loss of independence and increased mortality rates affecting individuals as a result of fragility fractures were highlighted by the Scorecard for Osteoporosis in Europe 2021 project (Kanis *et al.*, 2021). The substantial

economic, societal, and individual burden, along with the increasing prevalence of osteoporosis, emphasises the importance of understanding the factors which influence the likelihood of developing osteoporosis (Gordon *et al.*, 2017).

Bone mass during adulthood is determined by a combination of PBM achieved at skeletal maturity when bone accrual plateaus, and subsequent age-related bone loss (Eisman *et al.*, 1993; Hernandez, Beaupre and Carter, 2003; Gordon *et al.*, 2017). Evidence for the relevance of PBM in the future development of osteoporosis comes from longitudinal studies which have studied the extent to which bone mass tracks (shows continuity or stability) across the lifespan. In adult women[2] aged 30 to 94 years, femoral neck aBMD at baseline was strongly correlated (r = 0.83) with femoral neck aBMD 16 years later (Melton III *et al.*, 2000). Likewise, over a 6-year period, baseline and follow-up forearm aBMD was strongly correlated (r = 0.90 to 0.96) in females and males aged 45 to 84 years (Emaus *et al.*, 2006). The importance of PBM in determining osteoporosis in later life is further supported by theoretical computer modelling of the bone remodelling process, which predicted that a 10% increase in PBM can delay the onset of osteoporosis by 13 years (Hernandez, Beaupre and Carter, 2003). In comparison, a 10% change in bone loss was predicted to delay the onset of osteoporosis by 2 years (Hernandez, Beaupre and Carter, 2003). Although it has not yet been possible to track the same sample from PBM in young adulthood into old age, taken together these studies suggest that PBM is an important determinant in the risk of osteoporosis in later life. Therefore, bone accrual during growth, prior to PBM attainment, should be

[2] When referring to the sex or gender of participants in previous studies, biological sex is referred to with female/male and gender is referred to with girls/women/boys/men. If it is unclear whether the previous study measured sex or gender, the terminology presented in the previous studies is used.

considered in improving skeletal health across the lifespan. Before discussing bone growth during childhood and adolescence, consideration will be given to bone anatomy and physiology and the measurement of bone in this population.

2.2 Bone Anatomy and Physiology

The skeleton has multiple functions in the body, primarily providing structural support for the body and levers for muscles to facilitate movement, but also playing a role in mineral homeostasis, and facilitating bone marrow production and haematopoietic (blood-forming) cell development (Clarke, 2008; Fuchs, Warden and Turner, 2009; Grabowski, 2015). Bone is composed of mineral (around 65% by weight), an organic component (about 25% by weight), and water (remaining 10% by weight) (Morgan, Barnes and Einhorn, 2008; Burr and Akkus, 2014). The inorganic matrix stores around 99% of total body calcium, around 85% of total body phosphorus, and between 40 to 60% of the sodium and magnesium within the body (Fuchs, Warden and Turner, 2009). These ions form crystals, predominantly in the form of calcium hydroxyapatite (Fuchs, Warden and Turner, 2009; Burr and Akkus, 2014). The organic component consists of around 90% by weight type 1 collagen, with the remaining 10% made up of non-collagenous proteins and proteoglycans (Morgan, Barnes and Einhorn, 2008; Fuchs, Warden and Turner, 2009; Burr and Akkus, 2014).

Bones can be grouped according to their type (long, short, flat, irregular, sesamoid), location (axial, which includes the bones of the head and trunk, or appendicular, which includes the bones of the limbs and pelvic girdle), or composition (predominantly cortical, also known as compact or dense bone, or trabecular, also known as cancellous or spongy bone) (Morgan, Barnes and

Einhorn, 2008; Fuchs, Warden and Turner, 2009). Cortical bone makes up around 80% of skeletal mass, with the remaining 20% made up of trabecular bone, though the ratio of cortical to trabecular bone varies between bones and skeletal sites (Clarke, 2008; Goltzman, 2019). Cortical bone has low porosity and high matrix mass per unit volume, which means cortical bone has great compressive strength and predominantly contributes to the mechanical functions of the skeleton (Fuchs, Warden and Turner, 2009). It is therefore found primarily in the shafts of long bones of the appendicular skeleton (such as the femurs, radii and humeri) (Clarke, 2008; Fuchs, Warden and Turner, 2009). Trabecular bone, on the other hand, has high porosity, leading to reduced compressive strength (Clarke, 2008; Fuchs, Warden and Turner, 2009; Goltzman, 2019). However, trabecular bone does contribute to the mechanical function of bone by providing internal support, which allows loads on the bone to be evenly distributed, particular close to joints (Fuchs, Warden and Turner, 2009). For skeletal sites rich in trabecular bone, such as the femoral neck, the connectivity of trabeculae is particularly important in determining bone strength (Fuchs, Warden and Turner, 2009). Trabecular bone is also important in facilitating hematopoiesis and mineral homeostasis in bone (Fuchs, Warden and Turner, 2009).

2.2.1 Bone Cells

Cellular elements only account for a small amount of total skeletal mass (Fuchs, Warden and Turner, 2009). Osteoclasts are involved in bone resorption, which is essential for bone modelling and remodelling (Bellido, Plotkin and Bruzzaniti, 2014). Osteoclasts differentiate from hematopoietic stem cells through a process of recruitment to the bone surface, proliferation, and differentiation

(Goltzman, 2019; Bellido, Plotkin and Bruzzaniti, 2014). Receptor activator of the nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor 1 direct differentiation, whereas osteoprotegerin reduces differentiation by acting as a decoy receptor for RANKL (Bellido, Plotkin and Bruzzaniti, 2014). Following differentiation, osteoclasts attach to the surface of the bone, and resorb bone by acidifying and degrading the mineralized matrix (Fuchs, Warden and Turner, 2009; Bellido, Plotkin and Bruzzaniti, 2014). Once bone resorption is completed, osteoclasts undergo apoptosis, with both the activity and survival of osteoclasts regulated by RANKL (Fuchs, Warden and Turner, 2009; Bellido, Plotkin and Bruzzaniti, 2014).

Osteoprogenitor cells, from mesenchymal stem cell lineage, undergo three developmental stages to form osteoblasts, which are involved in bone formation (Heino and Hentunen, 2008; Buck and Dumanian, 2012; Amarasekara, Kim and Rho, 2021). In the first stage, osteoprogenitor cells proliferate and express collagen and bone proteins, and in the second stage, immature osteoblasts differentiate into mature osteoblasts, and secrete collagen and alkaline phosphatase to mature the extracellular matrix (Florencio-Silva *et al.*, 2015; Amarasekara, Kim and Rho, 2021). Osteoblast growth and differentiation is governed by a range of transcription factors which regulate several signalling pathways, the two main signalling pathways for osteoblast differentiation being those activated by wingless-related integration site (termed Wnts) and bone morphogenetic proteins and (Bellido, Plotkin and Bruzzaniti, 2014). In the third stage of osteoblast development, the matrix is mineralised (Amarasekara, Kim and Rho, 2021). Mature osteoblasts then either undergo apoptosis, become

bone-lining cells, or embed in the mineralized bone matrix as osteocytes (Bellido, Plotkin and Bruzzaniti, 2014).

Osteocytes, which are former osteoblasts embedded in the mineralized bone matrix, account for more than 90% of the cells within the matrix or on the bone surface (Bellido, Plotkin and Bruzzaniti, 2014). Osteocytes can modulate osteoclast and osteoblast function in response to mechanical and chemical cues (Fuchs, Warden and Turner, 2009; Bellido, Plotkin and Bruzzaniti, 2014). Osteocytes can direct and secret factors that influence osteoclastogenesis, through RANKL and osteoprotegerin signalling, and that influence osteoblastogenesis, through bone morphogenetic proteins and Wnts signalling (Bellido, Plotkin and Bruzzaniti, 2014).

2.2.2 Bone Modelling and Remodelling

Bone modelling is defined as either bone formation by osteoblasts (formation modelling) or bone resorption by osteoclasts (resorptive modelling), with the primary function being to increase bone mass and maintain or change bone shape (Allen and Burr, 2014). This occurs primarily in childhood, though continues throughout life, with the net effect of increasing bone mass (Allen and Burr, 2014). Bone modelling can only occur on a pre-existing bone surface, and bone tissue is selectively added or removed, altering bone geometry (Fuchs, Warden and Turner, 2009; Allen and Burr, 2014). As bone modelling is achieved by independent actions of osteoblasts and osteoclasts, the processes are termed ‘uncoupled’ (Fuchs, Warden and Turner, 2009). In contrast to modelling, bone remodelling involves sequential coupled osteoclast and osteoblast activity, with the primary function being to renew bone (Fuchs,

Warden and Turner, 2009; Allen and Burr, 2014). The process begins when pre-osteoclasts are activated to become osteoclasts, which then resorb a surface packet of bone (Goltzman, 2019). This is followed by differentiation of mature osteoclasts and the formation of new bone (Goltzman, 2019). This coupled action of osteoclast and osteoblasts is termed the bone multicellular unit, which remodel bone in sequence of activation, resorption then formation (Fuchs, Warden and Turner, 2009). Remodelling can be targeted, which is in response to mechanical forces or microdamage and replaces a specific packet of bone, or non-targeted, which involves the storage or release of minerals from unspecified locations in response to systemic hormones (Fuchs, Warden and Turner, 2009). Bone remodelling occurs throughout life, with the net effect is to maintain or slightly decrease bone mass (Allen and Burr, 2014). In healthy individuals, a complete remodelling cycle takes around 4 to 6 months, with resorption taking around 3 to 6 weeks, and formation for the remainder of the cycle (Fuchs, Warden and Turner, 2009; Allen and Burr, 2019).

2.3 Measuring Bone Health

Bone mass accrual during childhood and adolescence is commonly assessed with dual-energy X-ray absorptiometry (DXA), as endorsed by the International Society for Clinical Densitometry (Gunter, Almstedt and Janz, 2012; Guss, McAllister and Gordon, 2021). In order to differentiate between bone and soft tissue, two beams with different energy levels are transmitted by the DXA scanner, allowing the derivation of bone and soft tissue mass (Albanese, Diessel and Genant, 2003; Dimai, 2017). DXA directly measures BMC (g) and bone area (BA) (cm^2) and provides a calculated aBMD from the ratio of BMC to BA (g/cm^2) (Dimai, 2017; Khalatbari, Binkovitz and Parisi, 2021). In addition to

quantifying bone mass, DXA can also provide accurate measures of lean and fat mass (Kohrt, 1995). DXA is considered the gold-standard method for the non-invasive assessment of aBMD and BMC in children and adults, and for the diagnosis of osteoporosis in adults according to the WHO (World Health Organisation) criteria (Cummings and Melton, 2002; Dimai, 2017). The radiation dose from a DXA scan is similar to the background radiation during one day at sea level, which is very low, ranging from 1 to 10 μSv for a spine and hip examination (Damilakis *et al.*, 2013; Shepherd *et al.*, 2017). The reproducibility of DXA scans is better than for many laboratory tests, with a coefficient of variation (CV) from 1 to 5% depending on the site measured, though this level of precision is dependent on quality assurance factors (Adams and Bishop, 2008; El Maghraoui and Roux, 2008).

In clinical practice in adults, DXA-assessed BMC and aBMD are transformed into a T-score to describe the deviation from a sex- and ethnicity-matched young adult mean, which is used in the diagnosis of osteoporosis (Dimai, 2017). However, the assessment and interpretation of BMC and aBMD are more challenging in children and adolescents compared to adults, as BMC and aBMD measurements are affected by height and maturity status in paediatric populations (Gordon *et al.*, 2008; Khalatbari, Binkovitz and Parisi, 2021). DXA cannot provide true volumetric bone mineral density (vBMD), rather it provides a two-dimensional representation of a three-dimensional structure (Dimai, 2017; Khalatbari, Binkovitz and Parisi, 2021). As aBMD is derived from BA, depth is not incorporated into the measure, and aBMD becomes a two-dimensional representation of a three-dimensional structure (Khalatbari, Binkovitz and Parisi, 2021). Therefore, when two bones have the same vBMD but are different sizes,

the smaller one will have lower calculated aBMD (Khalatbari, Binkovitz and Parisi, 2021). This may lead to an underestimation of vBMD in smaller children (Bachrach *et al.*, 2016; Khalatbari, Binkovitz and Parisi, 2021). This is further complicated in growing bones, as the growth of bones is not uniform in three-dimensions, making the interpretation of repeated measures challenging (Binkovitz and Henwood, 2007). This highlights the importance of adjusting BMC and aBMD for size. Calculations have been proposed which provide a bone mineral apparent density (BMAD) (g/cm^3) as an estimate of vBMD, accounting for depth (Carter, Bouxsein and Marcus, 1992; International Society for Clinical Densitometry, 2019a; Khalatbari, Binkovitz and Parisi, 2021). BMAD, defined as the mineralised tissue mass per total tissue volume, is equal to BMC divided by an estimate of bone volume that is obtained from the DXA-derived area and other skeletal length measurements (Katzman *et al.*, 1991; Carter, Bouxsein and Marcus, 1992). However, these calculations have not been validated in children for total body measures (International Society for Clinical Densitometry, 2019a; Khalatbari, Binkovitz and Parisi, 2021). Therefore, the International Society of Clinical Densitometry recommends that total-body-less-head (TBLH) BMC and aBMD measurements should be adjusted for a sex-specific height-for-age z-score (Zemel *et al.*, 2010). T-scores should never be used with DXA assessments in children, as children are yet to reach their PBM, and therefore bone mineral deficits will be overestimated by T-scores in children (Guss, McAllister and Gordon, 2021). The terms osteopenia and osteoporosis should not be used to describe densitometry findings in the paediatric population. Instead, the phrase ‘low bone density’ should be used in paediatric DXA reports, defined as an aBMD z-score ≤ -2 (Binkovitz and Henwood, 2007; International Society for Clinical Densitometry, 2019a; Khalatbari, Binkovitz and

Parisi, 2021). For longitudinal growth studies, it has been recommended that BMC rather than aBMD is used, due to the variability in the size and shape of children's skeletons leading to a potential under- or over-estimation of vBMD when adjusting BMC for BA (Nelson and Koo, 1999; Baxter-Jones *et al.*, 2003).

In adults, DXA is used to measure the entire skeleton and specific sites (Dimai, 2017). Clinically this is important, as the risk of site-specific fractures can be better characterised by site-specific DXA measurements (Dimai, 2017). DXA measures of the lumbar spine, hip (total hip or proximal femur), and total body have been highlighted as clinically relevant sites for the diagnosis of osteoporosis, though peripheral skeletal sites, such as the radius, can also be scanned (Adams and Bishop, 2008; Bachrach *et al.*, 2016). The International Society for Clinical Densitometry recommend TBLH and the posterior-anterior spine (L1 to L4) as the preferred sites for DXA measurements of BMC and aBMD in children (Bachrach *et al.*, 2016; Guss, McAllister and Gordon, 2021). Unlike in adults, the forearm and proximal femur are not recommended as routine assessment sites in children, due to the changes in shape and size of bones and the transition from cartilage to bone and closure of growth plates as children grow (Guss, McAllister and Gordon, 2021). Further, in children younger than 13 years, it is difficult to identify the bony landmarks at the total hip or femoral neck (Bachrach *et al.*, 2016).

In addition to DXA, bone in paediatric populations can be assessed using quantitative computed tomography (CT), including central, peripheral, and high-resolution peripheral CT, quantitative ultrasound (QUS), and high-resolution magnetic resonance imaging (MRI) (Bachrach *et al.*, 2016; Khalatbari, Binkovitz

and Parisi, 2021). Although the CT- and MRI-based methods can provide vBMD measurements and assessments of bone microarchitecture, DXA remains the preferred method in clinical practice, because it is widely available, has paediatric reference data, is quick, has standardised scanning protocols, and has low radiation (Bachrach *et al.*, 2016; Khalatbari, Binkovitz and Parisi, 2021). Therefore, this literature review will largely reflect the majority of published paediatric studies and discuss DXA-derived outcomes, whilst referencing other methodologies as appropriate (Gunter, Almstedt and Janz, 2012).

2.4 Bone Development During Childhood and Adolescence

Childhood and adolescence are a period of particular importance in maximising bone accrual, as the skeleton undergoes rapid change through processes of growth, modelling and remodelling (Baxter-Jones *et al.*, 2011; Hart *et al.*, 2017). BMC increases during childhood and adolescence (Davies, Evans and Gregory, 2005; Baxter-Jones *et al.*, 2011; Zemel *et al.*, 2011). Longitudinal evidence indicates that BMC and aBMD track from childhood into adulthood, indicating the importance of childhood bone status in predicting PBM. In childhood, correlation coefficients in the range of 0.76 to 0.88 have been observed between BMC and aBMD z-scores across 3 years in females and males aged 6 to 16 years (Kalkwarf *et al.*, 2010), and across 7 years in females and males aged 8 to 17 years (Rønne *et al.*, 2018b). The results of these studies indicate that children with low BMC and aBMD are likely to continue to have low BMC and aBMD throughout growth (Kalkwarf *et al.*, 2010). This tracking continues into adulthood, with correlations in the range of 0.81 to 0.88 between aBMD measurements from age 16 to 25 years in females and males (Yang *et al.*, 2018). As bone measures track strongly from childhood through to young

adulthood, bone status during childhood strongly predicts PBM (Gordon *et al.*, 2017). This suggests that if bone can be augmented at a young age, this may track into adulthood.

Although evidence supports the tracking of BMC and aBMD throughout growth, studying the patterns of bone accrual during childhood and adolescence as it relates to PBM is challenging, due to variation in biological age in children of the same chronological age (Baxter-Jones *et al.*, 2011). Adolescents experience a growth spurt, along with changes in body size, shape and composition (Baxter-Jones *et al.*, 2011). The peak BMC velocity lags 0.7 years and 0.6 years behind the PHV in females and males respectively, and is strongly related to maturity timing (Iuliano-Burns, Mirwald and Bailey, 2001; Baxter-Jones *et al.*, 2011). The onset of puberty can vary by up to 4 to 5 years in healthy females and males, and is associated with a considerable increase in BMC (Baxter-Jones *et al.*, 2011). Therefore, in order to study patterns of bone accrual throughout childhood and adolescence, longitudinal studies are needed to align children based on biological rather than chronological age (Baxter-Jones *et al.*, 2011).

The University of Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) assessed BMC accrual longitudinally in females and males from age 8 to age 30, with the participants aligned on biological age, based on age of PHV calculated from repeated measures (Baxter-Jones *et al.*, 2011). The study found that during the five years surrounding PHV, 39% of adult total body BMC is accrued in females and males, 46% and 43% of adult lumbar spine BMC and adult total hip BMC is accrued in females and males respectively, and 33% of adult femoral neck BMC is accrued in females and males (Baxter-Jones *et al.*,

2011). Total body BA increased significantly from -4 to +4 years from PHV, and TBLH BMC increased significantly from -4 to +6 years from PHV, after which there were no further significant gains in BA or BMC (Baxter-Jones *et al.*, 2011). At the lumbar spine and total hip, no further development was observed after +3 years from PHV for BA, and after +4 years from PHV for BMC (Baxter-Jones *et al.*, 2011). At the femoral neck, BA development plateaued at +1 year after PHV in males and +2 years after PHV in females, and BMC accrual plateaued at +2 years after PHV in both sexes (Baxter-Jones *et al.*, 2011). PBM was estimated to occur around +7 years from PHV in females and males, which equates to an average age of 18 years and 20 years in females and males respectively (Baxter-Jones *et al.*, 2011). However, there is large variation in the timing of PBM, with differences based on sex and skeletal site, though a recent narrative review indicates that at most skeletal sites, total bone mineral mass does not significantly increase from the third to the fifth decade (Chevalley and Rizzoli, 2022). This emphasises the importance of the adolescent period in determining PBM, and potentially skeletal health across the lifespan (Gunter, Almstedt and Janz, 2012).

In addition to being a critical period for determining adult PBM, childhood and adolescence is also a period of increased fracture risk (Weaver *et al.*, 2016). The lifetime risk of sustaining a fracture in childhood is 27 to 40% in girls and 42 to 64% in boys, with a peak in childhood fracture incidence in early puberty, and the distal forearm being the most common fracture site (Donaldson *et al.*, 2008; Boreham and McKay, 2011). As peak bone mineralisation rates lag behind peak linear growth, there is a transient period when the skeleton is particularly vulnerable to fracture, as bone size increases before the corresponding

increase in bone density (Bailey, 1997; Heaney *et al.*, 2000; Frost, 2001; Weaver *et al.*, 2016). A review of the evidence highlighted that childhood BMC and aBMD is predictive of fracture risk during childhood, and children with greater BMC and aBMD are less likely to suffer fractures (Weaver *et al.*, 2016). Having greater BMC and aBMD in childhood may therefore have important implications for a child's current health, as well as their future health.

2.5 Determinants of Bone Health

Given the importance of childhood and adolescence in determining PBM and protecting against fractures, factors that influence BMC and aBMD during this period are of interest. The development of BMC and aBMD in childhood and adolescence is influenced by non-modifiable factors, such as genetics, sex and maturation, and modifiable factors such as nutritional variables and PA (Guo *et al.*, 1997; Janz *et al.*, 2001; Wells, 2003; Weaver *et al.*, 2016). Although 60 to 80% of the variation in bone mass is accounted for by genetic factors, the remaining 20 to 40% is modulated by environmental and hormonal factors (Weaver *et al.*, 2016). Further understanding of how modifiable factors influence bone accrual is important for maximising PBM and reducing the risk of osteoporosis. This literature review will first consider sex and maturation, as non-modifiable factors which influence bone health, followed by consideration of the role of PA, body composition and associated endocrine factors, and vitamin D in influencing BMC and aBMD during childhood and adolescence.

2.6 Sex and Maturation

The pattern of bone accrual during childhood and adolescence is dependent on various factors including sex and maturational timing. Sex differences in

absolute BMC at all maturity levels were largely explained by anthropometric differences between females and males in the PBMAS (Baxter-Jones *et al.*, 2003). However, in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, males gained BMC at faster rates than females from prepuberty up to 4 years after PHV, though this may be confounded by sex differences in size, as estimates were not adjusted for stature or lean mass gains (Elhakeem *et al.*, 2019). This suggests that sex differences in bone accrual are largely accounted for by sex differences in body size and highlights the importance of considering body size when examining sex differences in bone accrual.

A later pubertal timing, based on age of PHV or age of menarche, has consistently been associated with lower DXA bone outcomes in adulthood in females and males in retrospective studies (Finkelstein *et al.*, 1992; Kindblom *et al.*, 2006; Chevalley *et al.*, 2009). Similarly, in the longitudinal Bone Mineral Density in Childhood Study (BMDCS), age of onset of puberty was negatively associated with DXA bone measurements at age 16 in females and males (Gilsanz *et al.*, 2011). The PBMAS observed a sex-dependent effect of maturity timing on TBLH BMC development in a sample covering age 8 to 30 years (Jackowski *et al.*, 2011). In females, early maturers (those with an age of PHV > 1 year earlier than average), had consistently greater TBLH BMC than average maturers (those with an age of PHV within 1 year of average) (~ 62 g) and late maturers (those with an age of PHV > 1 year later than average) (~ 113 g) from adolescence to adulthood (Jackowski *et al.*, 2011). There were no differences in TBLH BMC from adolescence into adulthood between early, average and late maturing males (Jackowski *et al.*, 2011). The apparently contradictory findings

may be due to the longer follow-up in the PBMAS (Gilsanz *et al.*, 2011; Jackowski *et al.*, 2011). As males reach PBM approximately 2 years later than females, it is possible that late-maturing males continue to catch up in terms of bone mass, and this would therefore not be captured at age 16, as in the BMDCS (Gilsanz *et al.*, 2011; Jackowski *et al.*, 2011; Baxter-Jones and Jackowski, 2021). However, in the ALPSAC cohort, an older age at PHV was associated with consistently lower subsequent aBMD, in females and males up to age 25 years (Elhakeem *et al.*, 2019). It is possible that greater statistical power due to the substantially larger sample size in ALSPAC (n = 6389) compared to the PBMAS (n = 230) increased the ability to detect an effect in males (Table 2.1) (Jackowski *et al.*, 2011; Elhakeem *et al.*, 2019). Overall, these studies suggest delayed puberty may be a risk factor for reduced PBM. However, as shown in Table 2.1 it is important to consider that these findings are limited to North America and UK populations, in predominantly white samples, and may not be applicable to other populations and ethnic groups.

Table 2.1 Study characteristics of key longitudinal studies of bone development

Authors	**Study**	**Location**	**Sample Size**	**Participant Ethnicity**
Baxter-Jones *et al.* (2003) Jackowski *et al.* (2011)	Pediatric Bone Mineral Accrual Study	Saskatchewan, Canada	67 to 121 females and 85 to 109 males	White
Elhakeem *et al.* (2019)	Avon Longitudinal Study of Parents and Children	Avon, UK	3196 females and 3193 males	98% white, 2% nonwhite
Gilsanz *et al.* (2011)	Bone Mineral Density in Childhood Study	Los Angeles, California, US; Cincinnati, Ohio, US; Omaha, Nebraska, US; Philadelphia, Pennsylvania, US; New York, New York, US	78 females and 85 males	33 African American, 10 Asian, 90 Caucasian, 30 Hispanic

2.7 Physical Activity, Exercise, Sport and Bone

The following section will consider PA, and exercise and sport as subsets of PA, as modifiable determinants of bone health. PA is defined as any bodily movement produced by skeletal muscles that increases energy expenditure (Caspersen, Powell and Christenson, 1985). Exercise is defined as a subset of PA that is planned, structured, and repetitive, with the objective to maintain or improve physical fitness (Caspersen, Powell and Christenson, 1985). Sport is defined as a subset of exercise that can be undertaken individually or as part of a team, with participants adhering to a common set of rules or expectations with a defined goal (Khan *et al.*, 2012). This section will first describe the mechanisms by which PA influences bone, followed by a discussion of the relationship between sport and bone, exercise and bone, and PA and bone.

2.7.1 Mechanical Loading

PA, and therefore exercise and sport as subsets of PA, influence bone mass by mechanical loading (Frost, 2001; Janz *et al.*, 2001; Janz *et al.*, 2003). The relationship between mechanical loading and bone adaptation was proposed by Julius Wolff in 1892 (Frost, 1994):

> *"Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws"*
>
> Wolff, Maquet and Furlong (1986)

Although the basic tenets of Wolff's Law have since been suggested to contain some engineering and biological inaccuracies, the fundamental concept that bone adapts in response mechanical loads is now widely accepted (Frost,

2001; Fuchs, Warden and Turner, 2009). The Mechanostat hypothesis, proposed by Frost in 1987 (Frost, 1987), extended the idea that the skeleton adapts in order to be strong enough to withstand voluntary mechanical usage without breaking (Frost, 1998). Frost proposed a negative feedback loop, whereby strains on bones cause modelling and remodelling, and bone is removed when it is not needed mechanically (Frost, 2001). Crucially, although the biological mechanisms orchestrated by the Mechanostat hypothesis are helped or hindered by nonmechanical factors, such as hormones, genetics, and nutritional factors, the influence of mechanical loading on bone cannot be replaced by nonmechanical factors (Frost, 1998; Frost and Schönau, 2000). Mechanical loading, through impact loading, muscular contraction, and gravitational forces, via PA, exercise or sport, causes stress leading to strains in the bone, which directs the adaptive response of bone to mechanical loading (Frost, 2001; Fuchs, Warden and Turner, 2009; Hart *et al.*, 2017).

Stress is defined as force per unit area, and may be compressive (material becomes shorter), tensile (material is stretched) or shear (material slides relative to an adjacent region) (Turner and Burr, 1993; Heinonen, 2001). Even under simple loading schemes, compressive, tensile and shear stresses occur in combination, which are translated into tension, bending, shear and torsional mechanical outputs (Turner and Burr, 1993; Heinonen, 2001; Hart *et al.*, 2017). Stress applied to the bone causes strain (deformation) in the bone, and the relationship between stress and strain is termed the stress-strain curve (Figure *2.1*) (Turner and Burr, 1993; Heinonen, 2001). The stress-strain curve includes an elastic region, when strains are beneath the yield point and where bone material can elastically store and return applied stress, without microdamage

occurring (Hart *et al.*, 2017). In the plastic region, strains above the yield point deform bone leading to material damage (Hart *et al.*, 2017). However, all aspects of the strain environment, including strain magnitude, strain rate, strain frequency, strain distribution, number of loading cycles, and rest-recovery periods, contribute to determining bone's response to mechanical loading (Hart *et al.*, 2017; Pivonka, Park and Forwood, 2018). The current understanding of these factors is largely based on animal models, due to the complicated and invasive nature of accurately estimating or directly quantifying site-specific internally distributed mechanical loads (Lanyon and Rubin, 1984; Gross *et al.*, 1997; Mosley and Lanyon, 1998; Judex and Zernicke, 2000; Judex *et al.*, 2003; Judex *et al.*, 2007; Wallace *et al.*, 2014; Hart *et al.*, 2017).

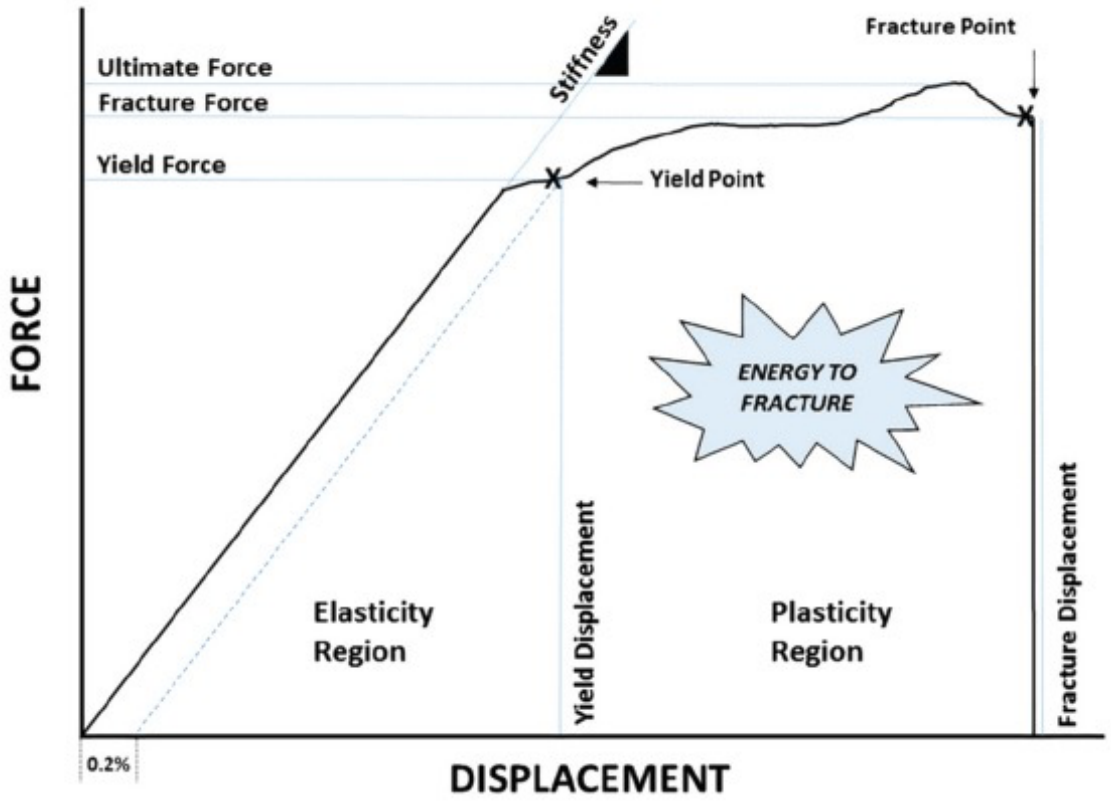

Figure 2.1 Stress-strain curve demonstrating the elastic region, yield point, and plastic region. Freely available from Hart *et al.* (2017) under the terms of the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported.

Strain magnitude is the most influential aspect of the strain environment in terms of bone adaptation and refers to the relative change in length of bone that occurs with mechanical loading (Fuchs, Warden and Turner, 2009; Hughes and Petit, 2010; Hart *et al.*, 2017). Strain can be expressed in terms of microstrain (με), with a one thousand microstrain meaning a deformation of 0.1 mm for a 10 cm long bone (0.1%) (Fuchs, Warden and Turner, 2009). The level of strain experienced within the bone may lead to net mineral loss, mineral homeostasis, net mineral gain, or damage formation, according to minimum effective strain thresholds proposed in the Mechanostat hypothesis (Figure 2.2) (Frost, 2001). If strains remain below the minimum effective strain remodelling threshold, then disuse remodelling occurs, and bone is permanently removed (Frost, 2001; Fuchs, Warden and Turner, 2009; Hughes and Petit, 2010). When strains fall

between the minimum effective strain remodelling threshold and the minimum effective strain modelling threshold, mineral homeostasis is maintained, resulting in the maintenance of bone (Frost, 2001; Fuchs, Warden and Turner, 2009). If the strains on the bones exceed the minimum effective strain modelling threshold, bone modelling occurs to increase bone strength (Frost, 2001; Fuchs, Warden and Turner, 2009). Strains exceeding the minimum effective strain microdamage threshold cause pathological overload, resulting in tissue damage (Hart *et al.*, 2017; Pivonka, Park and Forwood, 2018). However, strain magnitude is not linearly related to bone adaptation, the minimum effective strain thresholds are dependent on other loading characteristics, and as such other loading characteristics may influence the adaptive response to mechanical loading (Judex *et al.*, 2003; Wallace *et al.*, 2014; Hart *et al.*, 2017).

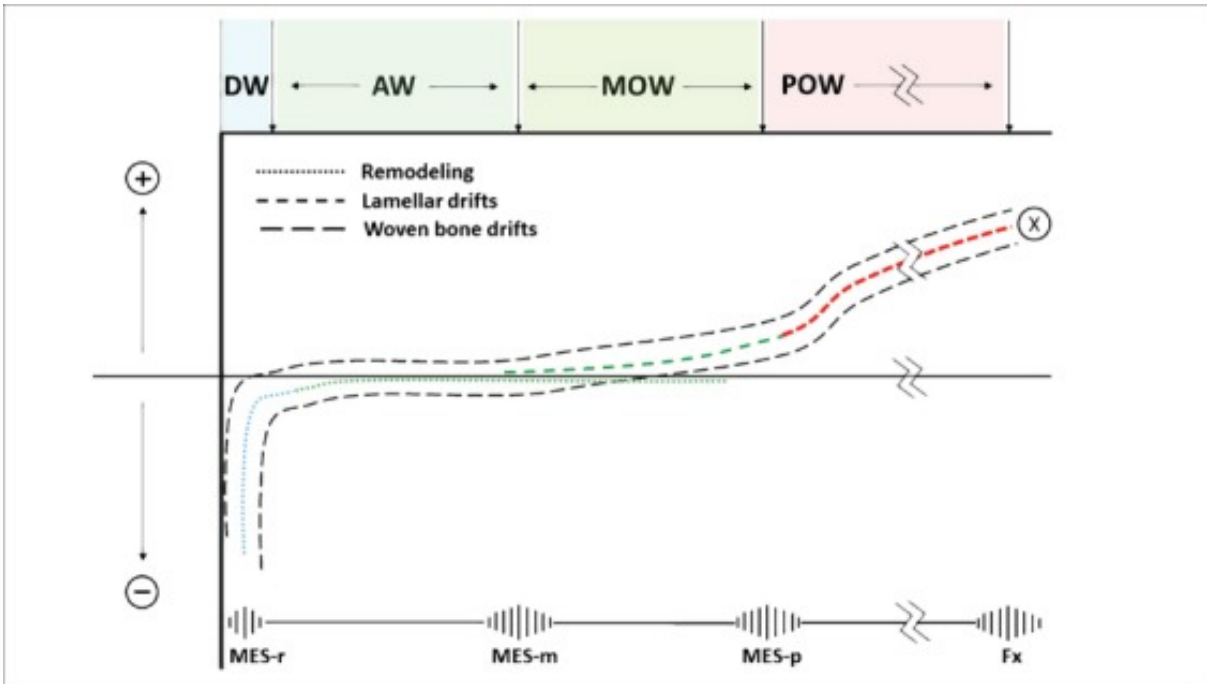

Figure 2.2 Mechanostat Theory: Modelling and remodelling effects on bone strength and mass. Freely available from Hart *et al.* (2017) under the terms of the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported.

DW, disuse window; AW, adapted window; MOW, mild overload window; POW, pathologic overload window; MES, minimum effective strain (r, remodelling; m, modelling; p, microdamage); Fx, fracture strain.

In addition to strain magnitude, dynamic loading is necessary for bone adaptation; the same strain magnitudes applied dynamically induce adaptation whereas equal strain magnitudes held statically are no different to disuse (Turner, 1998; Fuchs, Warden and Turner, 2009; Mellon and Tanner, 2012). Dynamic loading is determined by strain frequency and strain rate (Fuchs, Warden and Turner, 2009). Strain frequency refers to the number of applied cycles-per-second (Hart *et al.*, 2017). Strain frequency modulates the thresholds for adaptation; as strain frequency increases, the minimum effective strain threshold is adjusted downwards (Rubin *et al.*, 2002; Judex *et al.*, 2007; Forwood, 2008; Hart *et al.*, 2017). This means high magnitude and low frequency strains, and low magnitude and high frequency strains could elicit similar positive adaptations (Mellon and Tanner, 2012; Hart *et al.*, 2017). Strain rate refers to how quickly the strain occurs, as the change in magnitude per second of acceleration or deceleration of deformation (microstrain per second; μƐ/s) (Cullen, Smith and Akhter, 2001; Hart *et al.*, 2017). Strain rate has been positively related to bone adaptation, independently of strain magnitude (Judex and Zernicke, 2000; Mosley and Lanyon, 1998). Dynamic strains applied slowly yield minimal adaptations, whereas dynamic strains applied quickly lead to big adaptations (Lanyon and Rubin, 1984; Turner, 1998; Hart *et al.*, 2017). Further to strain frequency and strain rate, strain distribution refers to the spatial characteristic of strain magnitude, as the change in strain magnitude across a given volume of bone (microstrain per linear distance; ΔμƐ/d) (Hart *et al.*, 2017). In terms of strain distribution, irregular and unusual spatial distribution of strain is favourable for bone adaptation (Rubin and Lanyon, 1984; Gross *et al.*, 1997; Hart *et al.*, 2017). This highlights the need to consider other strain

characteristics alongside magnitude, as bone responds to a combination of magnitude and frequency, applied quickly and in an unbalanced distribution (Hart *et al.*, 2017). Further to the strain characteristics discussed above, strain modality has also been shown to be important, with torsional deformations key to bone strength development (Mittag, Kriechbaumer and Rittweger, 2018; Hart *et al.*, 2017).

The durational product of strain magnitude, rate and frequency, termed strain volume, should also be considered, as the duration of skeletal loading does not proportionally increase bone adaptation (Fuchs, Warden and Turner, 2009; Hart *et al.*, 2017). Instead, the relationship between strain volume and bone adaptation follows a law of diminishing returns, with a reduced bone responsiveness to mechanical loads as the number of daily loading cycles increases, whereby after 20 loading cycles the mechanosensitivity of bone is reduced by more than 95%, with almost no benefit after 100 loading cycles (Forwood, 2008; Fuchs, Warden and Turner, 2009; Hart *et al.*, 2017). As mechanosensitivity reduces as duration increases, a period of resensitisation is needed between bouts of loading, with mechanosensitivity returning in around 4 hours (Fuchs, Warden and Turner, 2009; Hart *et al.*, 2017). As such, a given strain volume delivered across bouts with rest periods will lead to greater bone adaptations than the same strain volume delivered in a single bout (Hart *et al.*, 2017; Pivonka, Park and Forwood, 2018). An osteogenic index, developed by Turner and Robling, accounts for the interdependent relationships between these different characteristics of loading, to estimate the osteogenic potential of different mechanical loading programs (Turner and Robling, 2003; Santos-

Rocha, Oliveira and Veloso, 2006; Forwood, 2008; Pivonka, Park and Forwood, 2018):

$$Osteogenic\ index = peak\ ground\ reaction\ force\ (body\ weight) \times \ln(loading\ cycles + 1)$$

The mechanical adaptation of bone in response to strains requires a biological system to sense the strains and communicate the loading information to effector cells (Duncan and Turner, 1995; Burger, Klein-Nulend and Cowin, 1998). This occurs through the process of mechanotransduction (Duncan and Turner, 1995) In bone, the process of mechanotransduction can be divided into four steps; 1) mechanocoupling, 2) biochemical coupling, 3) transmission of signal, 4) effector cell response (Duncan and Turner, 1995). In the first step, mechanocoupling, strains cause mechanical stretch and extracellular fluid flow, which is sensed by osteocytes and bone lining cells (Duncan and Turner, 1995). The mechanical stimuli are sensed, and mechanical energy is transduced to a mechanical signal that can be detected by cells (Duncan and Turner, 1995; Turner and Pavalko, 1998). In the next stage, biochemical coupling, the mechanical signal is further transduced into a biochemical signal, as the changes to the external environment are detected by the bone cell, leading to the generation of a subsequent biochemical signal (Stewart *et al.*, 2020; Turner and Pavalko, 1998). In the third step, the biochemical signal is transmitted between the sensor and effector cells (osteoblasts and osteoclasts) (Stewart *et al.*, 2020). In the final step, the cellular response is manifested as a tissue response, referred to as the effector cell response (Stewart *et al.*, 2020). The effector cell response

is dependent on the strain environment, as described above (Duncan and Turner, 1995).

2.7.2 How Physical Activity Loads the Bone

During PA, the skeleton is subjected to external forces from gravity and internal forces from muscle contraction (Turner and Burr, 1993; Heinonen, 2001). In order to study the mechanical loading associated with weight-bearing PA in humans, the ground reaction force (GRF) of an activity, measured using a floor-mounted force plate, is used and it is reflective of the magnitude of force on the skeleton (Turner and Robling, 2003; McKay *et al.*, 2005). A higher impact with the ground leads to a greater GRF (Heinonen, 2001). In children, peak GRFs of 1.2 x bodyweight have been observed during walking, 2.3 x bodyweight during running, and 3 to 5 x bodyweight during drop jumps (Janz *et al.*, 2003; McKay *et al.*, 2005; Gunter *et al.*, 2008). Increases in GRFs have been linearly related with strain in an instrumented femoral implant (Bassey, Littlewood and Taylor, 1997), and as such GRFs can provide a surrogate measure for strain, offering an indication of the types of activities that will stimulate an osteogenic response (Heinonen, 2001). As discussed above, strain rate, in addition to strain magnitude, is important stimulating bone adaptation. This can be reflected in the peak loading rate and peak acceleration (Rowlands *et al.*, 2014). In children, it has been suggested that activities with the most osteogenic potential have GRFs > 3.5 x bodyweight per leg, with peak force occurred in less than 0.1 seconds, such as during high-impact jumping (Gunter, Almstedt and Janz, 2012).

Without muscle activity, GRFs are transmitted from the foot through the lower limbs and hips, though the forces applied to the bone are primarily the result of muscular contraction (Heinonen, 2001). Muscles typically attach close to axes of motion, resulting in short lever arms, and subsequently large forces from muscular contraction must be generated in order to produce a required torque at the end of a level (i.e., bone) (Avin *et al.*, 2015). For example, the biceps brachii needs to generate a force over 10 times the weight of the forearm in order to produce elbow flexion, as the lever arm in the biceps brachii is around one tenth of the centre of mass of the forearm (Avin *et al.*, 2015). Further, the force in the Achilles tendon during standing and hopping is approximately three times greater than the GRF (Ireland, Rittweger and Degens, 2014). Although weight-bearing activities generate GRFs, as described above, which load the bones, given the short levers that muscles work against, internal forces due to muscle contractions may substantially exceed the forces on bones compared to external loads (Ireland, Rittweger and Degens, 2014).

As postulated by the Mechanostat theory (Section 2.7.1), the adaptation of bone to mechanical loading, and therefore PA, is governed by peak strains, and as discussed above, internal muscle forces account for the majority of peak strains during activity (Schoenau and Frost, 2002; Ireland, Rittweger and Degens, 2014). Therefore, the osteogenic potential of an activity should be determined by the maximal muscle force of an activity (Ireland, Rittweger and Degens, 2014). The highest muscular forces are from eccentric contractions, with high rates of muscle lengthening, which are typically seen during sudden application of a large external force, such as in high-impact exercises (Hill, 1938; Sousa *et al.*, 2007).

	Standing	Hopping
Body Mass [kg]	70	70
Ground reaction force [kN]	0.7	2.5
Torque [Nm]	84	300
Acceleration [g]	1	3
$Force_{AchillesT}$ [kN]	2.1	7.5
$Force_{Tibia}$ [kN]	2.8	10

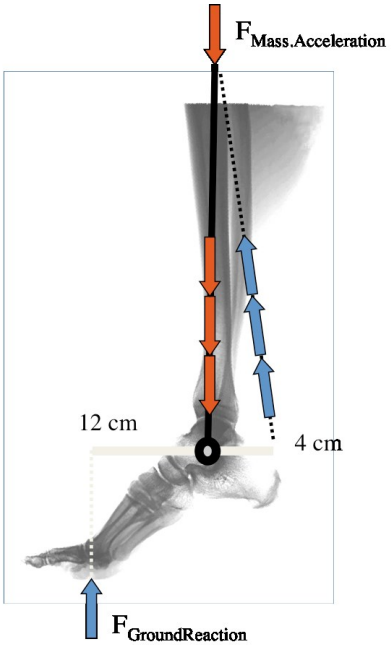

Figure 2.3 Ground reaction forces and tendon forces during standing and hopping. Reproduced from Ireland, Rittweger and Degens (2014) with permission from Springer Nature. The reproduced figure was reused from Rittweger (2007), and permission from Springer Nature has been obtained to reprint.

$Force_{AchillesT}$, achilles tendon force; $Force_{Tibia}$, force applied to the tibia.

The relevance of eccentric contractions compared to concentric contractions in generating an osteogenic response has been shown in adult females (Hawkins *et al.*, 1999). Following 18 weeks of training (concentric knee extension and flexion on one leg, and eccentric knee extension and flexion on the other leg), similar increases in muscle strength were observed between sides, but bone density only increased on the side that was trained eccentrically (Hawkins *et al.*, 1999). This is likely due to the greater peak force production with eccentric training compared to concentric training, leading to a greater strain magnitude on the bone (Hawkins *et al.*, 1999). The importance of internal muscle forces in bone adaptation is further highlighted in a study in male baseball players (throwers), which found greater skeletal differences between the dominant and nondominant upper extremities compared to matched controls (Warden *et al.*,

2009). Given that there is no external load applied to the upper limb when throwing a baseball, the findings of this study demonstrate that the loading of bones via internal muscular forces, due to rapid eccentric contractions, may generate an osteogenic response (Warden *et al.*, 2009). As discussed in Section 2.7.1, torsional loads are key for bone development. Torsional loads are exerted via muscles on the tibia during walking and running (Yang *et al.*, 2014). Further, side-to-side differences in bone strength of the serving arm humerus in tennis players may be explained by the large torsional stresses due to muscular action present during serving (Ireland *et al.*, 2015). This suggests that although typically the activities which tend to result in rapid eccentric contractions with torsional loads are weight-bearing in nature, it is the internal muscular forces, rather than external loading due to GRFs, which are responsible for the osteogenic effect of these activities.

In summary, PA loads the bone by applying stress to the bone, from GRFs or muscular contraction, leading to strains, which above a certain threshold elicit a modelling response leading to increase in bone mass. The next section will explore the relationship between free-living activities (exercise, sport, and PA) and bone health.

2.7.3 Exercise, Sport, and Bone

As discussed above, PA, and therefore exercise and sport as subsets of PA, through active loading and impact loading forces, causes strains on bone greater than needed for steady state remodelling (Frost, 2001; Janz *et al.*, 2001; Janz *et al.*, 2003). To meet this increasing load requirement, a modelling response causes BMC and aBMD to increase (Frost, 2001; Janz *et al.*, 2001).

The influence of sport and exercise on BMC and aBMD is demonstrated when considering BMC and aBMD in athletes compared to non-athletes. Studies comparing BMC and aBMD in athletes and non-athletes date back to the 1970s, with a wealth of evidence now indicating athletes who participate in impact-loading sports have greater bone density compared to athletes in non-impact sports and controls, demonstrating the principles of the Mechanostat theory discussed in Section 2.7.1 (Nilsson and Westlin, 1971; Andreoli *et al.*, 2001; Vlachopoulos *et al.*, 2017; Agostinete *et al.*, 2020). Men who were athletes, mean age 22 years, had greater bone density compared to non-athletes, and within the non-athletes, those who exercised regularly had greater bone density than those who did not (Nilsson and Westlin, 1971). Further, when athletes were stratified according to their sport, the swimmers did not differ to the controls (Nilsson and Westlin, 1971). Similar findings have been consistently observed in other studies. In males age 18 to 25 years, those who participated in judo or karate had greater aBMD than age-matched controls and water polo players, with no difference between the control and water polo players (Andreoli *et al.*, 2001). Adolescent male footballers had greater aBMD compared with swimmers, cyclists, and controls (Vlachopoulos *et al.*, 2017). In females and males age 10 to 17 years, in a comparison of 10 sports (soccer, basketball, volleyball, track and field, judo, karate, kung fu, gymnastics, baseball, and swimming) and a non-sport control group, soccer and gymnastics athletes had the greatest aBMD (Agostinete *et al.*, 2020). These studies indicate that high impact, weight-bearing activity is crucial for bone adaptation, as athletes in water-based sports did not appear to have an advantage in terms of aBMD compared to the controls. However, it is important to consider that these findings may be confounded by differences in years of practice and

training load, in terms of training frequency, intensity, duration (time) and type (the FITT principle).

Retrospective studies strengthen these findings further. In female athletes aged 42 to 50 years, with greater than 20 years of training, those who participated in netball or basketball had greater whole body aBMD than swimmers and non-sport controls, and those who participated in running or field hockey had greater whole body aBMD than controls (Dook *et al.*, 1997). In females, mean age 51 years, self-reported participation in ballet classes between the ages of 10 and 12 years was positively associated with a difference in aBMD between dancers and controls (Khan *et al.*, 1998). Prepubertal female gymnasts had greater whole body aBMD than prepubertal controls, with a similar magnitude of difference between retired gymnasts and controls aged 25 years, despite the lower frequency and intensity of exercise since retirement (Bass *et al.*, 1998). However, retired elite female ballet dancers did not differ in aBMD at weight-bearing sites to controls (Khan *et al.*, 1996). This was likely due to the multiple risk factors elite dancers are exposed to, in terms of low body weight status, low nutritional intake, and consequentially, menstrual disturbances (Khan *et al.*, 1996). These studies suggest that athletes who participate in high impact, weight-bearing sports, which likely elicit high GRFs and eccentric muscle contractions, have greater aBMD than water-based athletes and non-athlete controls, and that these differences persist over time, even after retirement.

As these studies suggest that loading in non-impact sports may not be sufficient to improve bone mass, intervention studies have sought to understand whether athletes in non-impact sports may improve their bone density by incorporating

impact-loading training (Vlachopoulos *et al.*, 2018). In adolescent males, a 9-month high-impact jumping intervention led a greater increase in BMC in swimmers and cyclists in the intervention group compared to the control, with no differences between the intervention and control group in footballers (Vlachopoulos *et al.*, 2018). These findings indicate that in male adolescent athletes, the addition of impact-loading training, generating forces on the skeleton due to high GRFs and eccentric contractions, to non-impact sports training can improve BMC (Vlachopoulos *et al.*, 2018). Although athlete studies have indicated that high impact loading is associated with improved BMC and aBMD, and that jump training improves BMC for non-impact athletes, the level of training athletes do is far above that in the general population, and the findings may not be applicable to the general population. Therefore, it is important to consider these relationships in non-athletic populations.

There have been several reviews of the effects of exercise interventions on bone health parameters in children and adolescents (Hind and Burrows, 2007; Behringer *et al.*, 2014; Gomez-Bruton *et al.*, 2017). A meta-analysis of controlled trials of the effect of weight-bearing activities on BMC and aBMD in children and adolescents found a small but significant effect on BMC and aBMD in favour of the intervention (Behringer *et al.*, 2014). Similarly, in a review of weight-bearing exercise interventions and bone mineral accrual in children and adolescents, 17 out of the 22 included studies found positive effects of exercise on bone parameters (Hind and Burrows, 2007). However, the interventions included in each of these reviews were diverse in terms of weight-bearing activities, and it was unclear what constituted the optimal exercise program for skeletal development in children (Hind and Burrows, 2007; Behringer *et al.*,

2014). Given that jump training elicits high GRFs, which are known to be osteogenic, Gómez-Bruton and colleagues undertook a systematic review of jumping interventions on bone health parameters (Gomez-Bruton *et al.*, 2017). Of the 26 studies which met the inclusion criteria, 24 found positive effects of the intervention in terms of aBMD, BMC and bone structure (Gomez-Bruton *et al.*, 2017). However, the exact volume, intensity and duration needed for jumping interventions to be effective remain unclear (Gomez-Bruton *et al.*, 2017). Although the evidence indicates that targeted weight-bearing interventions can elicit positive changes in BMC and aBMD in children and adolescents in the general population, the types of activities programmed in intervention studies may not reflect children's and adolescents' every day activity choices (Gunter, Almstedt and Janz, 2012). Therefore, from a public health perspective, habitual PA should be considered (Gunter, Almstedt and Janz, 2012).

2.7.4 Physical Activity and Bone Health

Considering the relationships between habitual, self-selected PA with BMC and aBMD is particularly relevant from a public health perspective, as the exercises included in intervention studies do not necessarily reflect the everyday activities of children and adolescents, and PA includes all parts of movement, though there are challenges with capturing all types of movement (Gunter, Almstedt and Janz, 2012). Further, whereas research has traditionally focused on PA, as the most active part of the day, there is now increasing interest in how the 24-hour day is distributed across the movement continuum, between sleep, sedentary time, and PA, termed movement behaviours, and how each of these components may influence health outcomes (Rollo, Antsygina and Tremblay,

2020). This section will consider the relationships between questionnaire-assessed and accelerometer-assessed PA and bone, discussing the implications of each measurement method, before considering the relationships between muscle and bone strengthening activity and bone. Following this, the other movement behaviours, sedentary time and sleep, and their relationship with bone, will be considered.

2.7.4.1 Questionnaire-Assessed Physical Activity and Bone

Classically, free-living research has used self- or parental-reported measures of PA when examining the relationships between PA and bone health. Early cross-sectional paediatric studies examining the relationships between questionnaire-assessed PA with BMC or aBMD found more active children, between the ages of 5 and 14 years, had greater BMC or aBMD than less active children (Slemenda *et al.*, 1991; Ilich *et al.*, 1998). The PBMAS, initiated in 1991, was the first longitudinal study of bone development in childhood (Bailey, 1997). A 6-year longitudinal analysis, with 53 females and 60 males aged 8 to 14 years at entry, found that the most active children, assessed with questionnaire, had greater total body bone mineral accrual at peak BMC velocity (409 g/year vs 331 g/year), accumulated more bone mineral in the 2 years surrounding peak BMC velocity (699 g vs 582 g), and had greater BMC at 1 year post peak BMC velocity (16% in females, 9% in males), compared with less active children, after adjustment for stature and body weight (Bailey *et al.*, 1999). The positive relationship between PA and bone mass persisted into adulthood (Baxter-Jones *et al.*, 2008b). The most active female and male adolescents had greater femoral neck BMC (9.5% in females and 8.9% in males) and total hip BMC (8.6% in females and 7.8% in males) at age 23 to 30 years, with males also having greater total body BMC (7.6%), compared to the least active

adolescents, controlling for adolescent BMC, and adult chronological age, biological age, stature, body weight, calcium intake and PA (Baxter-Jones *et al.*, 2008b). However, the differences were not significant at the total body in females, and at the lumbar spine in both sexes (Baxter-Jones *et al.*, 2008b). Similarly, the BMDCS found that questionnaire-assessed weight-bearing PA was positively associated with BMC accrual, adjusted for pubertal stage as assessed using Tanner's criteria, previous visit BMC, annualized height change, and previous visit age, in children and adolescents between ages 5 to 19 years, with a 3 to 7-year follow-up (Lappe *et al.*, 2015). The findings of the PBMAS and the BMDCS indicate that more active children have greater BMC, and that these positive associations persist into young adulthood (Baxter-Jones *et al.*, 2008b).

Although the PBMAS was pivotal in increasing understanding of the relationship between PA and bone accrual during childhood and adolescence, self- or parental-report measurements can lead to inaccuracy in estimating PA (Hildebrand and Ekelund, 2017). Consequently, the true relationship between PA and bone health may be masked or distorted (Hildebrand and Ekelund, 2017). The PBMAS used the Physical Activity Questionnaire for Children (PAQ-C) and the Physical Activity Questionnaire for Adolescents (PAQ-A), which has been validated against other self-report measures and an electronic motion sensor, adding strength to the findings (Baxter-Jones *et al.*, 2008b). However, even with validation, questionnaires may misrepresent PA due to recall bias, either intentional due to social desirability bias or unintentional due to poor recall, and the subjective nature of classifying intensity (Hildebrand and Ekelund, 2017). A comparison of associations between questionnaire-

assessed, using the PAQ-C, and device-captured PA with bone measures in children aged 11 years indicated that relationships were more likely to be detected with device-measured PA (Janz *et al.*, 2008). A bone-specific PA questionnaire (BPAQ) has been developed to record current and historical activity, with corresponding GRF loading values, which may be more sensitive to bone-specific activity (Weeks and Beck, 2008). The BPAQ was found to predict indices of bone strength whilst other PA questionnaires did not in adults, but this has not yet been explored in children (Weeks and Beck, 2008).

Although questionnaires are the most widely used method for assessing PA, and can assess the type of PA in addition to the intensity, duration and frequency of PA, questionnaires are limited in their ability to quantify the total volume of PA as well as accurately assessing intensity (Hildebrand and Ekelund, 2017). Further, as previously discussed, questionnaire assessment of PA is prone to recall bias (Hildebrand and Ekelund, 2017). Accelerometer assessments of PA can provide detailed information on the frequency, duration, and intensity of PA, overcoming some of the limitations associated with questionnaires (Hildebrand and Ekelund, 2017). As such, it is important to consider studies which have used accelerometers to capture PA.

2.7.4.2 Accelerometer Assessment of Physical Activity

Before discussing the relationships between accelerometer-assessed PA and bone, accelerometer methodology will be briefly considered. Accelerometers are generally worn on the wrist or hip, though can be placed at other body sites, and measure acceleration in one to three orthogonal planes (vertical, mediolateral and anteroposterior) (Rowlands, 2007). Although sometimes

termed an ‘objective’ assessment of PA, there are many decisions made by the researcher which may influence the accelerometer-derived estimates of PA (Rowlands, 2007). Epoch length, measurement period, wear criteria, and cut-points and other accelerometer-derived PA metrics will be discussed.

Epoch length refers to the time interval over which the accelerometer signal is integrated (Rowlands, 2007). Traditionally, a 60-second epoch was used, due to limitations in the memory size and the battery life of the accelerometers available at the time (Rowlands, 2007; Troiano *et al.*, 2014). However, with advancements in technology, accelerometers now have larger memory and battery capacities, making using a shorter epoch more feasible (Rowlands, 2007; Troiano *et al.*, 2014). Accelerometer output is influenced by epoch length, as a longer epoch has a smoothing effect on the data, leading to the dilution of PA at both extremes of the intensity continuum (Figure 2.4) (Rowlands, 2007; Edwardson and Gorely, 2010). The effect of epoch length is particularly important to consider when measuring PA in children, as their PA pattern is intermittent with rapid changes from rest to vigorous intensities, with bouts of vigorous PA (VPA) typically lasting less than 10 seconds (Baquet *et al.*, 2007). This means that the amount of PA, but particularly vigorous intensity PA, is dependent on the epoch length used, with epoch durations of 10-seconds or less recommended for capturing PA in children (Rowlands, 2007).

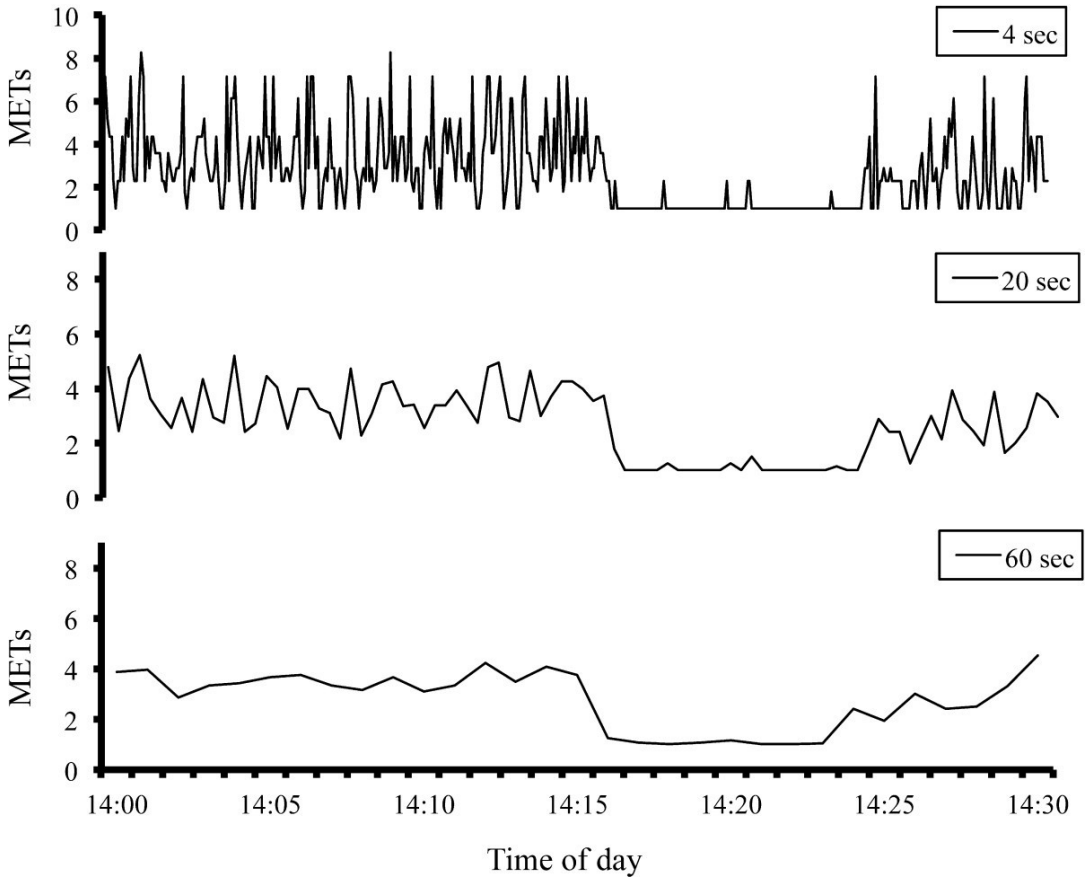

Figure 2.4 Accelerometer outputs as determined using three different epoch lengths. When averaged over longer epochs, the data is smoothed, and short bursts of high-intensity activity may be missed. Freely available from Ayabe *et al.* (2013) under the terms of the Creative Commons CC BY license.

As PA varies from day to day, and between weekdays and weekends, the accelerometer measurement period influences how reflective the data are of habitual PA (Rowlands, 2007). It is generally recommended that accelerometers are worn for 4 to 7 days, including both week and weekend days, to reflect differences between weekday and weekend PA patterns (Rowlands, 2007; Hildebrand and Ekelund, 2017). In addition, the researcher must decide what constitutes a valid day of measurement, as PA patterns vary across the day, and an overrepresentation of one part of the day may lead to bias in PA estimates (Rowlands, 2007). It has been suggested that 10 hours of wear time are needed per day, with 20 minutes of consecutive zeros indicating non-wear, but these criteria vary hugely between studies (Rowlands, 2007; Matthews *et*

al., 2012). The variability in criteria for what constitutes a valid day may influence the PA output variables, and makes comparisons between studies challenging (Rowlands, 2007).

Following data collection, decisions must be made about how to summarise the PA data. Traditionally, accelerometers only provided data expressed in counts, which are arbitrary and not comparable between accelerometer brands (Hildebrand and Ekelund, 2017). Accelerometer counts can then be summarised based on thresholds, or cut-points, to classify time spent in different intensities (i.e. sedentary, light, moderate and vigorous intensity) (Troiano *et al.*, 2014; Hildebrand and Ekelund, 2017). These cut-points are derived from calibration studies, which tend to measure accelerometer counts and oxygen consumption during treadmill walking and running, and other lifestyle activities, to provide thresholds for accelerometer counts associated with energy expenditure, based on the metabolic equivalent (MET) framework (Troiano *et al.*, 2014; Hildebrand and Ekelund, 2017). However, these cut-points are protocol and population specific, though there is also large variation in cut-points even when similar methodology and participant demographics are used (Trost *et al.*, 2011). As such, there remains no consensus on which cut-points to use, leading to challenges in making comparisons between studies using different cut-points (Trost *et al.*, 2011; Hildebrand and Ekelund, 2017; Rowlands *et al.*, 2019b). The use of different cut-points leads to variation in estimates of PA, as activity classed as moderate PA (MPA) in one study may be classed as VPA by a different study (Banda *et al.*, 2016; Haapala *et al.*, 2020; Brailey *et al.*, 2022). Applying PA cut-points also provides limited information as to how PA is accumulated within a given category, as the intensity continuum is

condensed into three or four broad categories (Colley and Tremblay, 2011; Janz *et al.*, 2014; Zymbal *et al.*, 2019; Rowlands *et al.*, 2020). For example, moderate-to-vigorous PA (MVPA) encompasses activities from brisk walking up to sprinting (Colley and Tremblay, 2011). As movement is accumulated across the whole day and across the full intensity continuum, it has been suggested that specific intensities of PA should be considered in the context of PA accumulated across the whole intensity spectrum in order to examine the relationships between PA with health outcomes (Rowlands *et al.*, 2020).

To overcome some of the limitations with the cut-point based approach to summarising PA data, Rowlands and colleagues have previously proposed using metrics which capture both the volume and intensity of the PA profile (Rowlands *et al.*, 2018). The total volume of PA is reflected in the average-acceleration over the waking day or 24-hour period, providing a proxy measure of PA volume that is not dependent on cut-points (Rowlands *et al.*, 2018). The intensity distribution of PA can be characterised with the intensity-gradient; the slope of a log-log regression between intensity and time accumulated in each intensity bin (Figure 2.5) (Rowlands *et al.*, 2018). Together, these metrics can be used to provide a description of the PA profile and allow the assessment of the mutually-adjusted associations of PA volume and intensity with health outcomes (Rowlands *et al.*, 2018). Further, translational metrics have been proposed, as the acceleration above which a person's most active X minutes are accumulated (MX) (Rowlands *et al.*, 2019a). This approach has advantages, as it allows MX metrics to be compared to a cut-point post hoc rather than collapsing the data initially, and allows post hoc translation in terms of various activities such as walking and running (Rowlands *et al.*, 2019a;

Rowlands *et al.*, 2019b). The MX metrics can be plotted on a radar plot, allowing a visual comparison of the PA profile of different groups (Figure 2.6) (Rowlands *et al.*, 2019a).

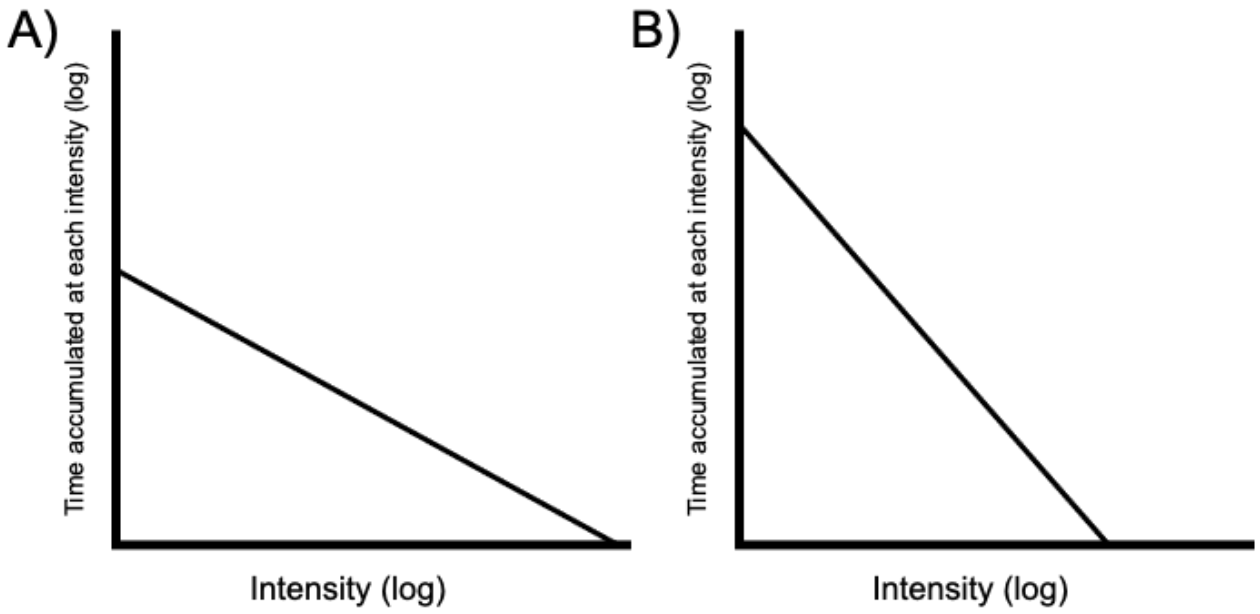

Figure 2.5 Examples of two different intensity-gradients. Panel A shows a shallower slope, indicating less time in very low intensities and more time in very high intensities, whereas Panel B shows a steeper slope, indicating more time in very low intensities and little to no time in very high intensities. Adapted from Rowlands *et al.* (2018).

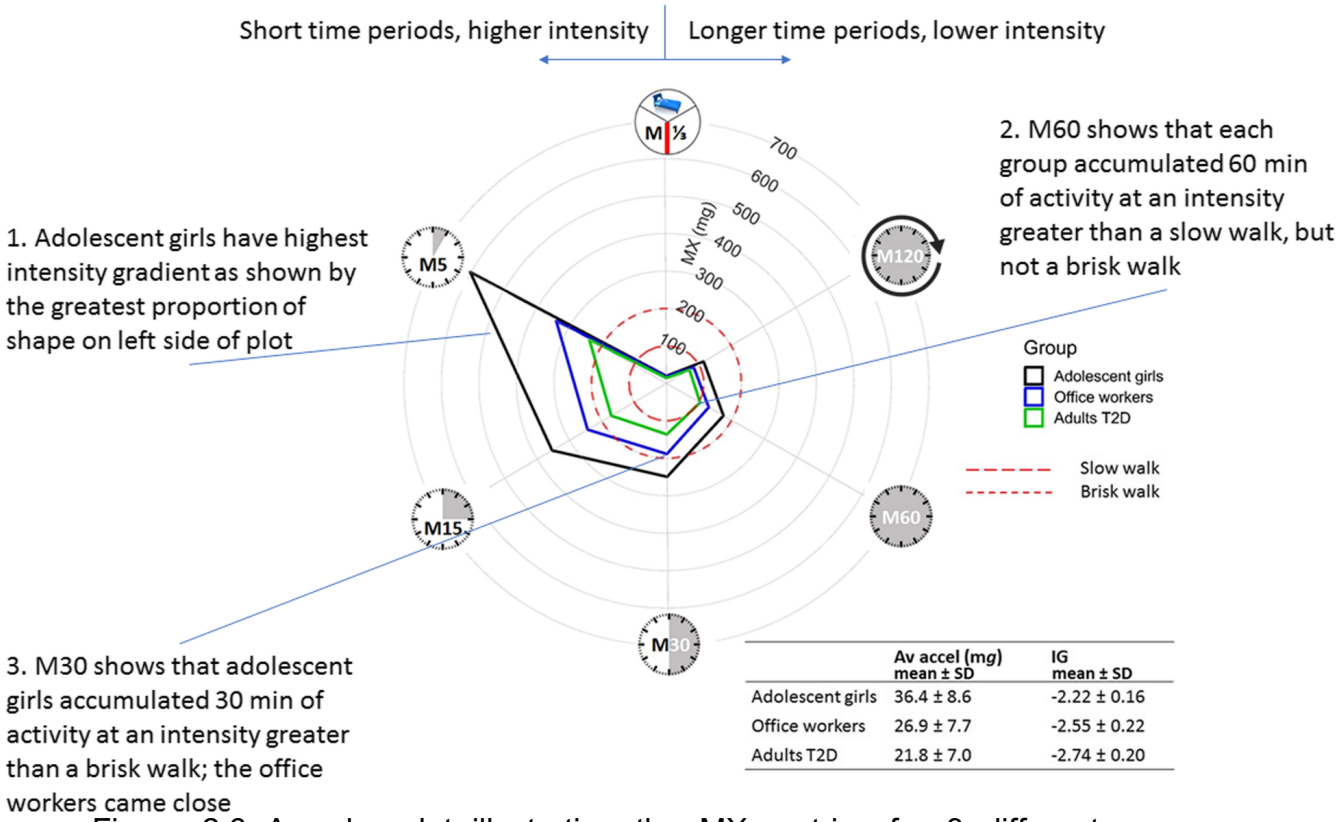

Figure 2.6 A radar plot illustrating the MX metrics for 3 different groups; adolescent girls, adult office workers, and adults with type 2 diabetes. The MX metrics show (clockwise) the acceleration above which the most active 8 hours (M $^{1}/_{3}$), 120 minutes (M120), 60 minutes (M60), 30 minutes (M30), 15 minutes (M15) and 5 minutes (M5) of the day are spent. Freely available from Rowlands *et al.* (2019a) under the terms of the Creative Commons Attribution 4.0 International License.

Av accel, average-acceleration; IG, intensity-gradient; T2D, type 2 diabetes; SD, standard deviation.

2.7.4.3 Accelerometer-Measured Physical Activity and Bone

Cross-sectional studies have consistently observed positive associations between accelerometer-measured PA with bone measures in children and adolescents (Janz *et al.*, 2001; Tobias *et al.*, 2007; Harvey *et al.*, 2012). In females and males aged 4 years, MVPA was positively associated with hip BA, BMC and aBMD, but not with bone indices at the full body and lumbar spine (Harvey *et al.*, 2012). In children aged 4 to 6 years, total PA was positively associated with hip (partial correlation coefficient = 0.25 in females and 0.20 in males) and spine BMC (partial correlation coefficient = 0.22 in females and 0.28 in males) in females and males, and with total body BMC in males (partial

correlation coefficient = 0.15), after adjustment for age, stature, and body weight (Janz *et al.*, 2001). In terms of aBMD, total PA was positively related to hip aBMD in females and males (partial correlation coefficient = 0.25 in females and 0.15 in males), to spine aBMD in females (partial correlation coefficient = 0.18), and not related to total body aBMD (Janz *et al.*, 2001). The associations between VPA with bone outcomes were of a similar magnitude, direction, and significance to those between total PA with bone outcomes (Janz *et al.*, 2001). In children aged 11 years, MVPA was positively associated with TBLH, upper-limb, and lower-limb BMC, area, and aBMD, adjusted for stature and body composition (Tobias *et al.*, 2007). In addition to MVPA, the authors also considered the independent associations of light PA (LPA), MPA and VPA (Tobias *et al.*, 2007). At the TBLH, all intensities of activity were positively associated with BMC and aBMD, while at the upper-limb, only LPA and VPA were associated with BMC and aBMD, and at the lower-limb, only LPA and MPA were associated with BMC and aBMD (Tobias *et al.*, 2007). These findings indicate that concurrent PA is beneficially related to bone outcomes in children and adolescents.

Evidence from the longitudinal Iowa Bone Development Study (IBDS) indicates that the positive associations between device-captured PA and bone measures persist over time. Baseline measurements were taken at age 5, with follow-up measurements at ages 8, 11, 12, 15, 17, 19 and 23 years. Children who had greater total PA at age 5 and age 8 years had greater total body and trochanter BMC at age 8 compared to children who had lower total PA at age 5 and age 8 years, adjusting for BMC at age 5, stature and body weight at age 8, and percent height change per year of age (Janz *et al.*, 2006). However, when

considering hip BMC, the difference was only significant in males (Janz *et al.*, 2006). These findings indicate that maintaining high levels of PA is important for bone accrual (Janz *et al.*, 2006). Further, MVPA at age 5 was positively associated with whole body, spine and hip BMC at age 8 and age 11 years with adjustment for age, stature, body weight, somatic maturity and MVPA at age 8 or age 11 years, in girls and boys (Janz *et al.*, 2010). A greater contribution was observed in boys (β for whole body BMC = 7.40 in females and 8.95 in males, β for spine BMC = 0.39 in females and 0.75 in males, β for hip BMC = 0.18 in females and 0.26 in males) (Janz *et al.*, 2010). However, when adjusting for BMC at age 5, the associations only remained significant in boys, though this may be because adjusting for baseline BMC would be expected to attenuate the effects, and the effect in boys was larger to start with. Children in the lowest quartile of MVPA age 5 (mean MVPA min/day, 9.6 in boys, 11.2 in girls) had lower whole body, spine and hip BMC at age 8 and age 11 compared to children in the highest quartile of MVPA at age 5 (45.4 in girls, 50.7 in boys), with adjustment for age, stature, body weight, somatic maturity and MVPA at age 8 or age 11 years. This suggests that PA may have a sustained effect, as well as a cumulative effect, on BMC accrual in pre- and early-pubertal children (Janz *et al.*, 2010).

In an analysis of the 12-year follow-up data from the IBDS, girls and boys who accumulated the most MVPA, assessed at ages 5, 8, 11, 13, 15 and 17 years, had greater total body BMC, hip BMC and hip aBMD at age 17, adjusted for stature and body weight, compared to their less-active peers, even after the decline in MVPA during adolescence (Janz *et al.*, 2014). Similarly, a longitudinal analysis of the ALSPAC cohort found that females who accumulated greater

amounts of MVPA in adolescence had greater hip aBMD at age 25 years compared to those with lower levels of MVPA in adolescence but higher levels in adulthood, and to those with consistently low levels of MVPA, adjusted for ethnicity, maternal education, stature, fat and lean mass indices assessed at age 10, and age at adult DXA scan (Elhakeem *et al.*, 2020). Males who accumulated greater amounts of MVPA from age 12 to 25 years had greater hip aBMD compared to those with consistently lower amounts of MVPA, adjusted for ethnicity, maternal education, stature, fat and lean mass indices assessed at age 10, and age at adult DXA scan (Elhakeem *et al.*, 2020). When considering trajectories of LPA, females who accumulated greater amounts of LPA from age 12 to 25 years had greater hip aBMD compared to those with consistently lower amounts of LPA, adjusted for the same covariates as with MVPA (Elhakeem *et al.*, 2020). However, in males, LPA trajectory group was not associated with hip aBMD at age 25 (Elhakeem *et al.*, 2020). In females, MVPA during adolescence was more important for adult hip aBMD than the levels of MVPA at age 25, and in males, MVPA in early adolescence was particularly important (Elhakeem *et al.*, 2020). These findings indicate that PA throughout childhood may contribute to determining PBM.

As discussed in Section 2.7.4.2, the accelerometer methodology may influence the observed associations between PA and bone health. A summary of the accelerometer methodology used in the studies discussed in this section is presented in Table 2.2. There is variation in terms of device and placement, with inconsistencies in measurement period, wear time criteria and cut-points used both between and within studies. Further, all studies which reported epoch length used a 60-second epoch. Although this is typical due to device limitations

at the time of data collection, this is not optimal, due to the transient nature of children's PA patterns (Rowlands, 2007). Therefore, although these studies show that higher levels of PA are associated with improved current and future BMC or aBMD in childhood and adolescence, it is difficult to precisely quantify PA and what constitutes specific intensities of PA differs between studies. This will be discussed in more detail in Section 2.7.4.3.1.

The following sections will consider various factors which may be important to consider when exploring the relationships between PA and bone; PA intensity, site-specific associations between PA and bone, and the influence of sex and maturation on the relationship between PA and bone.

Table 2.2 Summary of accelerometer methodology used in studies investigating the relationships between accelerometer-assessed physical activity and bone mass

Authors	Study Name	Sample	Accelerometer	Placement	Measurement Period	Wear Time Criteria	Epoch	Thresholds	Findings
Harvey et al. (2012)	Southampton Women's Survey	210 females and 212 males aged 4 years	Actiheart combined accelerometer and heart rate sensor	Chest	7 days wear time	Validity criteria not reported	60-seconds	20 cpm (low), 400 cpm (moderate), 600 cpm (vigorous), 800 cpm (very vigorous) broadly equal to 100, 2000, 3000, 4000 cpm Actigraph thresholds	MVPA was positively related to bone indices at the hip, but not the whole body or lumbar spine (no data on dose described).
Janz et al. (2001)	Iowa Bone Development Study	189 females and 179 males aged 4 to 6 years	Computer Science Application uniaxial accelerometer	Waist	Worn for 4 consecutive days including 1 weekend day,	required 8 hours of movement counts per day and at least 2 days of data per child	60-seconds	≥ 2972 cpm (VPA)	Those in the highest quartile (> 32 minutes for females and > 40 minutes for males) for average daily VPA had greater hip BMC (11.3% for females and 12.5% for males) than those in the lowest quartile (< 15 minutes for females and < 21 minutes for males).
Tobias et al. (2007)	Avon Longitudinal Study of Parents and Children	2336 females and 2121 males mean age 12 years	Actigraph uniaxial accelerometer	Hip	7 consecutive days	3 days with 600 mins recording each day	60-seconds	≥ 3600 cpm (MVPA), ≥ 6200 cpm (VPA)	MVPA was positively related with TBLH, upper-limb and lower-limb indices. A 100 cpm increase in MVPA was associated with 15 g greater TBLH BMC.
Janz et al. (2006)	Iowa Bone Development Study	199 females and 171 males, mean age 5 years at baseline and 9 years at follow-up	Actigraph uniaxial accelerometer	Hip	4 consecutive days	8 hours per day for at least 3 days	60-seconds	≥ 3000 cpm, equivalent to brisk walking	Mean PA predicted follow-up BMC at the hip, trochanter, spine and whole body in males, and the trochanter and whole body in females, explaining 1 to 2% of the variability in BMC (no data on dose described).
Janz et al. (2010)	Iowa Bone Development Study	185 females and 148 males, age 5 at baseline and age 8 and 11 years at follow-up	Actigraph uniaxial accelerometer	Hip	4 consecutive days, including 1 weekend day, at ages 5 and 8, 5 consecutive days, including both weekend days, at age 11	8 hours per day for at least 3 days	60-seconds	≥ 3000 cpm (MVPA)	Females and males in the highest quartile for MVPA (mean 45 mins MVPA/day) at age 5 had 4 to 14% more BMC at age 8 and 11 than those in the lower quartile for MVPA at age 5 (mean 11 mins MVPA/day).

Janz et al. (2014)	Iowa Bone Development Study	263 females and 267 males, measured at age 5, 8, 11, 13, 15 and 17 years	Actigraph uniaxial accelerometer	Hip	4 consecutive days, including 1 weekend day, at ages 5 and 8, 5 consecutive days, including both weekend days, at ages 11, 13, 15 and 17 years	10 hours per day for at least 3 days	60-seconds (age 15 and 17 data were reintegrated into 60-second epochs)	≥ 2296 (MVPA)	The most active females (85 mins MVPA/day at age 5 to 30 mins MVPA/day at age 17) from age 5 to 17 years had 200g greater TBLH BMC at age 17 years than the least active (40 mins MVPA/day at age 5 to 20 mins MVPA/day at age 17 years). The most active males (76 mins MVPA/day at age 5 to 50 mins MVPA/day at age 17) from age 5 to 17 years had 175 g greater TBLH BMC at age 17 years than the least active (46 mins MVPA/day at age 5 to 34 mins MVPA/day at age 17 years).
Elhakeem et al. (2020)	Avon Longitudinal Study of Parents and Children	1588 females and 981 males, measured at age 12, 14, 16 and 25 years.	Actigraph uniaxial accelerometer	Hip	7 days	3 days with >= 500 minutes per day	Not reported	Adolescence: 100 to 2296 cpm (LPA), > 2296 (MVPA) Adulthood: 100 to 2020 (LPA), > 2020 (MVPA)	In females, femoral neck BMD was 0.28 g/cm^2 greater at age 25 years in those with high levels of MVPA in adolescence compared to those with low levels in adolescence and adulthood. In males, femoral neck BMD was 0.38 g/cm^2 greater at age 25 years in those with high levels of MVPA in mid adolescence compared to those with low levels.

BMC, bone mineral content; BMD, bone mineral density; cpm, counts per minute; LPA, light physical activity; MVPA, moderate-to-vigorous activity; TBLH, total-body-less-head; VPA, vigorous physical activity.

2.7.4.3.1 Physical Activity Intensity

Although the evidence indicates that PA is important for bone outcomes throughout childhood and adolescence, it is unclear which intensity is most beneficial for bone health (Brailey *et al.*, 2022). The WHO recommends at least an average of 60 minutes of MVPA per day for children and adolescents aged 5 to 17 years, though this is not specifically for bone health (Chaput *et al.*, 2020). A 2020 systematic review aimed to describe and summarise the current evidence for accelerometer assessed LPA, MPA, VPA, MVPA and total PA on DXA-assessed bone outcomes in healthy children and adolescents (Bland *et al.*, 2020). Bland and colleagues (Bland *et al.*, 2020) found MPA, VPA and MVPA were positively related with bone outcomes in males. In females, although MPA, VPA and MVPA were shown to be beneficial, the evidence was less consistent (Bland *et al.*, 2020). However, the authors highlight that it is unclear how much and which intensity is most beneficial for bone (Bland *et al.*, 2020). In order to explore which intensity is most beneficial for bone, a 2021 systematic review compared whether the magnitude of association between MPA, VPA and MVPA with bone outcomes was consistently stronger for a particular intensity (Brailey *et al.*, 2022). Of all the association analyses included in the systematic review, chi-square tests provided very strong evidence that the proportion of significant associations were higher for VPA compared to MVPA and MPA (Brailey *et al.*, 2022). This suggests that VPA has a greater benefit for bone over MPA and MVPA.

It is important to note that both systematic reviews found some inconsistencies in the observed relationships between LPA, MPA, VPA and MVPA with bone outcomes (Bland *et al.*, 2020; Brailey *et al.*, 2022). These differences between

studies are likely due, at least in part, to differences in the accelerometer methodology used between studies (Brailey *et al.*, 2022). Of the studies included in the systematic review by Brailey and colleagues (Brailey *et al.*, 2022), a 60-second epoch was most common. However, as previously discussed, as children accumulate VPA in bouts less than 10-seconds in duration (Baquet *et al.*, 2007), the ability to capture VPA is limited when using a 60-second epoch, and periods of VPA may be misclassified as lower intensities (Janz *et al.*, 2003). This could lead to an underestimation of the relationships between VPA and bone, and an overestimation of the relationships between lower intensities of PA and bone, potentially explaining some of the conflicting findings discussed previously.

Furthermore, the cut-points applied to categorise PA intensity differ between studies. The limitations of cut-points were discussed in Section 2.7.4.2, but the variation in applied cut-points can lead to over- or underestimation of the importance of various PA intensities for bone health. It should also be considered that cut-points are justified on the basis of METs (Janz *et al.*, 2001; Tobias *et al.*, 2007; Trost *et al.*, 2011). Although metabolic energy expenditure is relevant for cardiometabolic health (Chaput *et al.*, 2020), mechanical loading is likely to be more important for bone outcomes (Janz *et al.*, 2003). Therefore, by categorising PA based on energy expenditure, the current literature may not reflect the exposures that are important for bone health. As discussed in Section 2.7.4.2, the intensity-gradient and average-acceleration metrics have been proposed as a cut-point free approach to summarising PA data. In the IBDS cohort, both intensity-gradient and average-acceleration were positively related with TBLH BMC in adolescents and adults (Rowlands *et al.*, 2020). This

indicates that accumulating PA volume at any intensity, or increasing intensity without increasing volume, could be beneficial for BMC (Rowlands *et al.*, 2020). However, this has yet to be explored in children.

2.7.4.3.2 Site Specific Associations with Physical Activity

The observed associations between PA with DXA-assessed bone outcomes appear to be dependent on the skeletal site. Drawing on evidence from systematic reviews, a higher proportion of associations between PA and bone parameters were significant at the hip compared to the lumbar spine and the total body (Bland *et al.*, 2020; Brailey *et al.*, 2022). During normal weight-bearing activity, the lower-limb and hip is loaded (Janz *et al.*, 2008). Therefore, it is expected that associations would be seen at those sites (Janz *et al.*, 2008). As positive associations were also observed at the whole body, perhaps this is because of the diversity in habitual activity patterns, resulting in varied loading patterns (Janz *et al.*, 2006; Bland *et al.*, 2020; Brailey *et al.*, 2022). Brailey and colleagues (Brailey *et al.*, 2022) highlight that the hip was the most common accelerometer placement in the studies included in their systematic review. When an accelerometer is placed at the hip, it more directly measures impact-loading at the hip (Gunter, Almstedt and Janz, 2012). This could result in the stronger associations observed between PA with DXA measures at the hip.

2.7.4.3.3 Sex Differences in the Associations Between Physical Activity and Bone

Although none of the studies that formally tested a PA by sex interaction support a sex difference in the PA with bone mass relationship (Bailey *et al.*, 1999; Tobias *et al.*, 2007; Harvey *et al.*, 2012), the associations between PA with BMC or aBMD consistently appear stronger or are only apparent in males

(Janz *et al.*, 2006; Tobias *et al.*, 2007; Harvey *et al.*, 2012; Bland *et al.*, 2020). This may reflect differences in movement behaviour during growth, as well as biological differences in terms of body composition and endocrine status, between females and males (Riddoch and Boreham, 1995; Baxter-Jones *et al.*, 2003; Klitsie *et al.*, 2013; Bland *et al.*, 2020). Therefore, stratifying analysis by sex is reasonable given the sex differences in biology and behaviour, and is important to provide data for future meta-analyses.

2.7.4.3.4 Maturity Dependent Associations Between Physical Activity and Bone

Some of the differences between studies may be due to maturity status of participants. In a 2020 systematic review of accelerometer-assessed PA with bone outcomes in children and adolescents, Bland and colleagues (Bland *et al.*, 2020) found that MVPA, VPA or MPA were consistently positively associated with bone outcomes in pre- and early-pubertal and peripubertal males, though in late- and post-pubertal males, concurrent PA did not influence DXA bone outcomes. However, PA in pre- and peripubertal years was associated with bone in late- and post-puberty (Bland *et al.*, 2020). As relationships between PA and bone were inconsistent in females, there was not enough evidence to indicate whether maturation influenced the PA-bone relationship in females (Bland *et al.*, 2020).

Of the studies which considered an interaction between maturity and PA, the analyses of the ALSPAC cohort at age 11 did not observe an interaction between MVPA and pubertal stage with bone measures, with the exception of an attenuation of the relationship between MVPA with upper-limb area-adjusted BMC with puberty (Tobias *et al.*, 2007). However, the sample in later pubertal

stages was considerably lower (25% of females and 20% of males stages 4 and 5), which could lead to attenuation of a possible interaction (Tobias *et al.*, 2007). Conversely, in a sample of 622 female and male children and adolescents between the ages of 10 and 17 years, the percentage of time in VPA was positively associated with TBLH BMC, and the magnitude of this association increased as maturation advanced (Rønne *et al.*, 2018a). This may be due to the different measure of maturation that was used, Rønne and colleagues (Rønne *et al.*, 2018a) calculated a continuous measure of age from PHV based on prediction equations (Moore *et al.*, 2015), whereas previous studies have used pubertal stages as described by Tanner (Tobias *et al.*, 2007). It is also possible that there are lower PA levels in late puberty, potentially meaning a small increase in PA is more potent. Overall, it appears that the skeleton is more sensitive to mechanical loading in pre- and early-puberty than in post-puberty (Pivonka, Park and Forwood, 2018). However, developing and maintaining favourable activity patterns in adolescence that carry into adulthood is also important (Bland *et al.*, 2020).

2.7.4.4 Muscle and Bone Strengthening Activity and Bone

As previously discussed, accelerometer-derived estimates of PA intensity are often based on energy expenditure and are limited in their ability to capture the type of PA (Trost *et al.*, 2011; Hildebrand and Ekelund, 2017). In addition to recommending at least an average of 60 minutes of MVPA per day for children and adolescents aged 5 to 17 years, the WHO also recommends that activities that strengthen muscles and bone should be incorporated at least 3 days a week (Figure 2.7) (Chaput *et al.*, 2020). Muscle-strengthening activities cause the body's muscles to work or hold against an applied force or weight, whilst

bone-strengthening activities produce a force on the bones of the body, commonly by impact with the ground, that promotes bone growth and strength (Piercy *et al.*, 2018). However, aerobic activities remain the primary focus of the guidelines, with a secondary focus on muscle and bone strengthening activities (Faigenbaum *et al.*, 2020). This is reflected in surveillance of adherence to the PA guidelines, which tends to focus solely on the MVPA component, as highlighted by a 2020 review which found that in the UK, none of the childhood PA surveys assessed muscle and bone strengthening activity (Strain *et al.*, 2020). Further, Weaver and colleagues (Weaver *et al.*, 2016) highlight that bone strengthening activity is not measured in the US Centers for Disease Control and Prevention Youth Risk Behaviour Surveillance System. The Active Lives Children and Young People Survey in England does include questions regarding activities which may reflect muscle and bone strengthening activities, such as sports and dance (Sport England, 2023). However, adherence to the PA guidelines is only presented as the percentage of children taking part in average of 60 minutes of MVPA per day (Sport England, 2023). This has led to the muscle and bone strengthening guidelines being termed the 'forgotten' guidelines (Strain *et al.*, 2016).

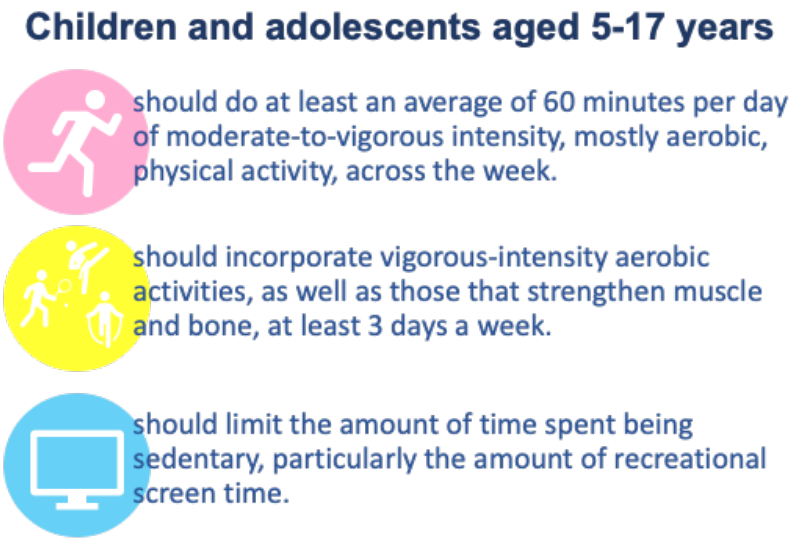

Figure 2.7 World Health Organisation Physical Activity Guidelines for children and adolescents aged 5 to 17 years.

The frequency recommendation for muscle and bone strengthening activity varies between national guidelines. Similar to the WHO guidelines, the Physical Activity Guidelines for Americans and the Canadian 24-Hour Movement Guidelines recommend muscle and bone strengthening activity 3 times a week for school-aged children and adolescents (Tremblay *et al.*, 2016; Piercy *et al.*, 2018; Chaput *et al.*, 2020). However, whilst the UK Physical Activity Guidelines recommends children and adolescents engage in a variety of types and intensities of PA across the week to develop muscular fitness and bone strength, it is highlighted that there was no strong evidence for specific numbers or durations of bouts (Department of Health and Social Care, 2019). The evidence cited by the WHO supporting the 3 times weekly frequency of muscle and bone strengthening activity highlights that dose-response is not conclusively known (Physical Activity Guidelines Advisory Committee, 2018). However, a systematic review on the determinants of PBM found that PA interventions designed to improve BMC and aBMD typically ranged from 2 to 5 sessions per week, including sports, games, dance, or high-impact exercises

such as jumping and hopping (Weaver *et al.*, 2016). The authors highlight that randomised controlled trials (RCT) indicate 3 days/week are needed to detect change, though there is overlap in the session frequency in interventions that show change and those that do not (Weaver *et al.*, 2016). Even so, the authors conclude that 3 days a week is a reasonable frequency dimension for bone strengthening activity based on successful RCTs (Weaver *et al.*, 2016). Despite this, further evidence is needed to support specific durations and frequencies of muscle and bone strengthening activities associated with favourable bone outcomes.

As previously discussed, and as highlighted in a 2020 rapid evidence review, weight-bearing sports, such as gymnastics and football, and jumping, are the types of activities associated with better bone outcomes (Public Health England, 2021). However, much of this evidence is based on youth athlete studies, whose level of training may be far above that of the general population, and intervention studies, in which the types of activities programmed may not reflect children's and adolescent's every day activity choices (Gunter, Almstedt and Janz, 2012). As such, little is known about the relationships between self-selected muscle and bone strengthening activities with BMC in the general population.

2.7.4.5 Sedentary Behaviour and Bone

Sedentary behaviour is defined as any waking behaviour with an energy expenditure ≤ 1.5 METs whilst sitting, reclining, or lying down (van der Ploeg and Hillsdon, 2017). Sedentary behaviour can be captured with accelerometers, which can assess total time spent sedentary, though are not generally able to

distinguish sitting, reclining or lying down from standing, or to provide information on the type of sedentary activity (Hardy *et al.*, 2013). Self- or parental-report methods are also used in the literature, and are able to provide contextual information, allowing the relationships of specific sedentary behaviours, such as TV viewing, with health outcomes to be examined (Hardy *et al.*, 2013).

In adults, extreme bed rest, which unloads the bone, resulted in increased bone resorption and decreased bone mass (Zerwekh *et al.*, 1998). However, this represents an extreme removal of loading, and it is unclear whether habitual sedentary behaviour has similar deleterious effects on bone (Koedijk *et al.*, 2017). A systematic review on sedentary behaviour and bone health in children and adolescents found moderate evidence for a negative association between accelerometer-assessed sedentary time with lower extremity bone outcomes that was independent of MVPA, though evidence was insufficient for questionnaire assessments of sedentary time with lower extremity bone outcomes (Koedijk *et al.*, 2017). There was insufficient evidence for an association between accelerometer- or questionnaire-assessed sedentary time with lumbar spine outcomes (Koedijk *et al.*, 2017). There was strong evidence to suggest no association between accelerometer-assessed sedentary time with total body bone outcomes, but insufficient evidence for questionnaire measures of sedentary time with total body bone outcomes (Koedijk *et al.*, 2017). This suggests total sedentary behaviour may be detrimental for bone at the lower extremity, but not at the total body (Koedijk *et al.*, 2017). However, the authors highlight a lack of high-quality evidence, with limited longitudinal studies (Koedijk *et al.*, 2017). Further, the insufficient evidence highlighted from

questionnaire studies mean that the relationships between specific types of sedentary behaviour with bone are poorly understood. As such, further research, in particular longitudinal studies with accelerometer and questionnaire measures of sedentary behaviour, are needed to further understand the relationship between sedentary behaviour with bone.

2.7.4.6 Sleep and Bone

It has been hypothesized that sleep may influence bone health, as bone turnover markers peak overnight, and anabolic endocrine factors, such as growth hormone, are sleep related (Swanson *et al.*, 2018; Chennaoui, Léger and Gomez-Merino, 2020). Sleep restriction has been shown to lead to lower levels of bone formation markers and unchanged or higher levels of bone resorption markers in adult males, which could ultimately lead to bone loss (Swanson *et al.*, 2021). However, there is limited research in children. Of the paediatric studies, one study found that in children aged 4 to 12 years, those in the tertile with greatest sleep duration, assessed by a survey undertaken by parents, had greater total BMC compared to those in the other tertiles (Casazza, Hanks and Fernandez, 2011). However, another study, using QUS measures of bone health, found that in a population sample of European children and adolescents from age 2 to 15 years there was no relationship between nocturnal sleep duration z-scores with bone stiffness index percentile (Cheng *et al.*, 2021). These conflicting findings may represent the differences in methodology for assessing bone, but further research is needed to understand the relationship between sleep with DXA-assessed bone outcomes.

2.8 Body Composition

Throughout childhood, lean, fat and bone mass increase substantially, with PHV followed by peak lean velocity, and then by peak fat velocity, peak weight velocity and peak BMC velocity (Iuliano-Burns, Mirwald and Bailey, 2001; Baxter-Jones *et al.*, 2003). Understanding how these tissues interact during growth is important in understanding the factors that may influence PBM. DXA allows a highly accurate assessment of lean mass and fat mass, in addition to BMC (Chaves *et al.*, 2022). As previously discussed, as DXA uses two different energy spectra to differentiate between two materials based on the attenuation of the beam, DXA can differentiate between bone and soft tissue, and between lean mass and fat mass (Buckinx *et al.*, 2018). These measurements can be performed for the whole body and for specific regions including the arms, legs, trunk and head, using specific well-defined cut lines, to provide estimates of regional lean and fat mass (Blake and Fogelman, 1997; Buckinx *et al.*, 2018; Shepherd *et al.*, 2017). As such, DXA can be used to explore the interrelationships between lean, fat and bone mass throughout growth.

2.8.1 Lean Mass and Bone

Lean mass and bone mass are closely positively associated throughout growth, with a strong positive association between total body peak lean mass velocity with total body peak BMC velocity (r^2 = 0.50) (Rauch *et al.*, 2004). The association between lean mass with bone mass is explained physiologically by the Mechanostat theory, which proposes that the skeleton continually adapts to the loads it is exposed to (Frost and Schönau, 2000; Frost, 2003; Rauch *et al.*, 2004). As the largest loads on the skeleton are from muscle contraction, it has

been suggested increases in lean mass, and therefore muscle force, may directly drive increases in bone mass (Rauch *et al.*, 2004).

As highlighted in a 2015 review article, the data from paediatric studies consistently support a positive relationship between DXA-assessed lean mass with DXA bone outcomes at various sites (Kindler, Lewis and Hamrick, 2015). A 2016 systematic review of observational and longitudinal studies found that lean mass was a consistent positive predictor of BMC in all 12 studies assessed, and of aBMD in all 7 studies assessed, in healthy children and adolescents from age one month to 20 years (Sioen *et al.*, 2016). In the PBMAS, lean mass accounted for 26% of the variation in total body BMC after adjusting for height, fat mass and sex at the age of PHV (Baxter-Jones *et al.*, 2003). Although these studies indicate positive associations between lean mass and bone mass, it is unclear whether the increased lean mass is causing the increased bone mass. An analysis of the PBMAS supports the hypothesis that muscle development drives bone development with longitudinal evidence, as the peak in lean mass velocity occurs four to six months before the peak in BMC velocity in female and male adolescents (Figure 2.8) (Rauch *et al.*, 2004). Further, lean mass reaches a plateau around 18 years of age, preceding PBM by months to years dependent on the skeletal region (Kindler, Lewis and Hamrick, 2015). Although causality cannot be assumed from observational studies, the temporal relationship between lean and bone mass development does support the application of the Mechanostat theory to the growing skeleton (Rauch *et al.*, 2004).

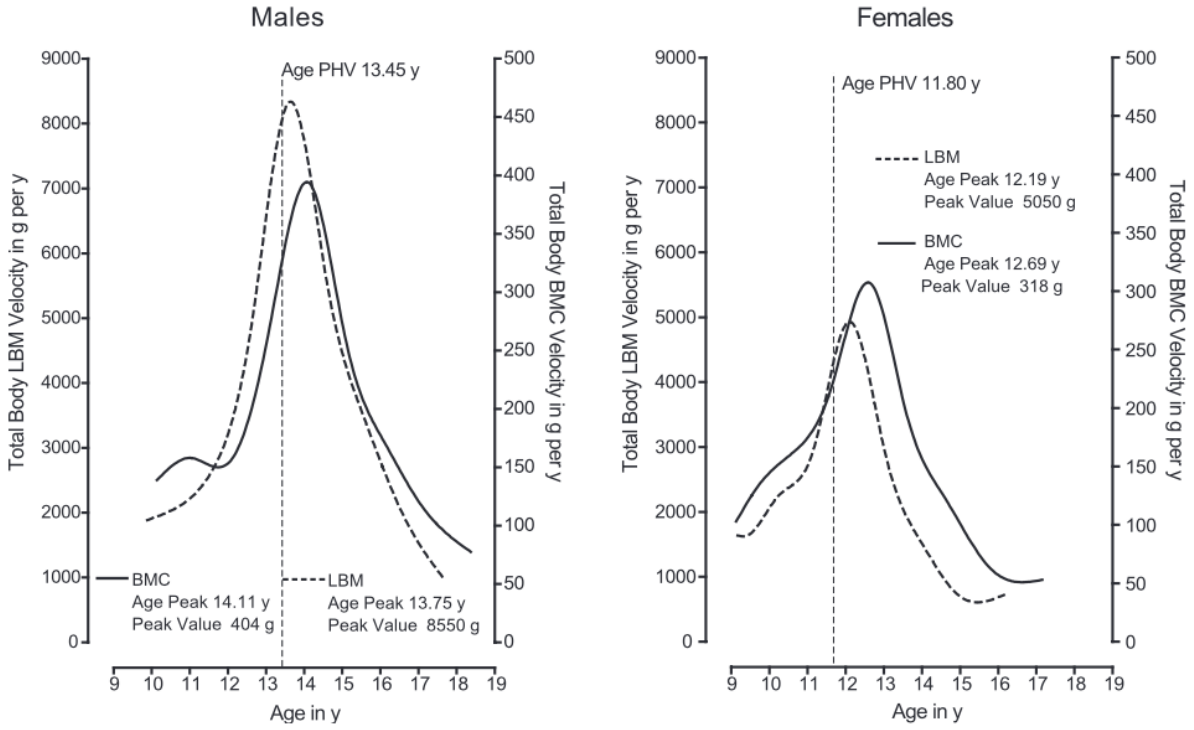

Figure 2.8 Velocities of total body lean mass and bone mineral content accretion in the years surrounding peak height velocity. The peak in lean mass accrual is closely followed by the peak in bone mineral content accrual. Reprinted from Rauch *et al.* (2004) with permission from Elsevier.

2.8.2 Lean Mass, Physical Activity and Bone

As PA is positively associated with increased lean mass (Baxter-Jones *et al.*, 2008a), and as lean mass is a strong determinant of bone mass (Baxter-Jones *et al.*, 2003), lean mass should be considered when examining the relationships between PA and bone. As muscles attach directly to bone, muscle forces mediate the relationship between PA and bone (discussed in Section 2.7.2) (Avin *et al.*, 2015). Although muscle, and as a proxy measure, lean mass, plays a critical role in the skeletal response to mechanical loading during PA, there is limited research considering lean and bone outcomes together (Zymbal *et al.*, 2019). A study of the IBDS cohort investigated the mediating effect of muscle on the relationship between PA and bone (Zymbal *et al.*, 2019). Females and males who accumulated higher levels of MVPA from age 5 to 17 years had

greater aBMD at the total proximal femur at age 17 years, in comparison to their less active peers (Zymbal *et al.*, 2019). Further, leg lean soft tissue explained 43 to 49% of the association between MVPA and femur aBMD in females, and 27 to 32% in males (Zymbal *et al.*, 2019). The findings from this study suggest that PA may increase muscle mass, which in turn increases the mechanical loads on bone, leading to greater bone mass (Zymbal *et al.*, 2019). Likewise, in male adolescent athletes mean age 14.1 years, lean soft tissue partially explained the association between training volume and aBMD in all body segments (upper-limbs, 58.37%; lower-limbs, 28.35%; spine, 33.80%; whole body, 41.82%) (Narciso *et al.*, 2020). Interestingly, the direct effect of training volume remained significant at all sites with the exception of the upper-limbs (Narciso *et al.*, 2020). This indicates the association between exercise and aBMD is influenced by lean mass more in areas of the body not commonly exposed to high impacts (Narciso *et al.*, 2020). As such, in order to better understand the associations of PA with bone health, consideration should be given to lean mass when investigating the relationships between PA and bone.

2.8.3 Fat Mass and Bone

Fat mass may influence bone mass by increasing mechanical loads on bone (Dimitri, 2018). However, as this may be either directly, or indirectly via increased lean mass, it is essential to adjust for lean mass when investigating the relationship between fat with bone. In prepubertal children aged 6 to 8 years, fat mass was positively associated with TBLH aBMD, after adjustment for lean mass (Soininen *et al.*, 2018). This observation extends to females and males with overweight and obese body weight status, who had greater BMC than children with normal body weight status, after controlling for lean mass, in a largely pre- and early-pubertal study sample (Leonard *et al.*, 2004b). The

ALSPAC study demonstrated positive associations between fat mass and TBLH BMC in largely pre- and early-pubertal girls and boys aged 9 years, after accounting for lean mass (Clark, Ness and Tobias, 2006). Longitudinally, fat mass at age 9 was positively associated with change in BMC over the following two years in prepubertal girls and boys independent of lean mass, though the body weight status of the children was not characterised (Clark, Ness and Tobias, 2006). This association became inverted in mid-pubertal girls, accounting for lean mass, perhaps indicating that the relationship between fat mass and bone mass changes during puberty (Clark, Ness and Tobias, 2006). Further, in females and males aged 12 to 17 years, fat mass was negatively associated with TBLH BMC, independent of lean mass (Gracia-Marco *et al.*, 2012). Overall, the findings suggest that fat mass is positively associated with bone measures, after adjusting for lean mass, in pre- and early-puberty (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006; Soininen *et al.*, 2018), with evidence that this association disappears (Clark, Ness and Tobias, 2006) or inverts (Gracia-Marco *et al.*, 2012) in late- or post-puberty.

This may reflect an interaction between fat mass and pubertal status, whereby the association between fat mass and TBLH BMC disappears or inverts during puberty (Dimitri, 2018). However, there was no interaction between fat mass and pubertal stage with change in TBLH BMC from age 9 years to 11 years in the ALSPAC cohort, though this may be limited by the low number of children in pubertal stage 3, as described by Tanner, in the sample population (Clark, Ness and Tobias, 2006). Even so, all of these studies controlled for age, stature and lean mass in their analyses, so it may be reasonable to suggest that pubertal status contributes to the differences observed between studies, though further

work is needed to understand the relationship between fat mass and skeletal development (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006; Gracia-Marco *et al.*, 2012; Soininen *et al.*, 2018). It is also possible that the association between fat mass and bone mass may be curvilinear, with a fat mass threshold, above which gains in fat mass negatively influence bone mass (Dimitri, 2018). As fat mass increases throughout childhood and early adolescence, before stabilising in males around the age of PHV and continuing to increase in females throughout adolescence, it is possible that the inverse relationship between fat mass and bone mass in adolescents is reflecting this potential curvilinear relationship (Baxter-Jones, 2017; Dimitri, 2018). However, this threshold is currently unknown, and may vary according to sex and pubertal stage (Dimitri, 2018).

2.8.4 Fat Mass, Physical Activity and Bone

Few studies have considered the interrelationships between fat mass, PA and bone. As PA is inversely associated with fat mass in children and adolescents (Ness *et al.*, 2007; Riddoch *et al.*, 2009), and as fat mass may be associated with bone mass, it is important to consider fat mass alongside PA and bone mass. In the ALSPAC cohort, in children aged 11 years, device-measured MVPA was positively associated with TBLH BMC, controlling for lean and fat mass (β = 14.92, representing the change in grams of BMC associated with 100 counts per minute average daily increase in activity level; $p < 0.001$) (Tobias *et al.*, 2007). However, the association between MVPA with TBLH BMC was not significant when only controlling for lean mass (β = -0.47, representing the change in grams of BMC associated with 100 counts per minute average daily increase in activity level; $p = 0.8$) (Tobias *et al.*, 2007). This finding emphasises

the importance of considering fat mass alongside lean mass when examining the relationship between PA with bone.

2.8.5 Fat Mass, Endocrine Factors and Bone

Skeletal growth and the response of bone to mechanical forces is dependent on endocrine regulation (Office of the Surgeon General, 2004). The endocrine factors that regulate skeletal growth include calcium-regulating hormones (parathyroid hormone, calcitriol and calcitonin), sex hormones (estrogen and testosterone), as well as other systemic hormones such as growth hormone and insulin-like growth factors, thyroid hormones and cortisol (Office of the Surgeon General, 2004). Previously, the association between fat mass and bone mass was thought to be biomechanical, whereby increased mass leads to increased forces on bones, which in turn leads to increased bone mass (Maurel, Jähn and Lara-Castillo, 2017; Dimitri, 2018). However, it is possible that systemic factors may be involved in mediating the relationship between fat mass and bone mass (Reid, 2010; Tobias, 2010). Adipokines, sex steroids, and insulin have been suggested as potential factors in mediating the fat mass and bone relationship (Tobias, 2010; Dimitri, 2019).

2.8.5.1 Adipokines

Leptin is an adipokine primarily produced by adipocytes, which has been positively associated with body fat percentage in children and adolescents (Blum *et al.*, 1997; Malina, Bouchard and Bar-Or, 2003; Dimitri, 2019). Leptin may influence bone metabolism, having opposing peripheral and central effects on bone (Dimitri, Wales and Bishop, 2011; Dimitri, 2019). *In vitro* and *in vivo* studies have demonstrated that leptin stimulates peripherally mediated osteogenic effects, by promoting osteoblast proliferation and differentiation

(Dimitri, Wales and Bishop, 2011; Dimitri, 2019). However, studies in mice have shown leptin has hypothalamically mediated antiosteogenic effects, through the inhibition of osteoblast proliferation (Dimitri, Wales and Bishop, 2011; Dimitri, 2019). Population studies in children and adolescents have demonstrated varying associations between leptin and DXA bone parameters. In females and males aged 10 to 15 years, leptin did not explain any additional variation in total or regional BMC and aBMD beyond age, fat mass, lean mass, insulin-like growth factor 1 and estradiol (Roemmich *et al.*, 2003). Similarly, in children aged 7 to 8 years, leptin was not associated with TBLH BMC (Garnett *et al.*, 2004). However, whilst leptin was not associated with aBMD in prepubertal females aged 6 to 8 years, after adjustment for age, stature, and fat mass, leptin was inversely associated with aBMD in males (Soininen *et al.*, 2018). Further, in children and adolescents aged 5 to 16 years, free leptin index was inversely associated with osteoprotegerin, though fat mass was not adjusted for (Dimitri, Wales and Bishop, 2011). The authors suggest that a reduction in osteoprotegerin may lead to increased osteoclastogenesis and increased bone resorption, potentially resulting in decreased bone mass. However, as bone mass was not measured by Dimitri and colleagues (Dimitri, Wales and Bishop, 2011), it is unclear whether the association between free leptin index and osteoprotegerin results in decreased bone mass, and it is unclear whether the relationship is independent of fat mass (Dimitri, Wales and Bishop, 2011). As such, the potential mediating role of leptin in the relationship between fat mass and bone in children and adolescents remains unknown.

Adiponectin is an adipokine that has been inversely associated with fat mass in children aged 9 years (Sayers *et al.*, 2010). Children and adolescents with

obesity also had lower levels of adiponectin compared to children and adolescents of normal body weight, of corresponding age and pubertal stage, supporting the previous finding that greater fat mass is associated with lower adiponectin (Böttner *et al.*, 2004). Mechanistically, drawing from evidence from murine studies and adult studies, it has been suggested that adiponectin may be a negative regulator of bone mass (Reid, 2010; Sayers *et al.*, 2010). In children, adiponectin has been inversely associated with TBLH BMC at age 9 years and with the change in TBLH BMC from age 9 years to 15 years in the ALSPAC cohort (n = 2754 to 4927), independently of lean and fat mass (Sayers *et al.*, 2010). However, in prepubertal children aged 6 to 8 years (n = 452), adiponectin was not associated with TBLH aBMD, independently of fat mass (Soininen *et al.*, 2018). It is possible that the smaller sample size in the study by Soininen and colleagues (Soininen *et al.*, 2018) limited their ability to detect a relationship between adiponectin and bone, or that differences in the maturational status of participants contribute to different observations (Sayers *et al.*, 2010). However, further studies are needed to establish whether adiponectin mediates the relationship between fat mass and bone in children and adolescents.

2.8.5.2 Sex Steroids

Sex steroids have been hypothesised as a potential mechanism by which fat mass may influence bone mass because adipose tissue acts in the conversion of steroid precursors to estrogens (Reid, 2010; Li, Papadopoulos and Vihma, 2015). In children aged 7 to 8 years, fat mass was positively associated with estradiol (Garnett *et al.*, 2004). Estrogens may positively influence bone mass by inhibiting osteoclast generation and inducing osteoclast apoptosis, as well as

inhibiting apoptosis of osteocytes and osteoblasts (Soyka, Fairfield and Klibanski, 2000; Bellido, Plotkin and Bruzzaniti, 2014). However, in children aged 7 to 8 years, estradiol was not associated with TBLH BMC, adjusted for lean and fat mass (Garnett *et al.*, 2004). Further, in children aged 8 years and aged 10 years, estradiol was not correlated with aBMD (Klein *et al.*, 1998). However, estrogen deficiency in adolescence, particularly in combination with undernutrition, can lead to low bone mass (Soyka, Fairfield and Klibanski, 2000). In females aged 13 to 20 years, an estrogen exposure score (based on age of menarche, pubertal status, menstrual cycle status, estradiol level, oral contraceptive use, and estrogen therapy), was positively correlated with spine and wrist aBMD (Dhuper *et al.*, 1990). However, as estrogen exposure score was also highly positively correlated with body weight, it is unclear whether the observed association is explained, at least in part, by body weight (Dhuper *et al.*, 1990). As such, it remains unclear whether estradiol is associated with BMC and aBMD in children and adolescents, independently of fat mass, and whether estradiol mediates the relationship between fat mass with BMC and aBMD.

Testosterone has been positively correlated with fat mass in children aged 7 to 8 years (r = 0.188, $p < 0.01$) (Garnett *et al.*, 2004), and may influence bone growth both directly and indirectly, through influencing muscle growth, and through conversion to estradiol via aromatization in adipose tissue (Office of the Surgeon General, 2004; Clarke and Khosla, 2009). However, in children aged 7 to 8 years, testosterone was not associated with TBLH BMC, adjusted for lean and fat mass (Garnett *et al.*, 2004). In males aged 10 to 18 years, testosterone was an important predictor of TBLH aBMD, after the influence of age, stature and body weight was excluded (Pomerants *et al.*, 2007). Further, in males,

testosterone at age 10 to 13 years was positively correlated with aBMD at age 16 to 19 years, though body size or composition was not adjusted for (Tamme *et al.*, 2019). Differences in the observed relationships between testosterone with BMC and aBMD between studies may be due to differences in the pubertal status of participants or in the covariates adjusted for. It is also possible that the relationship between testosterone with BMC and aBMD may be sex-specific, as the studies which observed a relationship were in males only (Pomerants *et al.*, 2007; Tamme *et al.*, 2019). However, further research is needed to assess whether testosterone is associated with BMC and aBMD, independently of fat mass, and whether testosterone mediates the relationship between fat mass with BMC and aBMD in children and adolescents.

Dehydroepiandrosterone sulphate (DHEAS) is an androgen precursor, which increases during adrenarche (Mantyselka *et al.*, 2018). Central adiposity has been positively associated with DHEAS, and children with obesity were more likely to have high DHEAS than normal body weight children in a population sample of prepubertal children aged 7 years (Corvalán, Uauy and Mericq, 2013). As both obesity and premature adrenarche are associated with advanced bone age, DHEAS has also been hypothesised as a potential mediator in the relationship between fat mass and bone mass (Sopher *et al.*, 2011). In prepubertal females and males of a healthy body weight, the positive relationship between DHEAS and aBMD was fully explained by lean mass and fat mass (Soininen *et al.*, 2018). Similarly, in children aged 7 to 8 years, DHEAS did not explain any additional variance in TBLH BMC beyond that of lean mass and fat mass (Garnett *et al.*, 2004). However, given the maturity-specific patterns DHEAS follows throughout puberty (Auchus and Rainey, 2004), it is

unknown whether these findings extend to adolescents at different pubertal stages. Further, it remains unclear whether DHEAS mediates the relationship between fat mass with BMC in children and adolescents.

2.8.5.3 Insulin

As obesity is associated with insulin resistance and hyperinsulinemia, and *in vivo* evidence from murine models indicates that insulin acts as an anabolic agent for bone, it has been suggested that insulin may mediate the association between fat mass and bone (Thrailkill *et al.*, 2005; Sayers *et al.*, 2010). However, population studies in children and adolescents have shown null (Dalskov *et al.*, 2016; Soininen *et al.*, 2018; Kindler *et al.*, 2019) or inverse (Lawlor *et al.*, 2012; Rønne *et al.*, 2019) relationships between measures of insulin or insulin resistance with bone. In females and males aged 6 to 8 years, the association between insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) with TBLH aBMD was explained by fat mass (Soininen *et al.*, 2018). Further, in children aged 7 to 15 years, HOMA-IR did not meet the criteria for mediation with fat mass and TBLH BMC (Kindler *et al.*, 2019), and in children aged 8 to 11 years, insulin was not associated with size-adjusted BMC, with adjustment for fat mass and lean mass (Dalskov *et al.*, 2016). However, in females and males aged 15 to 16 years, insulin was negatively associated with TBLH BMC after accounting for fat mass, though it did not mediate the fat mass and TBLH BMC relationship (Lawlor *et al.*, 2012). Further, HOMA-IR was inversely associated with TBLH BMC in males aged 10 to 17 years, with adjustment for maturity, stature, BA, baseline TBLH BMC, body weight and body fat percentage, though no association was shown in females (Rønne *et al.*, 2019). The conflicting findings may reflect differences in the maturational status of participants, as the studies which found an inverse relationship

between insulin and BMC tended to include older participants, and insulin sensitivity is known to decrease during puberty (Kelsey and Zeitler, 2016). However, further research would be valuable in establishing whether insulin mediates the relationship between fat mass with BMC and aBMD.

2.9 Vitamin D and Bone Health

2.9.1 Vitamin D Status and Bone

Vitamin D influences the development, maintenance and resorption of bone by regulating calcium and phosphate homeostasis (Institute of Medicine, 2011). Serum 25(OH)D is the clinically relevant marker of vitamin D status, reflecting vitamin D derived from foods, supplements, and endogenous synthesis (Hazell *et al.*, 2015). Although there is no consensus on the thresholds of serum 25(OH)D for deficiency, insufficiency and sufficiency, thresholds for deficiency have been proposed ranging from < 25 nmol/L to < 50 nmol/L, with levels from < 50 to < 75 nmol/L indicating insufficiency, and with levels above 50 nmol/L to 75 nmol/L indicating sufficiency (Holick *et al.*, 2011; Institute of Medicine, 2011; Arundel *et al.*, 2012; Society for Adolescent Health and Medicine, 2013).

As highlighted by a narrative review paper, a number of cross-sectional studies have investigated the associations between serum 25(OH)D and DXA-assessed bone parameters in children and adolescents (Moon *et al.*, 2014). In children aged 1 to 6 years, serum 25(OH)D was positively associated with forearm BMC and aBMD, with adjustment for age, height, body weight, sex, and sun index (Hazell *et al.*, 2015). Similarly, in children and adolescents aged 7 to 19 years, serum 25(OH)D was positively associated with aBMD of the lumbar spine (adjusted for height, body fat percentage, lean mass and PA score), total hip (adjusted for height, body fat percentage, and PA score), and whole body

(adjusted for height and PA score) (Pekkinen *et al.*, 2012). However, in girls aged 10 to 12 years there was no association between 25(OH)D with BMC or aBMD at any skeletal site, though it was unclear whether any adjustments were made (Cheng *et al.*, 2003). Several studies have examined differences in bone measures according to tertiles or thresholds for serum 25(OH)D (Moon *et al.*, 2014). In females aged 14 to 16 years, serum 25(OH)D concentrations ≤ 40 nmol/L were associated with unadjusted reduced forearm aBMD (Outila, Karkkainen and Lamberg-Allardt, 2001). In children aged 1 to 6 years, those with serum 25(OH)D concentration of > 75 nmol/L had greater forearm and whole-body BMC and aBMD, though there was no difference in lumbar spine measures, with adjustment for age, height, body weight, sex, and sun index (Hazell *et al.*, 2015). However, in girls aged 10 to 12 years, there were no differences total body, femur or lumbar spine BMC or aBMD between those with deficient (≤ 25 nmol/L), insufficient (26-40 nmol/L) and sufficient (>40 nmol/L) serum 25(OH)D status, though after adjustment for pubertal stage and body mass index (BMI), the deficient group had greater femur BMC than the sufficient group (Cheng *et al.*, 2003). Further, whilst females aged 12 and 15 years in the highest tertile for vitamin D status had greater forearm aBMD than those in the lowest tertile, there was no difference between groups in males, with adjustment for height, body weight, pubertal stage, PA, smoking, supplement use, and intake of alcohol, calcium and fruit (Cashman *et al.*, 2008). In girls aged 9 to 15 years, there were no differences in unadjusted lumbar spine aBMD by serum 25(OH)D tertile, though unadjusted femoral neck aBMD differed between groups in girls with advanced sexual maturation (Lehtonen-Veromaa *et al.*, 2002). The conflicting findings are likely due to the considerable variation in

participant age, geographic location, and the covariates considered in the analysis (Moon *et al.*, 2014).

Longitudinal studies of the associations between serum 25(OH)D with bone parameters in children and adolescents are scarce. One study found that baseline serum 25(OH)D was positively correlated with 3-year change in lumbar spine and femoral neck aBMD in girls aged 9 to 15 years (Lehtonen-Veromaa *et al.*, 2002). In girls with advanced skeletal maturation, 3-year change in lumbar aBMD was different across tertiles of serum 25(OH)D status, with the greatest change in the highest tertile, but no differences in 3-year change in femoral neck aBMD, and no differences in the less mature girls, with adjustment for baseline reproductive year, baseline bone values, change in height and body weight, mean calcium intake and mean PA (Lehtonen-Veromaa *et al.*, 2002). Conversely, in prepubertal females aged 4 to 8 years at baseline, serum 25(OH)D was inversely associated with the rate of BMC accrual at the lumbar spine, proximal femur, and forearm, over a nine-year period, adjusted for season and race (Breen *et al.*, 2011). Although the inverse relationship between serum 25(OH)D and BMC accrual seems contradictory with previous research which has found positive or null associations between serum 25(OH)D and bone measures, the authors suggest that low 25(OH)D status in children and adolescents may reflect the potential for greater bone gains in children with low baseline serum 25(OH)D compared to children with sufficient serum 25(OH)D levels (Breen *et al.*, 2011). This is supported by a meta-analysis of RCTs of the effects of vitamin D supplementation on aBMD and BMC in children and adolescents (Winzenberg *et al.*, 2011). Although vitamin D supplementation had no effect of total body BMC, there was a trend towards a greater effect in

children with baseline serum 25(OH)D levels ≤ 35 nmol/L (Winzenberg *et al.*, 2011). This indicates that whilst vitamin D supplementation, and therefore potentially serum 25(OH)D levels, may not be related with bone measures in children with greater levels of serum 25(OH)D, these relationships may become apparent in children and adolescents with lower levels of serum 25(OH)D.

2.9.2 Vitamin D Status, Physical Activity and Bone

In addition to the independent relationships between PA and serum 25(OH)D with bone mass, it is also possible that PA and vitamin D interact to influence bone mass, though research into the combined effects is limited. In female and male adolescents, those meeting the MVPA guidelines and with serum 25(OH)D ≥ 75 nmol/L had greater BMC than those meeting the MVPA guidelines with serum 25(OH)D < 75 nmol/L, controlling for age, sex, lean mass, calcium intake, and seasonality (Valtuena *et al.*, 2012). This suggests that sufficient 25(OH)D levels may improve BMC in active adolescents. However, these findings are based on a small subsample of adolescents with serum 25(OH)D ≥ 75 nmol/L (27 of the 100 participants were above this threshold), and fat mass was not adjusted for. In children aged 10 to 17 years, there was no interaction between vitamin D status and VPA with TBLH BMC, with adjustment for size, body composition, BMC at age 8 to 11 years, maturity, sex, and maturity-by-sex interaction (Rønne *et al.*, 2018a). This suggests that the relationship between VPA with TBLH BMC does not depend on vitamin D status, and vice versa. It is unclear what accounts for the different observations between studies, though differences in baseline levels of vitamin D and differences in age groups between studies may contribute to the different findings. A literature search did not reveal any studies which have considered

the effects of vitamin D supplementation in isolation and combined with exercise on bone health in children and adolescents, so there is no experimental evidence to draw on in understanding the potential interactions between vitamin D, PA and bone.

2.10 Thesis Objectives

Following an exploration of the importance of PBM, the measurement of bone health, and bone development in childhood and adolescence, this literature review has discussed the evidence regarding determinants of bone health. Specifically, this literature review considered sex and maturation, PA and other movement behaviours, body composition and associated endocrine factors, and vitamin D status. Whilst many studies have demonstrated positive relationships between PA and BMC or aBMD in childhood and adolescence, the methods of quantifying PA may not be the most appropriate when considering skeletal outcomes, and there is limited research considering the relationships between the other movement behaviours (sedentary time and sleep) and bone. Further research using different methods for summarising PA, including justification based on mechanical loading rather than energy expenditure and methods which account for the distribution of intensity across the day, would be beneficial. In addition, further exploration into the associations between the other movement behaviours, as well as specific muscle and bone strengthening activity, is warranted. This literature review has also highlighted the need for further research into the potential interaction between PA and vitamin D status with bone in childhood. Although the positive relationship between lean mass and BMC during growth is well-established, there remains inconsistencies in the research considering fat mass and bone in childhood. When considering fat mass and bone, further research into the role of associated endocrine factors

would be valuable in extending the current understanding of the relationships between fat mass and bone in childhood and adolescence. Furthermore, the importance of considering lean mass and fat mass when investigating the relationships between PA and bone has been highlighted.

Considering the previous literature and the gaps highlighted by this literature review, the objective of this thesis is to provide novel insights into PA, body composition and associated endocrine factors, and vitamin D status, as determinants of BMC and aBMD in children and adolescents.

The specific aims of each chapter are as follows:

1) **Chapter 4**: To investigate the sex-specific independent and interactive associations of PA intensity and serum 25(OH)D concentration on TBLH and lower-limb aBMD in prepubertal children.
2) **Chapter 5**: To determine the association between fat mass and TBLH BMC in females and males aged 9 to 11 years, and to assess the extent to which this relationship is mediated by insulin, leptin (free leptin index), adiponectin, DHEAS, testosterone and estradiol.
3) **Chapter 6**: To assess the associations of PA volume (average-acceleration) and intensity distribution (intensity-gradient) with TBLH BMC, lean mass, and fat mass in a population sample of pre- and early-pubertal children aged 9 to 11 years and to apply translational metrics to illustrate the profile of the PA volume and intensity distribution associated with improved BMC and lean mass, and reduced fat mass in this cohort.
4) **Chapter 7**: To assess whether MVPA, sport and exercise as a proxy measure of muscle and bone strengthening activity, sedentary time,

screen time, and sleep, are associated with TBLH BMC and TBLH lean mass cross-sectionally (at age 6 to 9 years, age 9 to 11 years, age 15 to 17 years), and longitudinally (from age 6 to 9 years and age 9 to 11 years to age 15 to 17 years).

Chapter 3 General Methods

3.1 Study Design and Participants

This thesis utilized data from baseline (2007 to 2009), 2-year follow-up (2009 to 2011), and 8-year follow-up (2016 to 2017) of the PANIC Study, which is an ongoing longitudinal study with a controlled lifestyle intervention in a population sample of Finnish children (ClinicalTrials.gov registration number NCT01803776). The PANIC Study was primarily carried out at the Institute for Biomedicine, University of Eastern Finland. The primary aims of the PANIC study were to investigate the risk factors and pathophysiological mechanisms for obesity, type 2 diabetes, and cardiovascular disease and the assess the long-term effects of a lifestyle intervention on these risk factors and pathophysiological mechanism. See Figure 3.1 for a schematic of the PANIC Study protocol.

Children aged 6 to 9 years who were registered for the first grade in one of the 16 public schools in Kuopio, Finland were invited to participate in the baseline examinations between October 2007 and December 2009. Children were eligible to participate in the study if they had no disability that could prevent their participation in the assessments or the intervention and had a caregiver who was able to communicate in Finnish to fill out the questionnaires and participate in the intervention. Of the 736 children invited to participate, 512 (70%) attended the baseline examinations. Of the 512 who attended baseline examinations, two children were excluded due physical disabilities, and six children withdrew from the study. The total sample from baseline was therefore 504 children. The participants did not differ in sex distribution, age, or BMI standard deviation score (SDS) from all children who started the first grade in

Kuopio between 2007 and 2009, based on data from standard school health examinations.

Of the 504 children included in baseline examinations, children were allocated to the intervention group (306 children) or control group (198 children). As the intervention included after-school exercise clubs, children were allocated according to schools to avoid contamination. Schools in the intervention and control group were matched according to school size and location (urban/rural) to minimise differences at baseline between the two groups. As a larger number of dropouts were expected in the intervention group, more children were allocated to the intervention group than the control group.

Children were invited to participate in 2-year follow-up examinations between 2009 to 2011. Altogether, 261 children from the intervention group and 177 children from the control group participated. The median (interquartile range (IQR)) of 2-year follow-up time was 2.1 (2.1 to 2.2) years in both groups (Sallinen *et al.*, 2022). The 8-year follow-up examinations were carried out in 2016 to 2017. Altogether, 169 adolescents from the intervention group and 108 adolescents from the control group participated. The median (IQR) of 8-year follow-up time was 8.3 (8.1 to 8.3) years for the intervention group and 8.1 (8.0 to 8.3) years for the control group (Sallinen *et al.*, 2022). Participants have since been invited to participate in 13-year follow-up examinations in 2021-2020. A summary of the study design and the variables used in each chapter of this thesis is presented in Table 3.1.

The study was conducted according to the ethical guidelines of the Declaration of Helsinki. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo (69/2006). The parents or caregivers of the children provided their written informed consent, and the children provided their assent to participation.

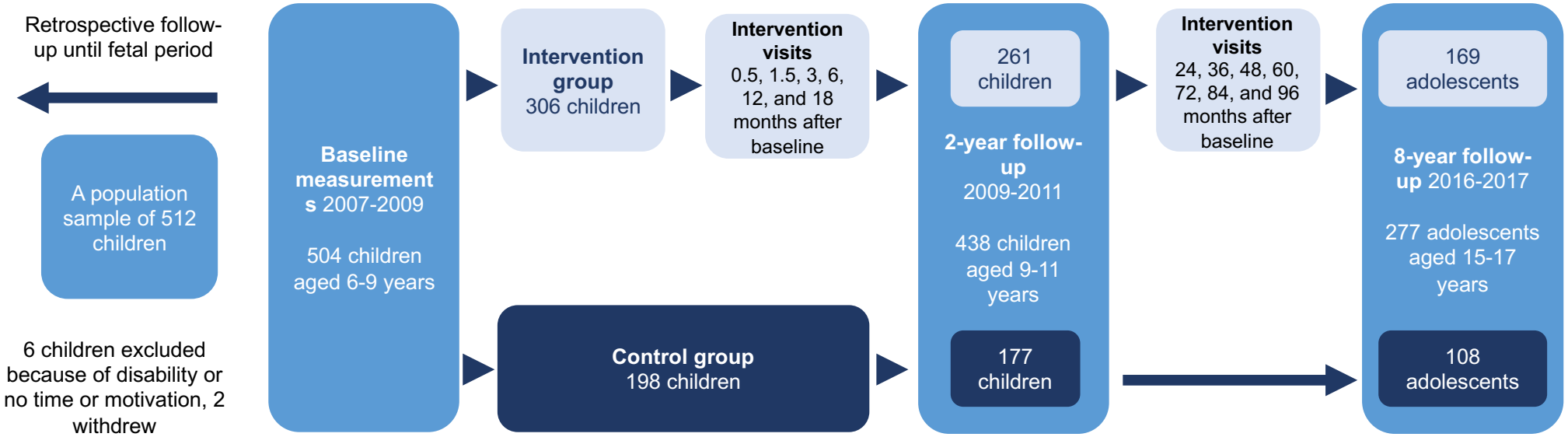

Figure 3.1 Schematic of Physical Activity and Nutrition in Childhood (PANIC) study protocol.

Table 3.1 Summary of study design and variables used in Chapters 4 to 7

	Chapter 4	Chapter 5	Chapter 6	Chapter 7
Study Design				
Cross-sectional	x	x	x	x
Longitudinal				x
Timepoint				
Baseline (age 6 to 9 years)	x	x		x
2-year follow-up (age 9 to 11 years)		x	x	x
8-year follow-up (age 15 to 17 years)				x
Anthropometric and Descriptive Characteristics				
Age	x	x	x	x
Sex	x	x	x	x
Pubertal status	x	x	x	x
Stature	x	x	x	x
Body weight	x	x	x	x
BMI	x	x	x	x
DXA Variables				
TBLH BMC	x	x	x	x
TBLH aBMD	x			
TBLH lean mass		x	x	x
TBLH fat mass		x	x	x
Upper-limb BMC			x	
Upper-limb aBMD				
Upper-limb lean mass			x	
Upper-limb fat mass			x	
Lower-limb BMC	x		x	
Lower-limb aBMD	x			
Lower-limb lean mass			x	
Lower-limb fat mass			x	
Physical Activity Variables				
Accelerometer-assessed physical activity	x		x	x
Accelerometer-assessed sedentary behaviour	x			x
Accelerometer-assessed sleep				x
Questionnaire-assessed physical activity				x
Questionnaire-assessed sedentary behaviour				x
Blood Biomarkers				
Seum 25(OH)D	x			
Serum insulin		x		
Plasma leptin		x		
Plasma soluble leptin receptor		x		
High-molecular-weight adiponectin		x		
DHEAS		x		
Testosterone		x		
Estradiol		x		

BMI, body mass index; TBLH, total-body-less-head; BMC, bone mineral content; aBMD, areal bone mineral density; 25(OH)D, 25-hydroxyvitamin D; DHEAS, dehydroepiandrosterone sulphate.

3.2 Intervention Details

The goals of the lifestyle intervention were to: 1) decrease the consumption of significant sources of saturated fat, in particular high-fat dairy and meat products; 2) increase the consumption of significant sources of unsaturated fat, in particular high-fat vegetable oil-based margarines, vegetable oils and fish; 3) increase the consumption of fruits and vegetables; 4) increase the consumption of significant sources of fibre, in particular whole grain products; 5) decrease the consumption of significant sources of sugar, in particular sugar-sweetened beverages, sugar-sweetened dairy products and candy; 6) decrease the consumption of significant sources of salt and the use of salt in cooking; 7) increase total PA by emphasising its diversity; 8) decrease total sedentary behaviour, in particular screen-based sedentary behaviour and 9) avoid excessive energy intake (Sallinen *et al.*, 2022).

During the first two years of the study, children and their caregivers received six 30-to-45-minute PA counselling sessions and six 30-to-45-minute dietary counselling sessions for children and their parents (Venalainen *et al.*, 2016; Viitasalo *et al.*, 2016). The sessions took place 0.5, 1.5, 3, 12, and 18 months after baseline. The children and their caregivers received individualised advice from an exercise medicine specialist and a clinical nutritionist on how to increase PA, decrease sedentary behaviour, and improve diet quality for the children. The children and their caregivers were provided with fact sheets on PA, sedentary behaviour, and diet quality, verbal and written information on opportunities to exercise in Kuopio, as well as exercise equipment and free admissions for indoor sports. Children were encouraged to participate in the after-school exercise clubs provided by the PANIC Study. Following the initial

two-year period, children in the intervention group were provided one 60-minute combined PA and diet counselling session per year until the 8-year follow-up examinations. The sessions took place 3, 4, 5, 6, and 7 years after baseline. The participants were able to attend the sessions at 3, 5, 6, and 7 years at the research site, with or without their caregivers. The session at 4 years was a group-based counselling session which took place in the schools. The intervention ended in December 2017. At baseline, the control group received general healthy lifestyle advice according to the Finnish recommendations.

3.3 Assessment of General Health and Medication Use

At baseline, 2-year follow-up and 8-year follow-up, general health was assessed by questionnaires completed by caregivers. The questionnaires included items on participants' chronic diseases, allergies and traumas diagnosed by a physician and detailed information on participants' medication use.

3.4 Assessment of Pubertal Status and Maturation

At baseline, 2-year follow-up and 8-year follow-up, pubertal status was assessed by a research physician according to stages described by Tanner (Tanner, 1986). A research physician performed a clinical examination and classified the females as having entered clinical puberty if their breast development had started and males if their testicular volume assessed by palpation and by an orchidometer was ≥ 4 mL, according to criteria described by Tanner (Tanner, 1986).

3.5 Anthropometry

At baseline, 2-year follow-up and 8-year follow-up, stature and body weight were measured by a research nurse. Stature was measured with a wall-mounted stadiometer, three times to an accuracy of 0.1 cm, with participants standing in the Frankfurt plane without shoes. Body weight was measured twice (InBody 720 bioelectrical impedance analysis (BIA) device, Biospace, Seoul, South Korea) to an accuracy of 0.1 kg, with participants having fasted for 12 hours, having emptied the bladder, and standing in light underwear. For stature and body weight, the mean of the values was used in analyses. BMI (kg/m^2) was calculated by dividing body weight (kg) by stature (m) squared. BMI-SDS was calculated using the Finnish reference values (Saari *et al.*, 2011). The BMI cut-offs of the International Obesity Task Force (IOTF) were applied to classify children as normal weight, overweight, or obese (used in Chapter 4) (Cole *et al.*, 2000; Cole *et al.*, 2007), which were later updated to reflect the more recent IOTF cut-offs to classify children and adolescents as thin, normal weight, or living with overweight or obesity as it related to their weight status (used in Chapters 5 to 7) (Cole and Lobstein, 2012).

3.6 Assessment of Bone Mineral Content, Bone Mineral Density and Body Composition

At baseline, 2-year follow-up and 8-year follow-up, TBLH BMC (kg), TBLH aBMD (g/cm^2), TBLH lean mass (kg) and TBLH fat mass (kg) were assessed by a trained and experienced research nurse using the Lunar Prodigy Advance DXA device (GE Medical Systems, Madison, WI, USA) and the Encore software, Version 10.51.006 (GE Company, Madison, WI, USA). These measurements were made in accordance with the instructions outlined by

manufacturers using standardized protocols. Participants were assessed in a non-fasted state, having emptied the bladder, and in light clothing with all metal objects removed. DXA provides valid and reliable data on BMC, aBMD and body composition in children and adolescents (CV 0.01 % – 4.37%) (Jaworski and Pludowski, 2013; International Society for Clinical Densitometry, 2019b). A quality assurance test was performed daily prior to patient measurements. The inspection ensured the functionality and accuracy of the measuring device and method, tested the setting of the high voltage, the longitudinal and transverse movement of the imaging arc, the shutter mechanism of the beams and the accuracy of the detector. The inspection was performed using a recording table and a standard. A standard is a piece corresponding to a tissue material. Furthermore, for device repeatability monitoring a weekly testing was performed. According to the quality assurance program, the aluminium phantom was measured once a week. The acceptable measurement result in repeatability monitoring must not deviate by less than 2% from the long-term average. The effective dose in whole body scans was 1 to 3 μSv.

TBLH BMC and aBMD are recommended by the International Society for Clinical Densitometry for use in paediatric participants (International Society for Clinical Densitometry, 2019b). Upper-limb and lower-limb BMC, aBMD, lean mass, and fat mass derived from the whole body DXA scan were also measured. The upper-limb and lower-limb is automatically defined using Encore software (GE Company, Madison, WI, USA), and an average of both sides was used. To allow comparisons with other populations, we used paediatric cross-calibration equations to account for differences in DXA outcomes between manufacturers (Shepherd *et al.*, 2012).

3.7 Assessment of Physical Activity, Sedentary Behaviour and Sleep

3.7.1 Accelerometery

At baseline, 2-year follow-up and 8-year follow-up, PA was assessed using Actiheart (CamNtech Ltd, Papworth, UK), a combined heart rate and movement sensor (Brage *et al.*, 2005; Corder *et al.*, 2007). The Actiheart is 7 mm thick with a 33 mm diameter, and contains a movement sensor, rechargeable battery and a memory chip, with a 100 mm wire running to a smaller clip (Brage *et al.*, 2005). The total weight is 8 g (Brage *et al.*, 2005). The device was attached to the chest with standard electrocardiogram (ECG) electrodes (Bio Protech Inc, Wonju, South Korea) and set to record heart rate and body movement in 60-second epochs (see Figure 3.2).

The Actiheart contains a piezoelectric element which measures acceleration with a frequency range of 1-7 Hz (3 dB) (Brage *et al.*, 2005). The accelerometer has a dynamic range of ±25 m/s^2 (±2.5 *g*), a 32 Hz sampling rate, with 0.2 m/s^2 (0.02 G) sensitivity per bit (Brage *et al.*, 2005). When exposed to a time-varying acceleration, the accelerometer generates a transient charge which produces a voltage signal (Brage *et al.*, 2005). The voltage signal is then converted into a binary signal by an 8-bit A/D converter, resulting in 256 distinctive levels of acceleration (128 positive and 128 negative levels) (Brage *et al.*, 2005). The binary signal is stored in a cache 32 times a second and summed over the 60-second epoch (Brage *et al.*, 2005). At the end of the 60-second epoch, the sum is divided by 16. The sum is then divided by 2, *N* number of times until the number is below 32, to give an integer, *Z,* and *N* (Brage *et al.*, 2005). The

resulting integer, *Z,* and the number of times, *N,* are then stored in a single byte in the non-volatile memory, and the cache is reset to zero (Brage *et al.*, 2005).

At age 6 to 9 years and age 9 to 11 years, the children were asked to wear the monitor continuously for a minimum of two weekdays and two weekend days, including during sleep and water-based activities, though some children wore the monitor for up to nine days (Collings *et al.*, 2017). At age 15 to 17 years, adolescents were requested to wear the monitor continuously for seven days, including sleep. In order to capture different PA patterns between weekends and weekdays, the wear period was scheduled to include an entire weekend (Rowlands, Pilgrim and Eston, 2008). Heart rate data were pre-processed using robust Gaussian Process regression (Stegle *et al.*, 2008), and individually calibrated to PA energy expenditure using a maximal exercise test on a cycle ergometer (Lintu *et al.*, 2014).

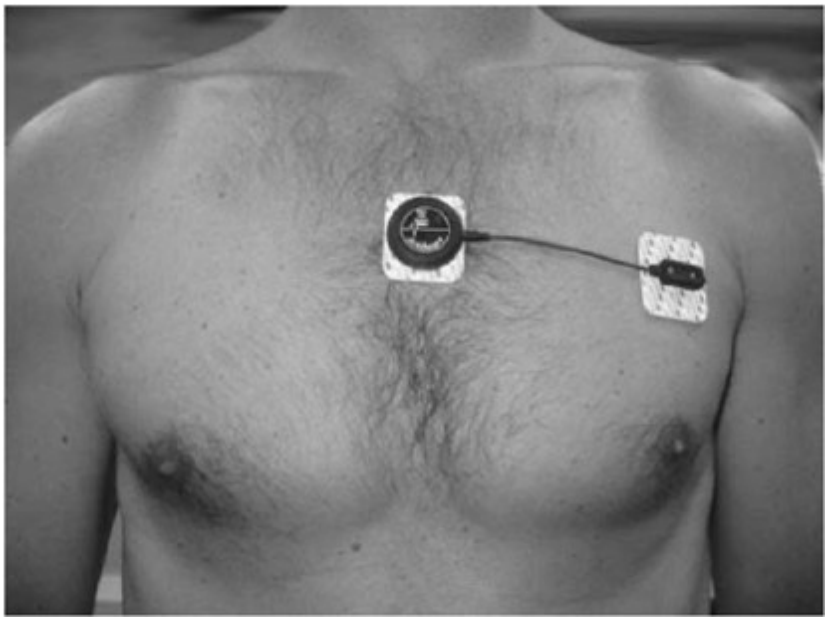

Figure 3.2 Actiheart combined heart rate and movement sensor placed on a human subject. Reprinted from (Brage *et al.*, 2005) with permission from Springer Nature.

3.7.1.1 Individual Calibration of Heart Rate to Energy Expenditure

In order to assess the individual heart rate and energy expenditure relationship, participants completed a maximal exercise test using the Ergoselect 200 K® electromagnetic cycle ergometer (Ergoline, Bitz, Germany) at baseline, 2-year follow-up and 8-year follow-up (Lintu *et al.*, 2014). Participants spent 3 minutes seated on the ergometer with no workload, followed by 3 minutes of cycling at 5 W. Participants cycled at a cadence of 70 to 80 revolutions per minutes for 1 minute at 20 W, and then workload was ramped at 1 W per 6 seconds until voluntary exhaustion. This was followed by 4 minutes cooling down at 5 W. Participants received verbal encouragement during the test to continue until exhaustion. The exercise test was considered maximal if the reason for terminating the test indicated maximal effort and maximal cardiorespiratory capacity. Heart rate was measured during the cycle test using an online ECG system (Cardiosoft v6.5 Diagnostic System, GE Healthcare Medical Systems, Freiburg, Germany). Oxygen consumption was measured in a subsample (n = 38 at baseline, n = 365 at 2-year follow-up, n = 215 at 8-year follow-up) to derive the energy cost of the cycle ergometer protocol (Lintu *et al.*, 2014; Lintu *et al.*, 2015; Tompuri *et al.*, 2016). Heart rate was plotted against the measured or estimated, based on a group estimate, energy cost of the cycling protocol, in order to calculate the calibration factors needed for individual calibration (MRC Epidemiology Unit, 2022b). The individual calibration factors (slope, intercept, and flex heart rate point) were carried forward to apply to the free-living data energy expenditure calculations (Collings *et al.*, 2017). Participants without a valid cycle test were assigned a group-level calibration derived from all valid cycle tests which represented the average heart rate to energy expenditure response for a given age, sex and sleeping heart rate (Collings *et al.*, 2017).

3.7.1.2 Processing of Physical Activity Data

Non-wear time was classified as zero-acceleration lasting > 90 minutes combined with non-physiological heart rate. Non-wear can be more easily determined using combined sensing data than when using an accelerometer alone, as heart rate can only assume a set range of values, whereas no acceleration looks the same in accelerometer data, regardless of whether the monitor is being worn (MRC Epidemiology Unit, 2022a). Activity estimates were adjusted during summarisation to minimise bias and error arising from diurnal imbalance (Brage *et al.*, 2013; Collings *et al.*, 2017). Criteria for a valid PA measurement were ≥ 48 hours of good-quality data with ≥ 32 hours of weekday data and ≥ 16 hours of weekend data as well as ≥ 12 hours of morning, noon, afternoon and evening wear time to protect against bias from over-representation from specific times of day and to optimise the diurnal bias minimisation procedure (Brage *et al.*, 2013; Collings *et al.*, 2017).

3.7.1.3 Modelling Intensity from Combined Heart Rate and Movement Data

The free-living heart rate and acceleration data were combined to estimate PA intensity, justified on the basis of energy expenditure (Brage *et al.*, 2004). Accelerometers only are subject to limitations for estimating energy expenditure based estimates of PA, as the accelerometry-PA intensity relationship is highly variable during different activities, such as walking on the level and incline, running at different speeds, cycling and during load-bearing activities (Brage *et al.*, 2004). On the other hand, the heart rate-PA intensity relationship, particularly during low intensity and sedentary behaviours, can be influenced by ambient temperature, hydration levels, illness, and mental stress (Brage *et al.*,

2004; MRC Epidemiology Unit, 2022b). As the errors associated which each method are not related, combining heart rate and accelerometry can lead to more precise estimates of PA intensity based on energy expenditure (Brage *et al.*, 2004). Intensity was modelled from the combined sensing signal using a branched equation framework (Brage *et al.*, 2004). The branched equation modelling estimates PA energy expenditure (PAEE) with different weightings for the accelerometry data and heart rate data depending on the intensity of the activity (Brage *et al.*, 2004). At low levels of movement and heart rate, mainly the accelerometry data determines PAEE, whereas at very high intensities only heart rate determines PAEE, with interim weightings in between (Brage *et al.*, 2004; MRC Epidemiology Unit, 2022a). The equivalent of 3.5 mL O_2/min/kg was used to define resting metabolic rate (Jetté, Sidney and Blümchen, 1990). For baseline, 2-year follow-up and 8-year follow-up, data were summarised into intensity bands; sedentary time was defined at ≤ 1.5 METs, LPA was defined as > 1.5 METs and ≤ 4 METs and MVPA was defined as > 4 METs (Troiano *et al.*, 2008; Trost *et al.*, 2011; Chaput *et al.*, 2020). The children and adolescents were defined as meeting the PA guidelines if they had at least an average of 60 minutes of MVPA per day over the measurement period, as described in the WHO PA guidelines (Chaput *et al.*, 2020).

The specific accelerometry exposures used in Chapters 4, 6 and 7 are described in the following chapters.

3.7.1.4 Sleep

Sleep duration was assessed from the Actiheart data by a trained exercise specialist and confirmed by an experienced researcher (Collings *et al.*, 2017).

The time of falling asleep was defined by the exercise specialist as a time point when accelerometer counts were zero with a heart rate plateau, and wake time was defined by an increase in accelerometer counts from zero and an increase in heart rate above plateau level. The children at age 6 to 9 years and age 9 to 11 years were defined as meeting the guidelines if their average daily sleep was between 9 and 11 hours, and the adolescents at age 15 to 17 years if their average daily sleep was between 8 and 10 hours (Tremblay, Carson and Chaput, 2016; Janssen, Roberts and Thompson, 2017; Roberts *et al.*, 2017; Leppänen *et al.*, 2022).

3.7.2 Questionnaires

3.7.2.1 Physical Activity

At baseline and 2-year follow-up, habitual PA and sedentary behaviour during a usual week were assessed using the PANIC Physical Activity Questionnaire filled out by the parents [33]. The PANIC Physical Activity Questionnaire was validated against the Actiheart data in a subsample of 38 children at the baseline examinations (Vaisto *et al.*, 2014). Total PA assessed by the PANIC Physical Activity Questionnaire positively correlated with total PA assessed by Actiheart (r = 0.39, p = 0.033) (Vaisto *et al.*, 2014). The types of PA in the questionnaire included unsupervised PA, organised sports, organised exercise other than sports, physically active school transportation, and PA during recess. Total PA was calculated by summing-up the amounts of different types of PA and was expressed in minutes per day. The compulsory 1.5 hours of physical education per week for all children aged 7–15 years in Finnish schools was included in total PA. The parents were also asked whether their children had participated regularly in any of 43 selected supervised and unsupervised PA.

At 8-year follow-up, habitual PA and sedentary behaviour were assessed using the PANIC Physical Activity Questionnaire for Adolescents and Adults. The questionnaire covered the previous 12 months to capture and control for seasonal variation in PA and sedentary behaviour. Adolescents were provided with a list of 42 activities, and asked whether they had participated in any of the activities, in how many months of the prior year, how times per week, and how many hours per session. The adolescents were also asked whether they had engaged in physical education (hours of physical education per week), PA during recess (number of 15-minute recesses being physically active per week), and physically active school transportation from a list of 6 activities.

At all timepoints, the questionnaire included assessment of types of sedentary behaviour during leisure time, including screen-based sedentary behaviour (watching TV and videos, using the computer and playing video games, using a mobile phone, playing mobile games), sedentary behaviour related to music (listening to music, playing music), sedentary behaviour related to academic skills (reading, writing), sedentary behaviour related to arts, crafts, and games (drawing, doing arts and crafts, playing board and card games), and sitting or lying for a rest. Times spent in each sedentary behaviour separately on weekdays and weekends were asked and were expressed in minutes per day. The amount of total sedentary behaviour was calculated by summing-up the times spent in each sedentary behaviour and was expressed in minutes per day, weighted by the number of weekdays and weekend days.

3.7.2.2 Screen Time

Screen time was assessed with the PANIC Physical Activity Questionnaire, which was filled out by the caregivers with their child (Leppänen *et al.*, 2022). The questionnaire asked for time spent watching television and videos, using computers and playing video and console games, and using mobile phones and playing mobile phone games (Leppänen *et al.*, 2022). Although self-report and proxy-report measures of screen time have mixed validity and reliability in the literature, the lack of gold standard method for assessing screen time makes it difficult to accurately assess validity and reliability, and self- and proxy-report measures are commonly used in the literature (Lubans *et al.*, 2011). The children and adolescents were defined as meeting the PA guidelines if their average daily screen time was no more than 2 hours (Roberts *et al.*, 2017; Tremblay, Carson and Chaput, 2016).

3.8 Biochemical Analyses

At baseline, 2-year follow-up and 8-year follow-up, venous blood samples were taken following a 12-hour overnight fast. Blood was immediately centrifuged and stored at –75°C until biochemical analyses. Serum 25(OH)D (nmol/L) concentration was analysed by LIAISON 25(OH)D TOTAL Assay (DiaSorin Inc., Stillwater, USA), a chemical luminescence immunoassay, using an automatic immunoanalyser (DiaSorin S.p.A.). Serum insulin (mU/L) concentration was measured by electrochemiluminescence immunoassay with the sandwich principle (Roche Diagnostics GmbH, Mannheim, Germany). Plasma leptin (ng/ml) concentration was analysed using a competitive radioimmunoassay (Multigamma 1261-001, PerkinElmer Wallac Oy, Turku Finland) and plasma soluble leptin receptor (ng/ml) concentration was analysed using an enzyme-

linked immunosorbent assay (ELISA) kit (Multicalc evaluation programme PerkinElmer Wallac Oy, Turku Finland). Free leptin index was calculated by dividing leptin with leptin receptor and multiplying by 100 and was used instead of leptin and leptin receptor in statistical analyses, as it has been suggested that free leptin index better reflects the physiological actions of leptin (Kratzsch *et al.*, 2002). Serum high-molecular-weight adiponectin (μg/ml) concentration was analysed using an ELISA kit, following specific proteolytic digestion of other multimeric adiponectin forms (Millipore, Billerica, MA, USA). Serum DHEAS (μmol/L) concentration was determined using an ELISA kit (Alpha Diagnostic International, San Antonio, TX, USA). Serum testosterone (pmol/L) and estradiol (pmol/L) were measured by using liquid chromatography-mass spectrometry (LC-MS), as explained in detail elsewhere (Häkkinen *et al.*, 2018). In the steroid analysis method, the lowest limit of quantitation of estradiol was 6.68 pmol/L, and therefore children with values below this were recorded as < 6.68 pmol/L. Although endocrine measures from a single fasted sample may not adequately reflect endocrine status across the day, a single fasted measure is common in epidemiological studies and in line with previous research (Garnett *et al.*, 2004; Lawlor *et al.*, 2012). The within-day and between-day CV for the biochemical assays are provided in Table 3.2.

Table 3.2 Within-day and between-day coefficients of variation for measurement of endocrine factors

	Within-day CV	**Between-day CV**
Seum 25(OH)D	8.2 to 11.0% (total variation)	
Serum insulin	1% to 4%	2% to 4%
Plasma leptin	2%	4%
Plasma soluble leptin receptor	7%	5%
High-molecular-weight adiponectin	2%	17%
DHEAS	9%	12%
Testosterone	4%	6%
Estradiol	6%	9%

CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulphate.

3.9 Statistical Analysis

Data were analysed using IBM SPSS Statistics for Mac software, Version 26.0 (IBM Corp, Armonk, NY, USA) and Stata/SE for Mac software, Version 16.1 and Version 17.0 (StataCorp LLC, College Station, TX, USA). For all analyses the alpha level for significance was set at 0.05.

For descriptive statistics and tests for sex differences in continuous variables, the distribution of the data was visually checked using histograms (Figure 3.3). When data met the assumption of normality (Example A, Figure 3.3) independent samples t-tests were used the assess sex differences, and when the assumption of normality was violated (Example B, Figure 3.3) Mann-Whitney U tests were used. For categorical variables, Fisher's exact test was used.

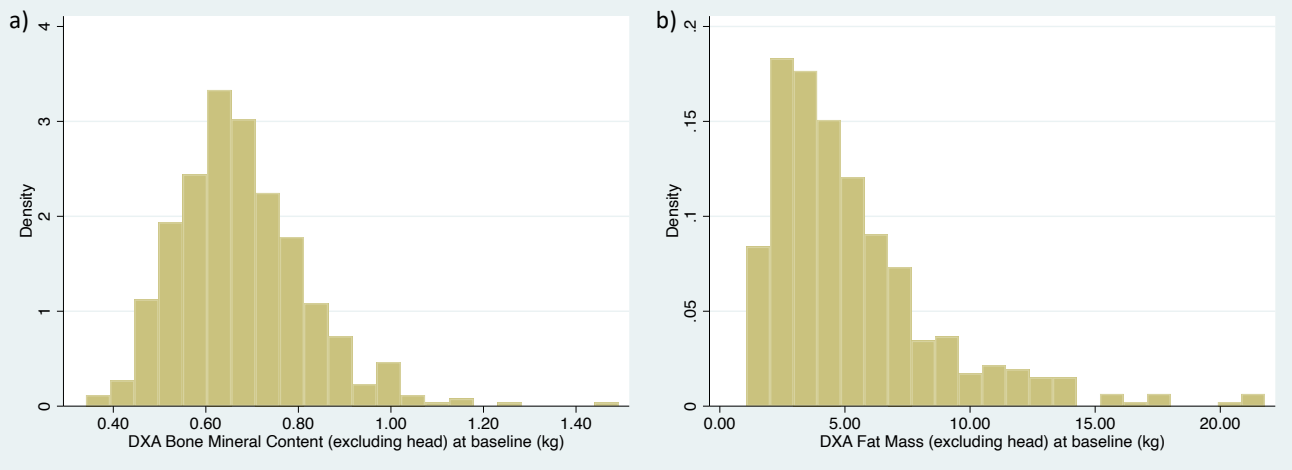

Figure 3.3 Examples of histograms used to visually check the assumption of normality. Example A shows total-body-less-head bone mineral content at baseline, where the assumption of normality was met. Example B shows total-body-less-head fat mass at baseline, where the assumption of normality was violated.

Linear regression was used in Chapters 4, 5, 6 and 7 to analyse the relationships between predictors and outcomes. Models were checked to meet the assumptions of normality, linearity, and homoscedasticity of residuals, and no multicollinearity. Normality was assessed with a histogram of residuals, a kernel density plot of residuals, a standardised normal probability-probability (P-P) plot and a quantile-quantile (Q-Q) plot (Figure 3.4) (UCLA: Statistical Consulting Group, 2021). For the histogram of residuals and the kernel density plot, deviation from the normal distribution bell curve indicates deviation from normality, and the for P-P and Q-Q plot, deviation from the 45 degree line indicates deviation from normality (UCLA: Statistical Consulting Group, 2021). However, it should be noted that in large sample sizes, where the number of observations per variable is > 10, the violation of the normality assumption does not noticeably impact regression model estimates (Schmidt and Finan, 2018).

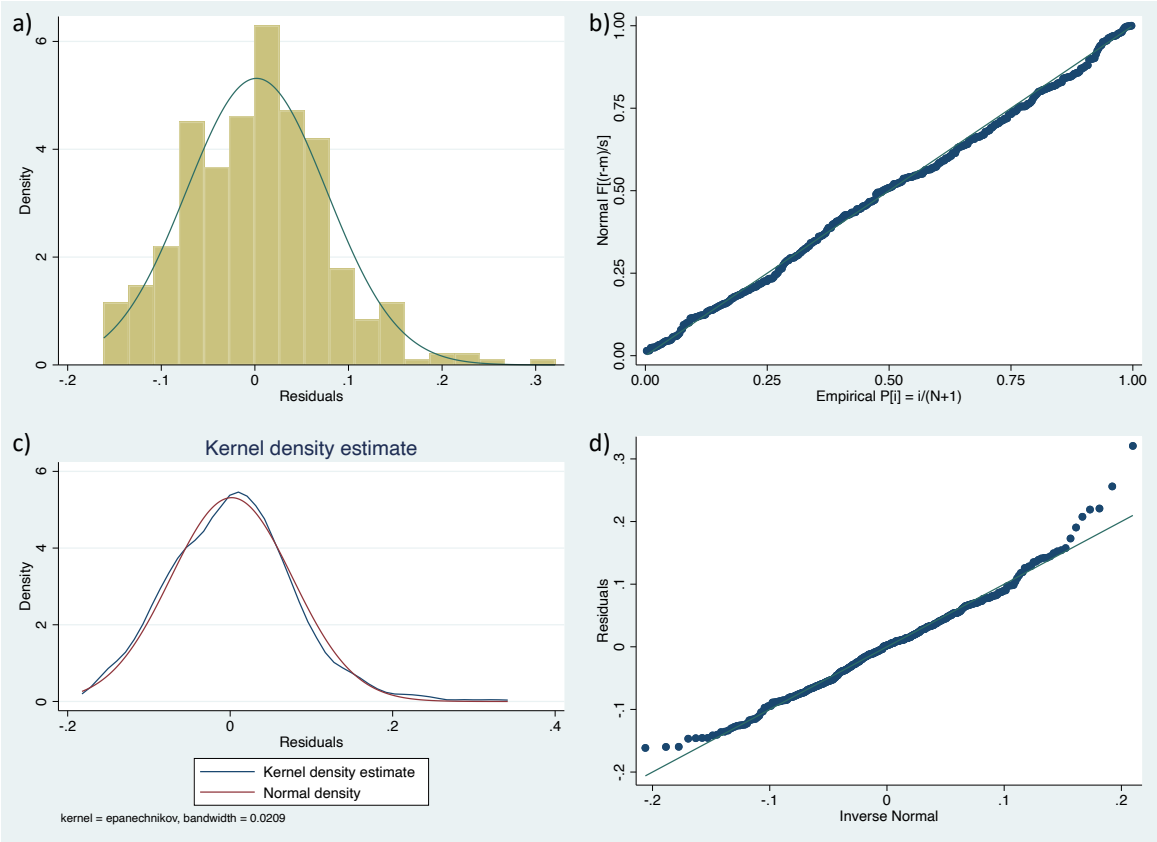

Figure 3.4 Examples of (a) histogram of residuals, (b) probability-probability (P-P) plot, (c) kernel density plot and (d), quantile-quantile (Q-Q) plot to check the assumption of normality of residuals.

Linearity and homoscedasticity of residuals were checked with a residuals versus fitted values plot (Figure 3.5) (Field, 2014). A curved relationship between the residuals and fitted values indicates non-linearity of residuals, and funnelling or fanning indicates the assumption of homoscedasticity is violated (Field, 2014).

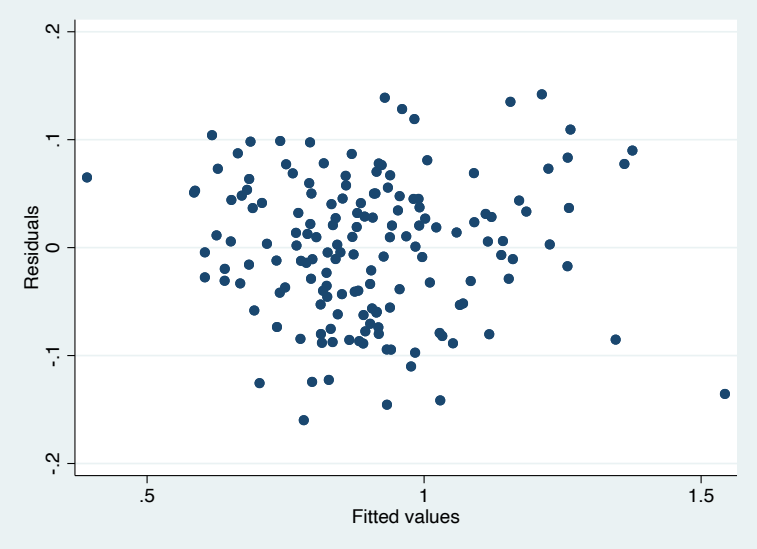

Figure 3.5 Example of a residuals versus fitted plot to check the assumption of linearity and homoscedasticity of residuals.

The variance inflation factor (VIF) was considered to test the assumption of no multicollinearity. If the largest VIF was greater than 10, and if the average VIF was substantially greater than 1, multicollinearity was considered problematic (Bowerman and O'Connell, 1990).

A detailed description of the specific statistical analyses used for each study is presented in Chapters 4 to 7.

Chapter 4 The Independent and Interactive Associations of Physical Activity Intensity and Vitamin D Status with Bone Mineral Density in Prepubertal Children

The following chapter has been published in full: Constable, A. M., Vlachopoulos, D., Barker, A. R., Moore, S. A., Soininen, S., Haapala, E. A., Väistö, J., Westgate, K., Brage, S., Mahonen, A., and Lakka, T. A. (2021) "The independent and interactive associations of physical activity intensity and vitamin D status with bone mineral density in prepubertal children: the PANIC Study." *Osteoporosis International.* https://doi.org/10.1007/s00198-021-05872-z

4.1 Abstract

Introduction: The sex-specific independent and interactive associations of PA intensity and serum 25(OH)D levels with aBMD were investigated in prepubertal children.

Methods: The participants were 366 prepubertal Finnish children (176 females, 190 males) aged 6 to 8 years. Linear regression analysed the associations of sedentary time, LPA, MPA, MVPA and VPA measured by accelerometery, and serum 25(OH)D with TBLH and lower-limb aBMD, measured by DXA.

Results: There was no interaction between PA intensity or serum 25(OH)D and sex with aBMD. MPA and MVPA were positively associated with TBLH and lower-limb aBMD (β = 0.11, 95% confidence interval (CI) 0.02-0.20, p = 0.01). Serum 25(OH)D was positively associated with TBLH and lower-limb aBMD (β = 0.09, 95% CI 0.01-0.18, p = 0.03). There were no interactions between PA intensity and serum 25(OH)D with aBMD.

Conclusion: Vitamin D status, MPA and MVPA levels in active prepubertal children were positively associated with aBMD. The influence of MVPA is due to the MPA component, though our findings regarding the role of VPA should be interpreted with caution, as shorter accelerometer epochs are needed to more accurately assess VPA. This study adds evidence to the promotion of MPA and behaviours to encourage optimal vitamin D status in supporting skeletal health in childhood, though these need not be used in conjunction to be beneficial, and a sex-specific approach is not necessary in prepubertal children.

4.2 Introduction

BMC and aBMD increase substantially during childhood and adolescence (Baxter-Jones *et al.*, 2011). In the four years surrounding the pubertal growth spurt, more than one quarter of adult bone is accrued (Baxter-Jones *et al.*, 2011). The timing of the pubertal growth spurt is sex-specific, with peak BMC velocity occurring around 8 months after PHV, on average at age 12.5 years in females, and at age 14.1 years in males, though there is considerable variation between individuals and populations (Bailey *et al.*, 1999). The pubertal period is a time of elevated fracture risk that is partly due to increases in linear length of long bones outpacing BMC and aBMD accrual over this period (Weaver *et al.*, 2016). Children who accrue more bone during prepuberty tend to have higher BMC and aBMD and a lower fracture risk in adolescence (Clark *et al.*, 2006). Further, the bone accrued during growth may track into adulthood, increasing PBM and therefore reducing the risk of osteoporosis in later life (Weaver *et al.*, 2016).

PA measured by accelerometery has been positively associated with aBMD in females and males aged 4 to 6 (Janz *et al.*, 2001) and 11 years (Tobias *et al.*, 2007) and aged 12.5 to 17.5 years (Gracia-Marco *et al.*, 2011). VPA, in addition to MVPA, has been positively associated with aBMD in adolescents (Gracia-Marco *et al.*, 2011). However, in children aged 11, MPA had a stronger influence on aBMD than LPA and VPA (Tobias *et al.*, 2007). As such, the individual associations of LPA, MPA, MVPA and VPA with aBMD are currently unclear. VPA was positively associated with hip aBMD but not with total body aBMD in females and males aged 4 to 6 (Janz *et al.*, 2001), suggesting that the associations of PA with aBMD may be more pronounced in weight-bearing bones. This is supported by athlete studies, which have observed greater aBMD in athletes who participate in impact loading sports compared to those who participate in active loading sports or healthy controls, suggesting mechanical loading is important for bone adaptation (Vlachopoulos *et al.*, 2017). Furthermore, previous studies have used samples of children at various pubertal stages (Tobias *et al.*, 2007; Gracia-Marco *et al.*, 2011). However, the associations of PA with aBMD may vary depending on age and pubertal status (MacKelvie, Khan and McKay, 2002), and it remains unclear whether the associations between PA with aBMD are sex-specific (Harvey *et al.*, 2012). It is therefore important to investigate the associations of PA intensity with total body and site-specific aBMD in prepubertal females and males.

Serum 25(OH)D has been associated positively with aBMD in cross-sectional studies among females and males aged 1.8 to 6 years (Hazell *et al.*, 2015) and 7 to 19 years (Pekkinen *et al.*, 2012). However, these studies did not address possible sex differences in these associations, so it is unknown whether the

relationships of serum 25(OH)D with aBMD differ between females and males. This requires further exploration, as serum 25(OH)D has been inversely associated with the rate of BMC accrual in prepubertal females (Breen *et al.*, 2011). Therefore, the relationships of serum 25(OH)D with aBMD in prepubertal females and males remain unclear.

In addition to their independent relationships with aBMD, it is also possible that PA and vitamin D interact to influence aBMD. One cross-sectional study found that adolescents meeting the PA guidelines (at least an average of 60 minutes MVPA per day) and having serum 25(OH)D ≥ 75 nmol/L had greater BMC than adolescents meeting the PA guidelines and having serum 25(OH)D < 75 nmol/L (Valtuena *et al.*, 2012). As male and female adolescents were not analysed separately in the previous cross-sectional study (Valtuena *et al.*, 2012), it remains unknown whether these associations differ depending on sex. This is of interest due to the sex-specific patterns of BMC and aBMD accrual during growth (Bailey *et al.*, 1999). It also remains unknown whether PA and serum 25(OH)D interact to influence aBMD in prepubertal children, as maturation independently affects aBMD (Baxter-Jones *et al.*, 2011).

Thus, the aim of this study was to investigate the sex-specific independent and interactive associations of PA intensity and serum 25(OH)D concentration on TBLH and lower-limb aBMD in prepubertal children.

4.3 Methods

4.3.1 Study Design and Participants

This study used cross-sectional baseline data of the PANIC Study, as described in Section 3.1. The children were included in these analyses if they had complete data for general health and pubertal status, anthropometric measures, DXA measurements, serum 25(OH)D, and PA measured by combined heart rate and movement sensor, and if they were prepubertal based on stages described by Tanner (Tanner, 1986). The children were excluded from the analyses if they currently or previously used oral corticosteroids, as this can independently affect aBMD (Weaver *et al.*, 2016). Altogether 366 prepubertal children (176 females, 190 males) were included in the analysis. The reduction in sample size from baseline examinations was largely accounted for by missing serum 25(OH)D data and invalid or missing PA data. Exclusion and inclusion criteria are displayed in Figure 4.1.

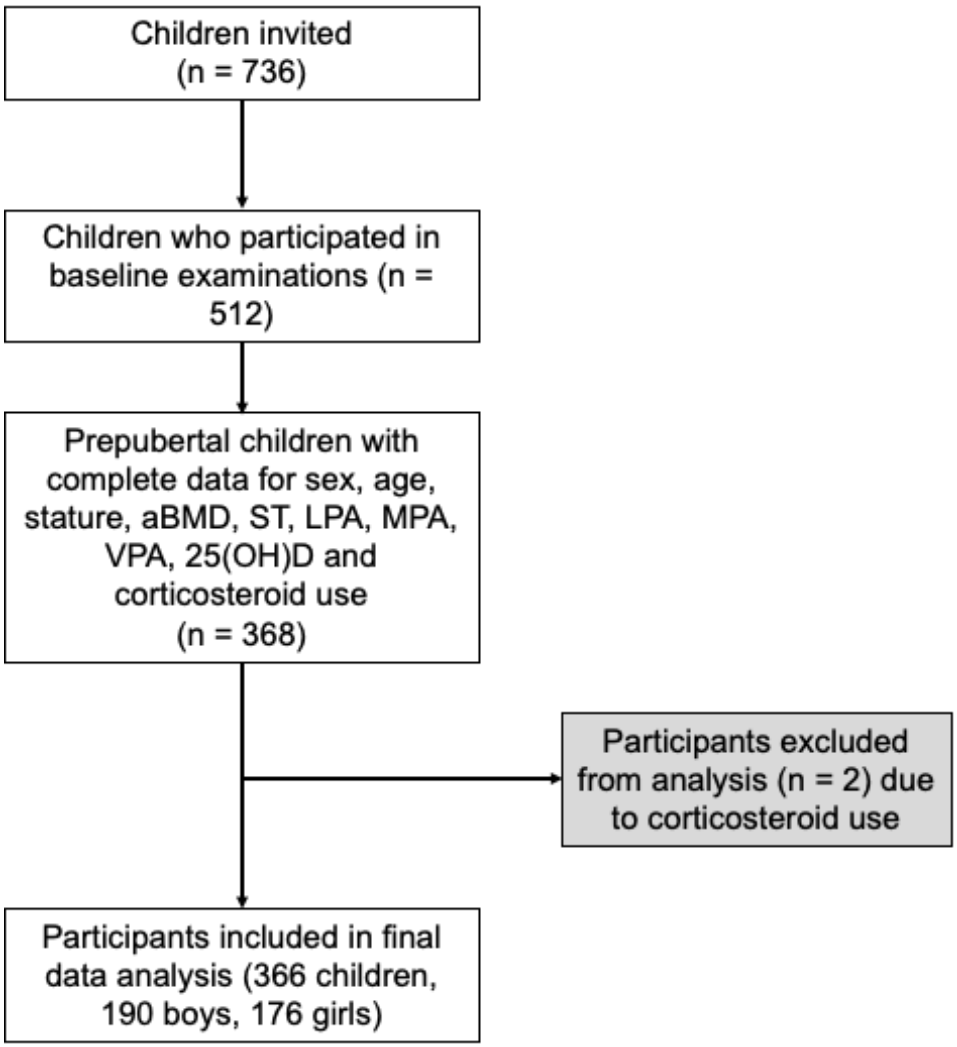

Figure 4.1 Participant flow chart.

aBMD, areal bone mineral density; ST, sedentary time; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; 25(OH)D, 25-hydroxyvitamin D.

4.3.2 Assessment of General Health and Pubertal Status

General health and pubertal status were assessed as described in Section 3.3 and Section 3.4.

4.3.3 Anthropometry

Stature and body weight were assessed as described in Section 3.5. BMI (kg/m^2) was calculated, and the BMI cut-offs of the IOTF were applied to

classify children as having normal weight, overweight, or obese weight status (Cole *et al.*, 2000; Cole *et al.*, 2007).

4.3.4 Assessment of Bone Mineral Density

TBLH and lower-limb aBMD (g/cm^2), and TBLH and lower-limb BMC (g) were measured as described in Section 3.6. The primary outcome variable for the present study was TBLH aBMD, as recommended by the International Society for Clinical Densitometry (International Society for Clinical Densitometry, 2019b). Lower-limb aBMD derived from the whole body DXA scan were also measured, as evidence indicates that the influence of PA on aBMD may be site-specific (Janz *et al.*, 2001). We included measurements of BMC to check whether the associations were broadly similar between PA and serum 25(OH)D with aBMD and BMC.

4.3.5 Assessment of Physical Activity and Sedentary Time

PA was assessed using Actiheart (CamNtech Ltd, Papworth, UK), as described in Section 3.7.1. For the present analyses, the uniaxial acceleration signal was used as the PA exposure, as it has been suggested that mechanical loading has a stronger influence on bone than metabolic loading (Janz *et al.*, 2003; Harvey *et al.*, 2012). The acceleration signal was summarised as a fraction of time spent in 25 acceleration thresholds (m/s^2) (0.075, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 6, 7, 8, 9) across the movement intensity continuum. These are the standard thresholds used in the Actiheart data processing pipeline, as the thresholds provide greater resolution at the low end of the intensity spectrum, where the data is denser, and less resolution at the high end of the intensity spectrum, where the data is sparser.

Data were summarised into intensity bands. Sedentary time was defined as ≤ 0.06 m/s^2, LPA was defined as > 0.06 m/s^2 and ≤ 0.75 m/s^2, MPA was defined as > 0.75 m/s^2 and ≤ 4.00 m/s^2, and VPA was defined as > 4.00 m/s^2 to categorise the data into the fractions of time spent in each intensity. The threshold for sedentary time is based on the threshold of ≤ 100 counts per minute validated and used in previous studies (Ekelund *et al.*, 2012; Ridgers *et al.*, 2012; Atkin *et al.*, 2013). Average sleep duration, described in Section 3.7.1.4, was subtracted from sedentary time to give sedentary time excluding sleep. The thresholds for PA are based on previous research which assessed uniaxial acceleration during treadmill walking and running at different speeds in children, measured at the trunk (Corder *et al.*, 2005) and the hip (Janz *et al.*, 2003). Walking at 3.2 to 5.2 km/hour was related with acceleration in the range of 0.75 to 1.5 m/s^2, and running at 8 to 12 km/hour was related with acceleration in the range of 4 to 6 m/s^2 (Janz *et al.*, 2003; Corder *et al.*, 2005). As the study by Janz *et al.* (2003) used Actigraph accelerometers, Actigraph counts were converted to Actiheart counts by dividing by 5, and then converted to m/s^2, as the International System of Units, by multiplying by 0.003 (Brage *et al.*, 2005; Harvey *et al.*, 2012).

The combined sensing data were used to characterise the proportion of children meeting the PA guidelines of at least an average of 60 minutes MVPA per day, as described in Section 3.7.1 (Tremblay, Carson and Chaput, 2016).

4.3.6 Serum 25(OH)D Assessment

Serum 25(OH)D concentration was assessed as described in Section 3.8. We applied cut-offs to the data based on thresholds for deficiency (< 25 nmol/L), insufficiency (25 nmol/L – 49.9 nmol/L) and sufficiency (> 50 nmol/L) described by the British Paediatric and Adolescent Bone Group and the Institute of Medicine (Institute of Medicine, 2011; Arundel *et al.*, 2012). As the threshold for sufficiency varies from > 50 nmol/L to > 75 nmol/L, we applied thresholds for 50 nmol/L – 74.9 nmol/ L, and ≥ 75 nmol/L (Holick *et al.*, 2011; Institute of Medicine, 2011).

4.3.7 Statistical Analysis

The IBM SPSS Statistics for Mac software, Version 26.0 (IBM Corp.), was used for the statistical analyses. Differences in age, stature, body weight, BMI, TBLH aBMD, lower-limb aBMD, sedentary time, LPA, MPA, MVPA, VPA and serum 25(OH)D between the included and excluded children were tested. Body weight, BMI, VPA and serum 25(OH)D had skewed distributions, whereas other continuous variables were normally distributed.

For normally distributed variables, the mean and SD were calculated for the total sample, and for females and males separately. Independent samples t-tests were used to test for sex differences. For skewed variables, the median and IQR were calculated for the total sample, and for females and males separately. Mann-Whitney U tests were used to test for sex differences. The Fisher's exact test was used to test for differences in categorical variables between sexes.

Linear regression was used to assess the associations of sedentary time, LPA, MPA, MVPA, VPA and serum 25(OH)D with TBLH aBMD and lower-limb aBMD. Interaction terms for sex with each PA intensity and serum 25(OH)D were computed from standardised values (Z-scores) to assess whether the association between PA, serum 25(OH)D and bone outcomes differed based on sex. These interaction terms were not significant, so all further analysis was carried out in the total sample. Analysis was also carried out split by sex, due to the sex differences in aBMD in our sample, and the sex-specific patterns of bone accrual during growth (Baxter-Jones *et al.*, 2011). The linear interaction of PA and serum 25(OH)D on TBLH aBMD and lower-limb aBMD was explored. For the total sample analyses, the predictor variable was entered with adjustment for age, stature, and sex. For testing the interactions, the main effects and the interaction term were entered with adjustment for age, stature, and sex. For the analyses stratified by sex, the same process was followed, though sex was not included as a covariate. Analyses were repeated with TBLH and lower-limb BMC as the outcome variables. Models were confirmed to meet assumptions of normality, linearity, and homoscedasticity of residuals. In all models, the VIF was < 5, and the average VIF was not considerably greater than 1, indicating that the model was robust (Alin, 2010). Standardised regression coefficients (ß) with 95% CI were reported and statistical significance was set at alpha level 0.05.

4.4 Results

4.4.1 Characteristics of Children

Descriptive characteristics of the 366 prepubertal children included in this study are provided in Table 4.1. These did not differ significantly from the children excluded from this study for age, stature, body weight, BMI status, TBLH aBMD, lower-limb aBMD, TBLH BMC, lower-limb BMC, sedentary time, LPA, MPA, MVPA, VPA and serum 25(OH)D. For the participants included in the study, stature, body weight, TBLH aBMD, TBLH BMC, lower-limb BMC, MPA and MVPA were greater in males than females, and LPA was lower in males than females. The proportion of children meeting the PA guidelines and the proportion of children in categories for serum 25(OH)D levels differed between females and males, though the mean levels of serum 25(OH)D did not differ between females and males.

Table 4.1 Descriptive characteristics of children

	Total		**Females**		**Males**		**P value for sex difference**
	n = 366		n = 176		n = 190		
	Mean / Median	SD / IQR	Mean / Median	SD / IQR	Mean / Median	SD / IQR	
Age (years)	7.7	0.4	7.6	0.4	7.7	0.4	0.083
Stature (cm)	128.8	5.5	127.7	5.4	129.9	5.4	**< 0.001**
Weight (kg)	26.0	23.6 – 29.0	25.2	23.1 – 28.7	26.9	24.1 – 29.9	**0.005**
BMI (kg/m^2)	15.6	14.7 – 16.0	15.6	14.6 – 16.8	15.8	14.7 – 17.1	0.251
IOTF Definition							
% (cases) normal weight	88.8 (325)		88.9 (169)		88.6 (156)		
% (cases) overweight	8.2 (30)		7.9 (15)		8.5 (15)		
% (cases) obese	3.0 (11)		3.2 (6)		2.9 (5)		
TBLH BMC (g)	675.2	130.2	649.5	125.2	699.0	130.6	**< 0.001**
TBLH aBMD (g/cm^2)	0.721	0.046	0.715	0.045	0.726	0.046	**0.019**
Lower-limb BMC (g)	318.9	65.3	310.7	64.2	326.6	65.5	**0.020**
Lower-limb aBMD (g/cm^2)	0.809	0.063	0.804	0.062	0.813	0.063	0.203
Proportion of children meeting MVPA guidelines (> 4 METs)							
% (cases) < 60 mins/day	20.5 (75)		15.8 (30)		25.6 (45)		
% (cases) ≥ 60 mins/day	79.5 (291)		84.2 (160)		74.4 (131)		
Sedentary time (minutes per day spent ≤ 0.06 m/s^2)	177	67	177	61	177	73	0.913
LPA (minutes per day spent > 0.06 m/s^2 and ≤ 0.75 m/s^2)	553	47	561	42	545	51	**0.002**
MPA (minutes per day spent > 0.75 m/s^2 and ≤ 4.00 m/s^2)	128	42	118	36	136	45	**< 0.001**
VPA (minutes per day spent > 4.00 m/s^2)	2	1 – 5	2	1 – 5	2	1 – 4	0.059
MVPA (minutes per day spent > 0.75 m/s^2)	131	43	122	37	139	47	**< 0.001**
25(OH)D (nmol/L)	65.7	52.3 – 79.1	63.7	52.3 – 74.9	67.4	52.2 – 83.4	0.081
25(OH)D thresholds							
% (cases) < 25 nmol/L	0.8 (3)		0.5 (1)		1.1 (2)		
% (cases) 25 nmol/L – 49.9 nmol/L	19.1 (70)		19.5 (37)		18.8 (33)		
% (cases) 50 nmol/L – 74.9 nmol/L	48.4 (177)		41.6 (79)		55.7 (98)		
% (cases) ≥ 75 nmol/L	31.7 (116)		38.4 (73)		24.4 (43)		

BMI, body mass index; IOTF, International Obesity Task Force; BMC, bone mineral content; aBMD, areal bone mineral density; METs, metabolic equivalents; MVPA, moderate-to-vigorous physical activity; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; 25(OH)D, serum 25-hydroxyvitamin D. Bold emphasis indicates statistical significance at $p < 0.05$.

4.4.2 Associations of PA Variables and Serum 25(OH)D with aBMD in Total Sample

Results from the regression analyses in the total sample are provided in Table 4.2. MPA and MVPA were positively associated with TBLH aBMD and lower-limb aBMD, adjusted for age, sex and stature. A 10-minute difference in either MPA or MVPA was associated with a 0.001 g/cm^2 higher TBLH aBMD and 0.002 g/cm^2 higher lower-limb aBMD. Serum 25(OH)D was positively associated with TBLH and lower-limb aBMD, adjusted for age, sex and stature. A 10 nmol/L difference in serum 25(OH)D was associated with 0.002 g/cm^2 higher TBLH aBMD and 0.003 g/cm^2 higher lower-limb aBMD. There were no interactions between PA variables and serum 25(OH)D with TBLH aBMD and lower-limb aBMD.

4.4.3 Associations of PA Variables and Serum 25(OH)D with aBMD in Females

Results from the regression analysis in females are provided in Table 4.3. MPA and MVPA were positively associated with TBLH and lower-limb aBMD, adjusted for age and stature. A 10-minute difference in either MPA or MVPA was associated with a 0.002 g/cm^2 higher TBLH and lower-limb aBMD. There were no associations between serum 25(OH)D with TBLH aBMD and lower-limb aBMD. There were no interactions between PA variables and serum 25(OH)D with TBLH and lower-limb aBMD.

Table 4.2. Determinants of aBMD in females and males aged 6 to 8 years

	TBLH aBMD		**Lower-limb aBMD**	
	β (95% CI)	*p*	**β (95% CI)**	*p*
Sedentary time[a]	-0.053 (-0.138 to 0.032)	0.221	-0.066 (-0.151 to 0.019)	0.126
LPA[a]	-0.042 (-0.129 to 0.044)	0.336	-0.018 (-0.105 to 0.068)	0.680
MPA[a]	**0.113 (0.026 to 0.199)**	**0.011**	**0.11 (0.023 to 0.196)**	**0.013**
VPA[a]	0.074 (-0.011 to 0.159)	0.089	0.075 (-0.011 to 0.16)	0.086
MVPA[a]	**0.115 (0.029 to 0.201)**	**0.009**	**0.112 (0.025 to 0.198)**	**0.011**
25(OH)D[a]	**0.093 (0.007 to 0.178)**	**0.034**	**0.094 (0.008 to 0.18)**	**0.032**
Sedentary time*25(OH)D[b]	0.03 (-0.057 to 0.116)	0.499	0.033 (-0.053 to 0.119)	0.449
LPA*25(OH)D[b]	-0.006 (-0.094 to 0.081)	0.884	-0.016 (-0.104 to 0.072)	0.714
MPA*25(OH)D[b]	-0.003 (-0.089 to 0.082)	0.937	0.005 (-0.081 to 0.09)	0.917
VPA*25(OH)D[b]	-0.075 (-0.16 to 0.011)	0.087	-0.065 (-0.151 to 0.021)	0.138
MVPA*25(OH)D[b]	-0.008 (-0.094 to 0.077)	0.850	0 (-0.085 to 0.086)	0.997

The values are standardised regression coefficients (β), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models. Bold emphasis indicates statistical significance at $p < 0.05$.

[a] adjustment for age, sex and stature.

[b] adjustment for age, sex, stature and the main effects of PA intensity and 25(OH)D.

TBLH, total-body-less-head; aBMD, areal bone mineral density; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity; 25(OH)D, serum 25-hydroxyvitamin D.

Table 4.3. Determinants of aBMD in females aged 6 to 8 years

	TBLH aBMD		**Lower-limb aBMD**	
	β (95% CI)	*p*	**β (95% CI)**	*p*
Sedentary time[a]	-0.104 (-0.22 to 0.017)	0.093	-0.106 (-0.225 to 0.014)	0.083
LPA[a]	0.033 (-0.089 to 0.154)	0.600	0.044 (-0.077 to 0.165)	0.477
MPA[a]	**0.141 (0.019 to 0.264)**	**0.024**	**0.132 (0.01 to 0.253)**	**0.034**
VPA[a]	0.067 (-0.056 to 0.191)	0.282	0.061 (-0.062 to 0.183)	0.328
MVPA[a]	**0.142 (0.019 to 0.264)**	**0.023**	**0.132 (0.01 to 0.254)**	**0.034**
25(OH)D[a]	0.053 (-0.07 to 0.176)	0.394	0.029 (-0.093 to 0.151)	0.634
Sedentary time*25(OH)D[b]	0.089 (-0.032 to 0.207)	0.151	0.081 (-0.04 to 0.202)	0.189
LPA*25(OH)D[b]	-0.076 (-0.203 to 0.051)	0.238	-0.076 (-0.201 to 0.05)	0.237
MPA*25(OH)D[b]	-0.05 (-0.172 to 0.073)	0.424	-0.034 (-0.156 to 0.088)	0.580
VPA*25(OH)D[b]	-0.093 (-0.217 to 0.03)	0.138	-0.076 (-0.199 to 0.047)	0.225
MVPA*25(OH)D[b]	-0.055 (-0.177 to 0.067)	0.374	-0.039 (-0.161 to 0.083)	0.527

The values are standardised regression coefficients (β), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models. Bold emphasis indicates statistical significance at $p < 0.05$.

[a] adjustment for age and stature.

[b] adjustment for age, stature and the main effects of PA intensity and 25(OH)D.

TBLH, total-body-less-head; aBMD, areal bone mineral density; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity; 25(OH)D, serum 25-hydroxyvitamin D.

4.4.4 Associations of PA Variables and Serum 25(OH)D with aBMD in Males

Results from the regression analysis in males are provided in Table 4.4. Serum 25(OH)D was positively associated with lower-limb aBMD, adjusted for age and stature. A 10 nmol/L difference in serum 25(OH)D was associated with 0.003 g/cm^2 higher lower-limb aBMD. There were no associations between any PA variable with TBLH aBMD and lower-limb aBMD. There were no interactions between PA variables and serum 25(OH)D with TBLH aBMD and lower-limb aBMD.

We repeated all analyses with TBLH and lower-limb BMC as the outcome variables, and found the associations were broadly similar to those with TBLH and lower-limb aBMD as the outcome variable, with the exception of serum 25(OH)D, which was not associated with any measures of BMC.

Table 4.4. Determinants of aBMD in males aged 6 to 8 years

	TBLH aBMD		**Lower-limb aBMD**	
	β (95% CI)	*p*	**β (95% CI)**	*p*
Sedentary time[a]	-0.015 (-0.138 to 0.109)	0.814	-0.034 (-0.157 to 0.088)	0.582
LPA[a]	-0.098 (-0.219 to 0.024)	0.115	-0.065 (-0.187 to 0.057)	0.294
MPA[a]	0.09 (-0.031 to 0.212)	0.145	0.088 (-0.033 to 0.21)	0.154
VPA[a]	0.078 (-0.044 to 0.2)	0.209	0.083 (-0.039 to 0.204)	0.182
MVPA[a]	0.093 (-0.028 to 0.214)	0.132	0.092 (-0.03 to 0.213)	0.138
25(OH)D[a]	0.121 (-0.001 to 0.242)	0.051	**0.139 (0.019 to 0.26)**	**0.024**
Sedentary time*25(OH)D[b]	-0.016 (-0.141 to 0.109)	0.800	-0.001 (-0.125 to 0.123)	0.991
LPA*25(OH)D[b]	0.067 (-0.058 to 0.193)	0.293	0.043 (-0.083 to 0.168)	0.504
MPA*25(OH)D[b]	0.044 (-0.079 to 0.166)	0.482	0.048 (-0.074 to 0.17)	0.436
VPA*25(OH)D[b]	-0.053 (-0.181 to 0.075)	0.419	-0.043 (-0.17 to 0.085)	0.509
MVPA*25(OH)D[b]	0.04 (-0.083 to 0.162)	0.525	0.044 (-0.077 to 0.166)	0.472

The values are standardised regression coefficients (β), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models. Bold emphasis indicates statistical significance at $p < 0.05$.

[a] adjustment for age and stature.

[b] adjustment for age, stature and the main effects of PA intensity and 25(OH)D.

TBLH, total-body-less-head; aBMD, areal bone mineral density; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity; 25(OH)D, serum 25-hydroxyvitamin D.

4.5 Discussion

This study is the first to address the sex-specific independent and interactive associations of accelerometer measured PA intensity and serum 25(OH)D with aBMD in prepubertal children. Positive associations of serum 25(OH)D, MPA and MVPA with TBLH aBMD and lower-limb aBMD were observed in the total sample, and these associations did not differ between sexes. The magnitude of the association was similar for serum 25(OH)D, MPA and MVPA, though serum 25(OH)D did not interact with PA intensity to determine aBMD. Our findings imply that these are independent determinants of aBMD in prepubertal children.

Reference values from the BMDCS study indicate that TBLH aBMD in the 50th percentile of non-Black children aged seven were 0.61 g/cm^2 for females and 0.58 g/cm^2 for males (Zemel *et al.*, 2011). After applying cross-calibration equations to allow comparison between DXA manufactures, the median levels of TBLH aBMD in our study were similar to the reference values, with 0.59 g/cm^2 for females, and 0.60 g/cm^2 for males (Shepherd *et al.*, 2012). Altogether 79.5% of our sample met the recommended average 60 minutes of MVPA per day, based on the combined sensing data (Tremblay, Carson and Chaput, 2016). The International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE) found that the average percentage of children aged 9 to 11 years from 12 countries meeting the PA guidelines was considerably lower than this (44%) (Roman-Vinas *et al.*, 2016). It is difficult to draw comparisons between studies, as the prevalence of children meeting the PA guidelines varies dependent on the PA intensity thresholds used (Migueles *et al.*, 2019). Even so, it is likely that our sample were more active than the ISCOLE sample

in Finland, with 61% of children assessed meeting the PA guidelines compared to the 79.5% in our sample (Roman-Vinas *et al.*, 2016).

The threshold for vitamin D deficiency varies from 25 to 50 nmol/L, and a review of evidence from the Institute of Medicine suggests that there are risks to bone health in children at serum 25(OH)D concentration < 30 nmol/L (Holick *et al.*, 2011; Institute of Medicine, 2011; Lamberg-Allardt *et al.*, 2013). Although living in a northern country is considered a risk factor for vitamin D deficiency (Lamberg-Allardt *et al.*, 2013), only < 1% of our sample had serum 25(OH)D below 25 nmol/L, with 21% below 50 nmol/L. Previous research in this sample showed that vitamin D intake was higher than in other European countries, which may explain the relatively high levels of 25(OH)D in this study (Soininen *et al.*, 2016). The majority of milk products as well as margarines available in Finland are fortified with vitamin D, and consumption of milk products was the main determinant of serum 25(OH)D in our study population (Soininen *et al.*, 2016). At the time of our data collection, vitamin D supplement use was recommended for this age group from October to March for children who did not use fortified milk products (Soininen *et al.*, 2016). Vitamin D intake from supplements was low: about 40% of the children did not use supplements at all, and many of those who used supplements did not use them regularly (Soininen *et al.*, 2016). The recommendations for both vitamin D intake from food and supplements and the level of vitamin D fortification in milk products and margarines in Finland has now been increased, and a wider range of other fortified products are now available (National Nutrition Council, 2010; National Nutrition Council, 2014).

4.5.1 Physical Activity Intensity and aBMD

The positive associations of PA with aBMD we found in the total sample and in females are in agreement with the results of previous population studies in children and adolescents (Janz *et al.*, 2001; Tobias *et al.*, 2007; Harvey *et al.*, 2012; Elhakeem *et al.*, 2020). MPA and MVPA were positively associated with TBLH and lower-limb aBMD in the total sample and in females. The ALSPAC study reported that MVPA was positively associated with TBLH aBMD and lower-limb aBMD in children aged 11 (10% females and 40% males prepubertal), and MPA was shown to have a stronger influence on TBLH aBMD and lower-limb aBMD than LPA and VPA (Tobias *et al.*, 2007). Similarly, the positive association we observed between MVPA with aBMD was due to the MPA component, highlighting the importance of MPA compared to VPA for aBMD. Findings from the Southampton Women's Survey (SWS) study showed no associations between MVPA and total body bone indices in children aged four, though MVPA was positively related to hip BMC and aBMD in females and males (Harvey *et al.*, 2012). These differences could be due to heterogeneity in the age and pubertal status of the participants in these studies. In younger children, such as those in the SWS study, the smaller body size and the lower BMC may limit the ability to detect a relationship between MVPA and total body bone indices (Harvey *et al.*, 2012). The importance of MVPA has also been demonstrated longitudinally from age 12 to 25 years by the ALSPAC study (Elhakeem *et al.*, 2020). Greater levels of MVPA in early- or mid-adolescence associated with greater femoral neck aBMD in early adulthood, though as the independent roles of MPA and VPA were not examined and TBLH measures were not included direct comparisons cannot be drawn (Elhakeem *et al.*, 2020).

Even so, our findings support those of the ALSPAC study, highlighting the importance of MVPA for bone health.

As it is mainly mechanical loading which is thought to increase aBMD (Weaver *et al.*, 2016), we expected that VPA, resulting in increased mechanical loads on the bone, would have a stronger positive association with aBMD than MPA. The method of capturing PA may have influenced our findings (Tobias *et al.*, 2007). Although all but 15 children recorded some VPA (data not presented), as we used a 60-second epoch the ability to detect short bursts of VPA is reduced (Baquet *et al.*, 2007), leading to a potential misclassification of VPA as MPA. This could affect our findings, by overestimating the association between MPA with aBMD and underestimating the association between VPA with aBMD. There are also differences in the methods used to process accelerometer data between studies. Whereas we justified our PA intensity thresholds on the basis of acceleration, as did the SWS, the ALSPAC study justified PA intensity thresholds on the basis of METs (Tobias *et al.*, 2007; Harvey *et al.*, 2012). Theoretically, using the acceleration data may strengthen the relationships of PA measures with aBMD, as mechanical loading may be more important for bone health than metabolic energy expenditure (Janz *et al.*, 2003). However, as bouts of VPA typically last less than 10 seconds in children (Baquet *et al.*, 2007), the 60-second epoch may limit our ability to capture the high-intensity mechanical loading which is likely important for aBMD (Janz *et al.*, 2003). Furthermore, GRFs generated during jumping, which are thought to have an osteogenic effect, were not associated with accelerometer counts in children (Janz *et al.*, 2003). This may further weaken the observed association between VPA and aBMD. This may also explain why the associations between lower-

limb aBMD and TBLH aBMD with MPA and MVPA were of equal magnitude. Although we expected to observe stronger associations between PA intensity and aBMD in weight-bearing bones, we were likely limited in our ability to capture the high-intensity mechanical loading which may have site-specific effects (Janz *et al.*, 2003). This may have resulted in the associations between PA and lower-limb aBMD being underestimated.

Although we did not find significant associations between MPA and MVPA with aBMD in males only, the associations followed the same direction and a similar magnitude to those in the total sample and in females only. Our ability to detect these associations in males is likely limited by our reduced statistical power when we stratified our analyses by sex. Previous studies have found the associations between PA intensity and aBMD to be similar between females and males (Janz *et al.*, 2001; Tobias *et al.*, 2007). In children aged 11 years the associations between PA variables and TBLH and lower-limb aBMD were similarly positive between females and males, with no interaction between PA intensity and sex with aBMD (Tobias *et al.*, 2007). Further, although a formal sex-by-activity interaction was not tested, the IBDS reported positive associations between total PA and VPA with total body and site-specific BMC and aBMD in females and males (Janz *et al.*, 2001). It is therefore unlikely that our findings reflect sex differences in the associations between PA intensity and aBMD. Our findings indicate that PA intensity, specifically MPA, is important to bone health in prepubertal children. However, given the aforementioned methodological limitations, it is possible that the influence of VPA was underestimated. Further research with assessment of PA using shorter epochs

and with comparison of acceleration-only thresholds with thresholds based on METs is needed to quantify the associations of VPA with aBMD.

4.5.2 Vitamin D Status and aBMD

We found a positive association between serum 25(OH)D and TBLH and lower-limb aBMD in the total sample, and between serum 25(OH)D and lower-limb aBMD in males only. Previous research has also reported positive associations between serum 25(OH)D and whole body aBMD in females and males aged 7 to 19 years (Pekkinen *et al.*, 2012), though these analyses were not stratified by sex, so direct comparisons cannot be drawn. Even so, as the associations between serum 25(OH)D with TBLH aBMD and lower-limb aBMD are a similar direction and magnitude in females and males, it is likely that the smaller sample size when we stratified by sex may have affected our ability to detect an association in females only. This is supported by experimental data, with meta-analyses indicating that sex did not modify the effect of vitamin D supplementation on bone health in children and adolescents age 10 to 17 years (Winzenberg *et al.*, 2011).

Our findings should be considered in context of the relatively low prevalence of vitamin D deficiency in our sample. In the aforementioned meta-analysis, the authors suggested that the positive influence of vitamin D on bone is less likely to occur in children with sufficient serum 25(OH)D (Winzenberg *et al.*, 2011; Weaver *et al.*, 2016). Therefore, it is possible that stronger positive associations would be observed in a sample with greater prevalence of vitamin D deficiency.

4.5.3 Physical Activity Intensity, Vitamin D Status and aBMD

We did not observe an interaction between PA intensity and serum 25(OH)D with TBLH and lower-limb aBMD in our prepubertal sample, in the total sample or in females and males separately. However, in adolescents, MVPA and serum 25(OH)D interact to influence BMC (Valtuena *et al.*, 2012). In physically active adolescents, high levels of serum 25(OH)D were positively associated with BMC. This association was not present in physically inactive adolescents in the same study. The differences in observed interactions may be due to the differences in pubertal status of the samples, differences in the methods of processing the accelerometery data and differences in baseline PA levels between the studies. Further longitudinal research would be valuable in assessing how these associations change throughout puberty.

4.5.4 Translation of Findings

Despite positive associations between serum 25(OH)D, MPA and MVPA with TBLH and lower-limb aBMD, the translation of the regression model estimates into a more meaningful metric requires consideration. We found an increase of MPA or MVPA by 10 minutes per day, as indicated by our study, was associated with a change in TBLH aBMD that equates to 2 to 3% of the annual change in TBLH aBMD in children aged 7 (Kalkwarf *et al.*, 2007). However, given the limitations with the 60-second epoch, discussed above and expanded on in Section 4.5.5, our study cannot provide an accurate estimate of PA. Therefore, the translation of these findings is to provide an idea of the effect size, rather than to make specific recommendations regarding the amounts of PA associated with a change in aBMD. Our study indicates a 10 nmol/L increase in serum 25(OH)D equates to 5 to 6% of the annual change in TBLH

aBMD in children aged 7 (Kalkwarf *et al.*, 2007). However, there are clearly other factors of more importance in determining aBMD in prepubertal children, such as lean mass and fat mass (Soininen *et al.*, 2018), and longitudinal research is needed to examine how these associations track into adulthood.

4.5.5 Strengths and Limitations

Strengths of this study include the population-based sample of children, the analysis of females and males separately, the objective measurement of vitamin D status from blood samples and the measurement of bone outcomes, including the lower-limbs, by DXA.

There are several limitations that should be taken into account when interpreting the results. The assessment and interpretation of aBMD measurements are not simple in growing children. The determinants of vBMD remain unclear, as it is not possible to obtain true measures for it from DXA. Although aBMD is a partly body size-corrected measure for vBMD, it may underestimate the vBMD of short children and overestimate the vBMD of tall children. Therefore, the International Society for Clinical Densitometry recommends adjusting TBLH aBMD using height z-score (International Society for Clinical Densitometry, 2019b). We used TBLH aBMD measured by DXA, as recommended by the International Society for Clinical Densitometry, and additionally lower-limb values which are less commonly used in children (International Society for Clinical Densitometry, 2019b). To account for body size, we adjusted the data for age, sex and stature, which are all components of height z-score.

As previously discussed, we used the 60-second epoch to capture PA. As children's MPA and VPA commonly occurs in short bouts (< 10 seconds), both of them may have been underestimated, possibly resulting in the attenuation of the associations of MPA and VPA with aBMD (Baquet *et al.*, 2007). Further to the epoch length, the dynamic range of the Actiheart (±2.5 *g*), may lead to an underestimation of high-intensity activity. Peak acceleration during everyday PA, such as running and jumping, may exceed 5 *g* at the hip and the wrist (Rowlands and Stiles, 2012). As the peak acceleration of these activities exceeds the dynamic range of the Actiheart, high-intensity activities, which may be meaningful for bone outcomes, could be missed. Although stature and age were controlled for, as in previous studies (Janz *et al.*, 2001; Tobias *et al.*, 2007; Valtuena *et al.*, 2012), residual confounding remains a potential limitation in all observational studies. Future research should use shorter epochs for investigating the relationships of the whole PA intensity spectrum with aBMD. The cross-sectional study design means that evidence for causal relationships cannot be provided and there is also a possibility of bidirectional relationships.

Although children had not participated in the intervention before collecting the baseline data, the reasons for not participating in the study were not asked, and a possibility of selection bias exists whereby more motivated families interested in health issues may be more eager to participate in this kind of study. This may be one of the reasons why the children in our study sample were more active than other cohorts in Finland and in other European countries (Roman-Vinas *et al.*, 2016). However, our sample did not differ in age, sex distribution, or BMI-SDS from all children who started the first grade in the city of Kuopio in 2007 to 2009 based on data from the standard school health examinations, indicating

our sample were representative of children of the same age in Kuopio, Finland. Even so, as our sample were more active and had lower levels of vitamin D deficiency than other cohorts both in Finland and in other European countries, it is unknown how these observations extend to children with low levels of PA and deficient vitamin D status (Roman-Vinas *et al.*, 2016; Soininen *et al.*, 2016). This offers a potential direction for future research. As there are clearly other factors of more importance in determining aBMD in this sample, further research considering the mediating role of hormonal factors and body composition may extend the understanding of determinants of aBMD in prepubertal children (Soininen *et al.*, 2018). Investigating these associations longitudinally would be valuable in understanding the role of maturation in these relationships and exploring whether these associations track beyond prepuberty.

4.6 Conclusion

In conclusion, the results of our study indicated that serum 25(OH)D, MPA and MVPA were positively associated with aBMD in active prepubertal children with sufficient vitamin D status, though these factors did not interact with each other, and these associations were not dependent on sex. The positive association between MVPA with aBMD was due to the MPA component, though as we were limited in our ability to capture short duration PA, it is possible that the importance of VPA was underestimated. Further research with shorter accelerometer epochs is needed to explore the associations of VPA with aBMD in prepubertal children. This study adds evidence to the promotion of MPA and behaviours to encourage optimal vitamin D status in supporting skeletal health in childhood, though our findings indicate that these strategies do not need to

be used in conjunction to be beneficial, and a sex-specific approach is not needed in prepubertal children.

Chapter 5 The Mediating Role of Endocrine Factors in the Positive Relationship Between Fat Mass and Bone Mineral Content in Children Aged 9 to 11 Years

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5.1 Abstract

Introduction: We aimed to investigate whether the relationship between fat mass and BMC is mediated by insulin, leptin, adiponectin, DHEAS, testosterone and estradiol in children aged 9 to 11 years.

Methods: We used cross-sectional data from the PANIC study (n = 230 to 396; 112 to 203 females). Fat mass and BMC were assessed with DXA. Endocrine factors were assessed from fasted blood samples. We applied the novel 4-way decomposition method to analyse associations between fat mass, endocrine factors, and BMC.

Results: Fat mass was positively associated with BMC in females (ß = 0.007 to 0.015, 95% CI 0.005 to 0.020) and males (ß = 0.009 to 0.015, 95% CI 0.005 to 0.019). The relationship between fat mass and BMC was mediated by free leptin index in females (ß = -0.025, 95% CI -0.039 to -0.010) and males (ß = -0.014, 95% CI -0.027 to -0.001). The relationship between fat mass and BMC

was partially explained by mediated interaction between fat mass and free leptin index in males (ß = -0.009, 95% CI -0.013 to -0.004) and by interaction between fat mass and adiponectin in females (ß = -0.003, 95% CI -0.006 to -0.000).

Conclusion: At greater levels of adiponectin and free leptin index, the fat mass and BMC relationship becomes less positive in females and males respectively. The positive association between fat mass with BMC was largely not explained by the endocrine factors we assessed.

5.2 Introduction

Increased fat mass may increase bone mass through greater mechanical load on bones (Dimitri, 2018). As BMC tracks from prepuberty through to adulthood, exploring the association between fat mass and BMC in pre- and early-pubertal children is important in understanding the factors that influence PBM, and potentially lifelong skeletal health (Kalkwarf *et al.*, 2010; Dimitri, 2018). In prepubertal females and males of largely normal weight status, fat mass was positively associated with aBMD independently of lean mass (Soininen *et al.*, 2018). Further, it has been shown that females and males with overweight and obese weight status had greater BMC than children with normal weight status after controlling for lean mass, in a predominantly pre- and early-pubertal sample (Leonard *et al.*, 2004b). Longitudinally, fat mass at age 9 was positively associated with change in BMC over the following two years in prepubertal girls and boys independently of lean mass, though the weight status of the children was not characterised (Clark, Ness and Tobias, 2006). The relationship between fat mass with bone mass is further supported by analysis with a Mendelian randomization approach in children aged 9 years, which found

support for a positive causal relationship between fat mass and bone mass (Timpson *et al.*, 2009). Although evidence seems to indicate positive associations of fat mass with BMC and aBMD after controlling for lean mass in pre- and early-pubertal females and males with normal, overweight and obese weight status, the mechanisms underlying these associations are unclear. A review published in 2018 concluded that in early childhood fat mass may have a positive effect on developing bone, but in time this association attenuates and reverses so that especially excess fat mass with unfavourable metabolic changes may lead to detrimental effects on skeletal structure and strength (Dimitri, 2018).

In addition to the increased mechanical load on bones due to fat mass, adipose tissue is a regulator of endocrine function and secretes several hormones and cytokines that may influence bone metabolism both positively and negatively (Reid, 2010; Dimitri, 2018). Insulin, leptin, DHEAS, testosterone and estradiol have been positively (Garnett *et al.*, 2004), and adiponectin negatively (Soininen *et al.*, 2018) associated with fat mass in largely normal weight prepubertal children. Insulin may increase bone mass by stimulating osteoblast differentiation (Torres-Costoso *et al.*, 2017). Leptin has a direct anabolic effect on bone, by driving the differentiation of bone marrow stem cells into osteoblasts, and an antiosteogenic indirect effect on bone, mediated via the sympathetic nervous system (Dimitri, 2018). Adiponectin may increase bone by driving osteoblast growth and inhibiting osteoclastogenesis, but also likely has negative indirect effects on bone, possibly by influencing the action of other hormones (Williams *et al.*, 2009). Androgens and estrogens have a protective effect on bone by decreasing osteoclastogenesis, preventing osteoblast

apoptosis and stimulating osteoclast apoptosis (Clarke and Khosla, 2009). Although the associations between these factors with bone have been investigated in clinical and adult populations, it is unclear whether these factors influence bone in healthy children (Welt *et al.*, 2004; Thrailkill *et al.*, 2005; Richards *et al.*, 2007; Cauley, 2015).

Previous studies have hypothesised that the positive association between fat mass and bone mass in healthy pre- and early-pubertal children is mediated by endocrine factors, namely leptin and estradiol (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006). Of the studies which have considered endocrine factors, the positive association of fat mass with BMC was unchanged after controlling for markers of insulin resistance in female and male adolescents aged 15 years (Lawlor *et al.*, 2012). This indicates that markers of insulin resistance do not mediate the association between fat mass and bone mass in adolescents. However, lean mass was not controlled for, and these findings cannot be extrapolated to younger children (Lawlor *et al.*, 2012). In children and adolescents aged 8 to 14 years with overweight weight status and varying pubertal status, leptin was negatively associated with BMC, independently of weight, subcutaneous abdominal tissue, and intra-abdominal adipose tissue (Afghani and Goran, 2009). However, lean mass was not controlled for, and these findings may not extend to children with healthy weight status, as leptin levels are positively associated with fat mass, and it has been suggested that the association between leptin with bone may be curvilinear (Dimitri, 2018). In females and males aged 9 years and females and males aged 15 years, adiponectin was negatively associated with BMC, controlling for fat mass and lean mass (Sayers *et al.*, 2010). This suggests an independent association of

adiponectin with BMC in children and adolescents, though it is not clear whether adiponectin partially mediates the association between fat mass and BMC (Sayers *et al.*, 2010). As such, the mediating role of insulin, leptin, adiponectin, DHEAS, testosterone and estradiol in the relationship between fat mass with BMC in a population sample of healthy pre- and early-pubertal remains unclear.

Although the results of these studies suggest that endocrine factors may be important in the association between fat and bone, there are currently few and contradictory data on endocrine factors (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006; Sayers *et al.*, 2010; Lawlor *et al.*, 2012). Studies to date have tended to use the 'difference method' for mediation analysis, which compares the association between the predictor and the outcome with and without taking the influence of potential mediators into account (Lawlor *et al.*, 2012; VanderWeele, 2016). This method is limited in that it does not allow for interaction between exposures and mediators (VanderWeele, 2016). Therefore, in cases where exposure-mediator interaction exists, but is not accounted for in the model, effect estimates are biased (VanderWeele, 2016). These limitations can be addressed by applying the 4-way decomposition method, which decomposes the total association between an exposure with an outcome into a controlled direct association, a reference interaction, a mediated interaction, and a pure indirect association (VanderWeele, 2014).

The aim of this study was to determine the association between fat mass and TBLH BMC in a population sample of females and males aged 9 to 11 years, and to assess the extent to which this relationship is mediated by insulin, leptin

(free leptin index), adiponectin, DHEAS, testosterone and estradiol using the 4-way decomposition method. We hypothesised that fat mass would be positively associated with TBLH BMC, and that this association would be partially mediated by insulin, free leptin index, adiponectin, DHEAS, testosterone and estradiol.

5.3 Methods

5.3.1 Study Design and Participants

This study used cross-sectional data from the 2-year follow-up of the PANIC Study, as described in Section 3.1. For the present analyses, we excluded children who were currently taking or had previously taken oral corticosteroids, as this could influence BMC (Weaver *et al.*, 2016). Complete data on the main variables used in the present analyses were available for 230 to 396 children (112 to 203 females, 118 to 193 males). Inclusion and exclusion criteria are displayed in Figure 5.1.

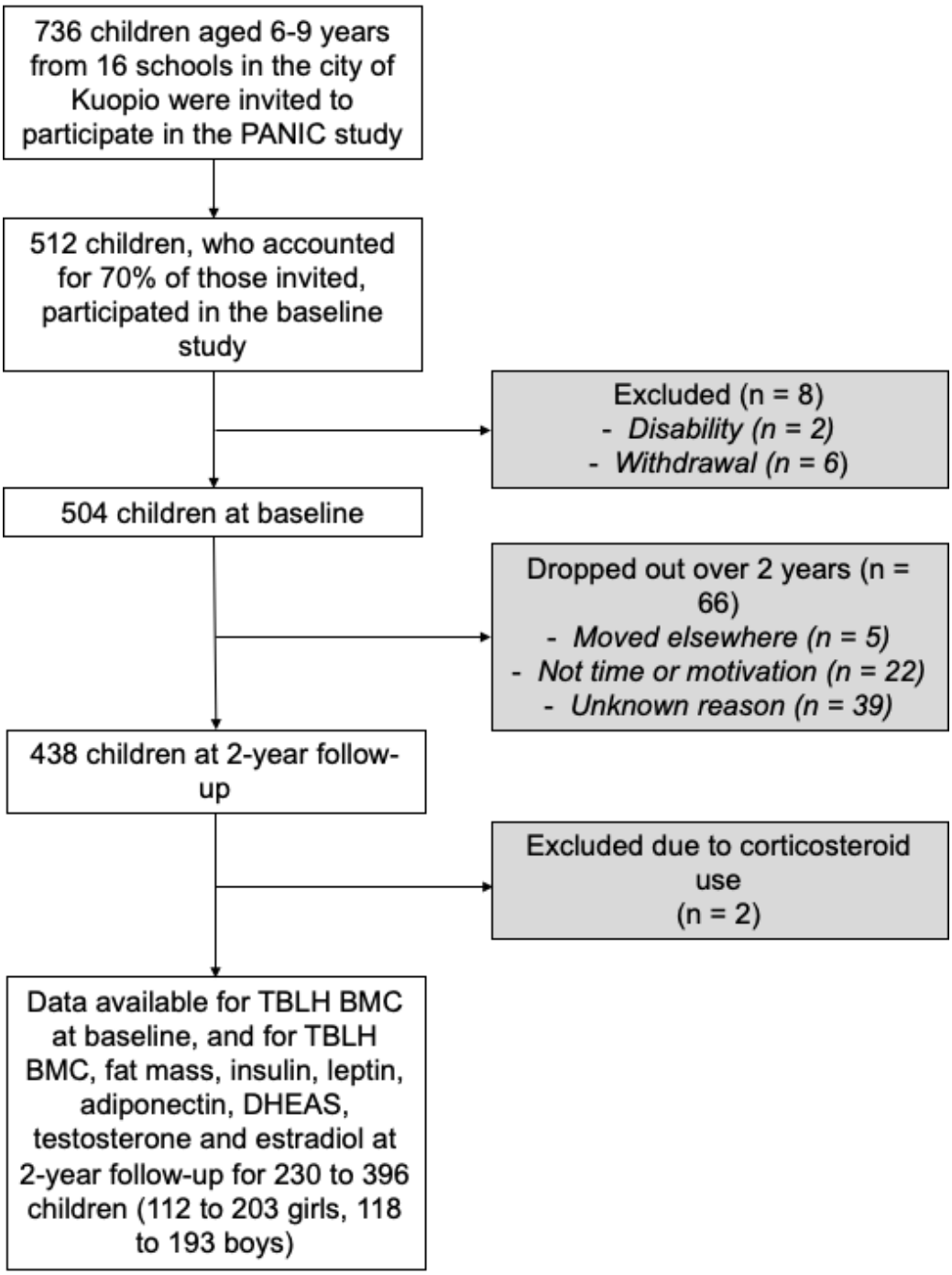

Figure 5.1 Participant flow chart.

PANIC, Physical Activity and Nutrition in Children; TBLH BMC, total-body-less-head bone mineral content; DHEAS, dehydroepiandrosterone sulphate.

5.3.2 Assessment of General Health and Pubertal Status

General health and pubertal status were assessed as described in Section 3.3 and Section 3.4.

5.3.3 Anthropometry

Stature and body weight were assessed as described in Section 3.5. BMI (kg/m^2) was calculated, and the BMI cut-offs of the IOTF were applied to classify children as underweight, normal weight, overweight, or obese weight status (Cole and Lobstein, 2012).

5.3.4 Assessment of Bone Mineral Content and Body Composition

TBLH BMC (kg), fat mass (kg) and lean mass (kg) were measured as described in Section 3.6. The primary outcome variable was TBLH BMC, as evidence indicates that for pre- and early-pubertal children BMC is a more accurate and reliable measure than aBMD (Wren *et al.*, 2005).

5.3.5 Biochemical Analyses

Serum insulin, plasma leptin concentration, plasma soluble leptin receptor concentration, free leptin index, serum high-molecular-weight adiponectin concentration, serum DHEAS concentration, serum testosterone and estradiol were measured as described in Section 3.8.

5.3.6 Statistical Analyses

Analyses were performed with Stata/SE for Mac software, Version 16.1 (StataCorp LLC, College Station, TX, USA). Differences in age, stature, BMI categories and pubertal status based on stages described by Tanner (Tanner, 1986) between the included and excluded children were tested. Summary statistics were calculated for the total study sample, and for females and males

separately. Independent samples t-tests, Mann-Whitney U tests and Chi-squared tests were used to test for sex differences.

All variables were mean-centred prior to entry into regression models, and free leptin index was log-transformed to account for the curvilinear relationship with fat mass. We tested whether fat mass, insulin, free leptin index, adiponectin, DHEAS, testosterone and estradiol interacted with sex to influence TBLH BMC. As the insulin by sex by fat mass interaction and the adiponectin by sex by fat mass interaction were statistically significant, and as there was limited estradiol data for males, further analyses were stratified by sex. We used the 4-way decomposition statistical approach with the *Med4Way* command in Stata/SE, which allows the total relationship between fat mass and BMC to be separated into four components: a controlled direct association (association due to neither mediation or interaction), a reference interaction (association explained by interaction only), a mediated interaction (association explained by mediation and interaction), and a pure indirect association (association due to mediation only) (see Figure 5.2) (VanderWeele, 2014; Discacciati *et al.*, 2018).

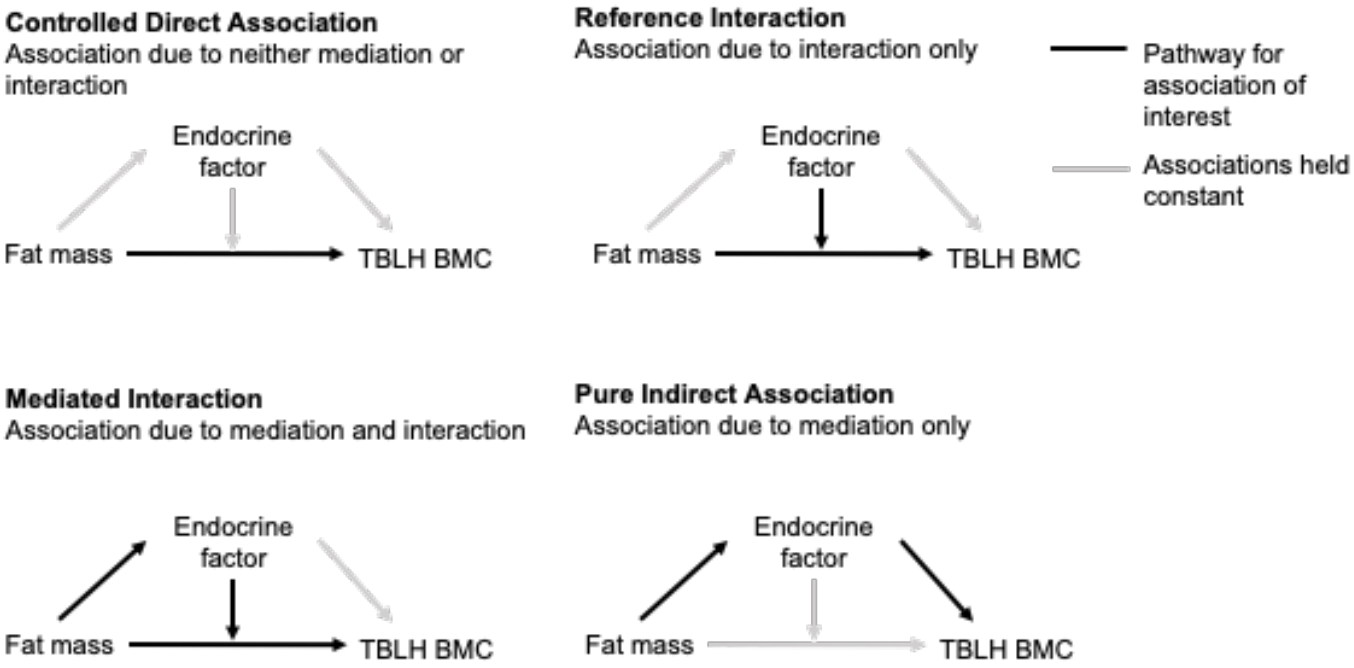

Figure 5.2 Components of the 4-way decomposition, as described by VanderWeele (2014), of the total association between fat mass with total-body-less-head bone mineral content.

TBLH BMC, total-body-less-head bone mineral content.

Two multivariable-adjusted regression models were established; an outcome model and a mediator model. Outcome models included fat mass, each endocrine factor, and an interaction term for fat mass and each endocrine factor as independent variables, and TBLH BMC as the dependent variable. Mediator models included fat mass as the independent variable and each endocrine factor as an outcome. As we used a continuous mediator, the mediator must be fixed at some level for the decomposition to be computed at (Bean *et al.*, 2019). When the mediator is fixed at a given level, some of the effect of the interaction is captured by the controlled direct association. Therefore, the reference interaction should be interpreted as the proportion of the total association due to interaction between fat mass and each endocrine factor that is not captured by the controlled direct association and not mediated (Bean *et al.*, 2019).

The models and 4-way decomposition of effects are expressed as follows (VanderWeele, 2014; Lee *et al.*, 2018):

Outcome Model: E [Y | *a, m, c*] = $\theta_0 + \theta_1 a + \theta_2 m + \theta_3 am + \theta_4$'$c$

Mediator Model: E [M | *a, c*] = $\beta_0 + \beta_1 a + \beta_2$'$c$

Where *a* is TBLH fat mass (i.e., exposure), M and *m* are the endocrine factors (i.e., mediators), *c* is a set of multiple potential confounders, and Y is TBLH BMC (i.e., outcome).

Based on the two regression models listed above, the controlled direct association, the pure indirect association, and the mediated interaction are as follows:

E [**Controlled Direct Association** (m*) | c] = ($\theta_1 + \theta_3 m$*)($a - a$*)

E [**Mediated Interaction** | c] = $\theta_3 \beta_1 (a - a^*) (a - a^*)$

E [**Pure Indirect Association**| c] = ($\theta_2 \beta_1 + \theta_3 \beta_1 a^*$) ($a - a$*)

Where m* is a fixed mediator level at the median, a* is the median level of TBLH fat mass, *a* is the 75th percentile of TBLH fat mass and c is a set of multiple potential confounders (age, stature, pubertal status, lean mass, baseline TBLH BMC).

The reference interaction is the difference between the pure direct association, given by E [**Pure Direct Association** | c] = ($\theta_1 + \theta_3(\beta_0 + \beta_1 a^* + \beta_2$'$c$)) ($a - a$*), and the controlled direct association:

E [**Reference Interaction** (m*) | c] = $\theta_3 (\beta_0 + \beta_1 a^* + \beta_2$'$c - m^*) (a - a^*)$

Estimates for the 4-way decomposition were computed fixing fat mass at the median and 75th percentile and endocrine factors at medians (Table 5.1)

All models were adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC (Leonard *et al.*, 2004b; Schoenau *et al.*, 2004). The alpha value for statistical significance was set as 0.05. Effect estimates (ß), 95% CI and p-values were reported. It is common to report results of mediation analyses as the proportion of the association between the exposure and outcome which is mediated. However, in cases where the mediator-outcome association is in the opposite direction to the exposure-outcome association, to report the proportion mediated would be nonsensical, and is therefore not included.

Models were confirmed to meet assumptions of linearity, normality, and homoscedasticity of residuals. In 96% of males, estradiol levels were under the lowest limit of quantitation, and therefore the analysis of estradiol could not be performed for males. As 73% of females had measurable levels of estradiol, the 27% of the samples with estradiol levels below the lowest limit of quantitation were included in analyses with the estradiol level coded as the lowest limit of quantitation. Given the nature of mediation analysis, multicollinearity is expected. In all models, all VIF values were less than 10, and most were less than 5, indicating acceptable levels of multicollinearity (Bowerman and O'Connell, 1990). Influential outliers were assessed using Cook's distance, and cases with Cook's distance substantially greater than the rest were considered outliers (Fox, 1991). Outliers were observed in all models, so analyses were run with and without outliers to check whether the inclusion of outliers altered the results. For free leptin index, the inclusion of the eight children (3 females, 5 males) who were outliers altered the results. As such, the alternative analysis strategy excluding these children is also presented. For all other endocrine

markers, the exclusion of outliers did not alter the results, so results are only presented including outliers.

Table 5.1 Fixed values used in *Med4Way* analysis

	Females	**Males**
Fat mass reference value (median value)	7.01	6.48
Fat mass actual value (75th percentile value)	11.40	10.67
Insulin (median value)	5.75	4.88
Log-transformed free leptin index (median value)	2.86	2.53
Adiponectin (median value)	7.87	7.89
DHEAS (median value)	0.69	0.74
Testosterone (median value)	217.52	186.71
Estradiol	6.68	.
Age (median value)	9.73	9.80
Stature (median value)	140.10	141.90
Pubertal status (median value)	Prepubertal	Prepubertal
Lean mass (median value)	20.48	23.03
Baseline TBLH BMC (median value)	0.64	0.68

Continuous values were mean-centred for entry into analysis.

DHEAS, dehydroepiandrosterone sulphate; TBLH BMC, total-body-less-head bone mineral content.

5.4 Results

5.4.1 Characteristics of Children

Children included in this study did not differ from the children excluded from this study for age, stature, pubertal status, and body weight status (Table 5.2). For the children included in this study, stature, body weight, TBLH BMC and TBLH

lean mass were lower in females than males, whereas TBLH fat mass, insulin, free leptin index, testosterone and estradiol were greater in females than males (Table 5.3). The proportion of pubertal children was greater in females than males.

Table 5.2 Differences in participant characteristics between included and excluded children

	Included children	**Excluded children**	**P value for group difference**
	n = 396	n = 108	
	Mean (SD) / Median (IQR)	Mean (SD) / Median (IQR)	
Age (years)	9.76 (0.43)	9.68 (0.45)	0.246
Stature (cm)	140.60 (6.29)	139.15 (6.27)	0.161
Weight (kg)	9.76 (9.45 to 10.02)	9.60 (9.39 to 9.98)	0.111
BMI-SDS	-0.05 (-0.93 to 0.62)	-0.52 (-1.02 to 0.34)	0.166
Pubertal Status			
% (cases) prepubertal	75.8 (300)	91.7 (22)	0.074
% (cases) pubertal	24.2 (96)	8.3 (2)	
IOTF Definition			
% (cases) normal weight	82.8 (328)	82.9 (34)	0.414
% (cases) overweight	13.6 (54)	17.1 (7)	
% (cases) obese	3.6 (14)	0 (0)	

BMI-SDS, body mass index standard deviation score; IOTF, International Obesity Task Force.

Number of children (n) varies for different variables.

n = 437, 396 included and 41 excluded: age, stature, weight, BMI-SDS, IOFT definition; n = 420, 396 included and 24 excluded: pubertal status.

Table 5.3 Descriptive characteristics of children

	Total	**Females**	**Males**	**P value for sex difference**
	n = 396	n = 203	n = 193	
	Mean (SD)/Median (IQR)	Mean (SD)/Median (IQR)	Mean (SD)/Median (IQR)	
Age (years)	9.8 (0.4)	9.7 (0.4)	9.8 (0.4)	0.063
Stature (cm)	140.6 (6.3)	139.9 (6.5)	141.3 (6.0)	**0.021**
Weight (kg)	33.0 (29.4 to 38.9)	32.5 (28.7 to 37.4)	34.3 (29.9 to 34.5)	**0.028**
BMI-SDS (kg/m^2)	-0.11 (1.06)	-0.13 (1.03)	-0.08 (1.09)	0.639
Pubertal Status				
% (cases) prepubertal	75.8 (300)	65.5 (133)	86.5 (167)	**< 0.001**
% (cases) pubertal	24.2 (96)	34.5 (70)	13.5 (26)	
IOTF Definition				
% (cases) thin and normal weight status	82.3 (326)	83.7 (170)	80.8 (156)	0.447
% (cases) overweight and obese weight status	17.7 (70)	16.3 (33)	19.2 (37)	
TBLH BMC (kg)	0.92 (0.80 to 1.05)	0.88 (0.77 to 1.03)	0.94 (0.83 to 1.08)	**0.001**
TBLH lean mass (kg)	21.82 (2.96)	20.77 (2.78)	22.93 (2.75)	**< 0.001**
TBLH fat mass (kg)	6.76 (4.33 to 11.13)	7.01(5.04 to 11.40)	6.48 (3.78 to 10.67)	**0.012**
Insulin (mU/L)	5.3 (3.8 to 7.8)	5.8 (4.1 to 8.3)	4.9 (3.3 to 7.1)	**0.001**
Free leptin index	15.3 (8.9 to 33.8)	17.6 (11.6 to 45.0)	12.6 (6.4 to 25.1)	**< 0.001**
Adiponectin (µg/ml)	8.6 (4.1)	8.3 (3.7)	8.9 (4.6)	0.180
DHEAS (µmol/L)	0.7 (0.4 to 1.1)	0.7 (0.4 to 1.1)	0.7 (0.4 to 1.2)	0.536
Testosterone (pmol/L)	198.7 (137.5 to 291.9)	217.5 (154.3 to 323.9)	186.7 (118.4 to 264.6)	**0.014**
Estradiol (pmol/L)[a]	< 6.7 (< 6.7 to 9.3)	9.4 (< 6.7 to 19.4)	**<** 6.7 (**<** 6.7 to **<** 6.7)	**< 0.001**
% (cases) with quantifiable levels (> 6.7 pmol/L)	37.8 (87)	73.2 (82)	4.2 (5)	

BMI-SDS, body mass index standard deviation score; IOTF, International Obesity Task Force; TBLH, total-body-less-head; BMC, bone mineral content; DHEAS, dehydroepiandrosterone sulphate. Bold emphasis indicates statistical significance at $p < 0.05$.

[a] the quantitation limit of the assay is 6.7 pmol/L

Number of children (n) varies from 230 to 396 for different variables;

n = 396, 203 females and 193 males: age, stature, body weight, BMI-SDS, pubertal status, IOFT definition, TBLH BMC, TBLH lean mass, TBLH fat mass; n = 380, 194 females and 186 males: insulin; n = 377, 191 females and 186 males: adiponectin, n = 376, 190 females and 186 males: free leptin index, n =

374, 190 females and 184 males: DHEAS, n = 231, 113 females and 118 males: testosterone, n = 230, 112 females and 118 males: estradiol.

5.4.2 Fat Mass

Fat mass was positively associated with TBLH BMC in females (ß = 0.007 to 0.015, 95% CI = 0.005 to 0.020, $p < 0.001$) and males (ß = 0.009 to 0.015, 95% CI = 0.005 to 0.019, $p < 0.001$) when adjusting for either insulin, free leptin index, adiponectin, DHEAS, testosterone or estradiol (Table 5.4). When fat mass changed from the median to the 75th percentile, the difference in TBLH BMC explained by the controlled direct association was positive in females (ß = 0.034 to 0.068, 95% CI = 0.023 to 0.089, $p < 0.001$) and males (ß = 0.034 to 0.064, 95% CI = 0.023 to 0.081, $p < 0.001$) in all models with either insulin, free leptin index, adiponectin, DHEAS, testosterone or estradiol (Table 5.5).

5.4.3 Insulin

Fat mass was positively associated with insulin in females (ß = 0.260, 95% CI = 0.120 to 0.400, $p < 0.001$) and males (ß = 0.406, 95% CI = 0.317 to 0.496, $p < 0.001$) (Table 5.6). Insulin was not associated with TBLH BMC, and there was no interaction between fat mass and insulin with TBLH BMC (Table 5.4). None of the total association between fat mass and TBLH BMC was mediated or moderated by insulin in females or males (Table 5.5).

5.4.4 Free Leptin Index

Fat mass was positively associated with free leptin index in females (ß = 0.186, 95% CI = 0.167 to 0.205, $p < 0.001$) and males (ß = 0.177, 95% CI = 0.162 to 0.193, $p < 0.001$) (Table 5.6). Free leptin index was negatively associated with TBLH BMC in females (ß = -0.031, 95% CI = -0.049 to -0.014, $p < 0.001$) and

males (ß = -0.022, 95% CI = -0.039 to -0.004, p = 0.013) (Table 5.4). There was an interaction between fat mass and free leptin index with TBLH BMC in males only (ß = -0.003, 95% CI = -0.004 to -0.001, p < 0.001). When fat mass changed from the median to the 75th percentile, the difference in TBLH BMC explained by the mediated interaction was negative in males (ß = -0.009, 95% CI = -0.013 to -0.004, p = 0.031) (Table 5.5). The difference in TBLH BMC explained by the pure indirect association was negative in females (ß = -0.025, 95% CI = -0.039 to -0.010, p = 0.001) and males (ß = -0.014, 95% CI = -0.027 to -0.001, p = 0.032) (Table 5.5). The alternate analysis strategy excluding outliers is presented in Table 5.7.

5.4.5 Adiponectin

Fat mass was not associated with adiponectin in females or males (Table 5.6). Adiponectin was negatively associated with TBLH BMC in females only (ß = -0.002, 95% CI -0.004 to -0.000, p = 0.036), and there was an interaction between fat mass and adiponectin on TBLH BMC in females only (ß = -0.001, 95% CI = -0.001 to -0.000, p < 0.001) (Table 5.4). When fat mass changed from the median to the 75th percentile, the difference in TBLH BMC explained by the reference interaction was negative in females (ß = -0.003, 95% CI = -0.006 to -0.000, p = 0.031) (Table 5.5). None of the total association between fat mass and TBLH BMC was mediated or moderated in males.

5.4.6 DHEAS

Fat mass was not associated with DHEAS in females or males (Table 5.6). DHEAS was not associated with TBLH BMC, and there was no interaction between fat mass and DHEAS with TBLH BMC in females or males (Table 5.4).

None of the total association between fat mass and TBLH BMC was mediated or moderated by DHEAS in females or males (Table 5.5).

5.4.7 Testosterone

Fat mass was positively associated with testosterone in males only (ß = 10.310, 95% CI = 0.089 to 20.531, p = 0.048) (Table 5.6). Testosterone was not associated with TBLH BMC, and there was no interaction between fat mass and testosterone with TBLH BMC in females or males (Table 5.4). None of the total association between fat mass and TBLH BMC was mediated or moderated by testosterone in females or males (Table 5.5).

5.4.8 Estradiol

Fat mass was not associated with estradiol in females (Table 5.6). Estradiol was not associated with TBLH BMC, and there was no interaction between fat mass and estradiol with TBLH BMC in females (Table 5.4). None of the total association between fat mass and TBLH BMC was mediated or moderated by estradiol in females (Table 5.5). Analysis in males was not performed because they had values below the lowest limit of quantitation of estradiol (6.68 pmol/L).

A summary of the significant mediation and moderation effects is presented in Figure 5.3.

Table 5.4 Associations between fat mass, endocrine factors, and total-body-less-head bone mineral content (outcome model)

Endocrine Factors	Variables included in the Model	Females		Males	
		ß (95% CI)	p-value	ß (95% CI)	p-value
Insulin	Fat mass	**0.008 (0.006 to 0.010)**	**< 0.001**	**0.009 (0.007 to 0.012)**	**< 0.001**
	Insulin	0.001 (-0.002 to 0.003)	0.479	0.000 (-0.003 to 0.003)	0.816
	Fat mass X Insulin	0.000 (0.000 to 0.001)	0.050	-0.000 (-0.001 to 0.000)	0.096
Free Leptin Index[a]	Fat mass	**0.015 (0.012 to 0.020)**	**< 0.001**	**0.015 (0.011 to 0.019)**	**< 0.001**
	Free Leptin Index	**-0.031 (-0.049 to -0.014)**	**< 0.001**	**-0.022 (-0.039 to -0.004)**	**0.013**
	Fat mass X Free Leptin Index	-0.001 (-0.002 to 0.001)	0.267	**-0.003 (-0.004 to -0.001)**	**< 0.001**
Adiponectin	Fat mass	**0.007 (0.005 to 0.010)**	**< 0.001**	**0.009 (0.007 to 0.011)**	**< 0.001**
	Adiponectin	**-0.002 (-0.004 to -0.000)**	**0.036**	-0.001 (-0.003 to 0.001)	0.179
	Fat mass X Adiponectin	**-0.001 (-0.001 to -0.000)**	**< 0.001**	0.000 (-0.000 to 0.000)	0.758
DHEAS	Fat mass	**0.009 (0.006 to 0.011)**	**< 0.001**	**0.008 (0.007 to 0.010)**	**< 0.001**
	DHEAS	0.005 (-0.008 to 0.017)	0.481	0.006 (-0.005 to 0.016)	0.275
	Fat mass X DHEAS	0.002 (-0.001 to 0.004)	0.142	0.001 (-0.001 to 0.003)	0.217
Testosterone	Fat mass	**0.009 (0.007 to 0.013)**	**<0.001**	**0.008 (0.005 to 0.010)**	**< 0.001**
	Testosterone	-0.000 (-0.000 to 0.000)	0.465	0.000 (-0.000 to 0.000)	0.560
	Fat mass X Testosterone	0.000 (-0.000 to 0.000)	0.826	0.000 (-0.000 to 0.000)	0.783
Estradiol	Fat mass	**0.010 (0.007 to 0.013)**	**< 0.001**	.	.
	Estradiol	0.000 (-0.000 to 0.001)	0.234	.	.
	Fat mass X Estradiol	0.000 (-0.000 to 0.000)	0.059	.	.

All models adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC. Bold emphasis indicates statistical significance at $p < 0.05$.

TBLH BMC, total-body-less-head bone mineral content; DHEAS, dehydroepiandrosterone sulphate.

[a] Free Leptin Index log-transformed using the natural log.

Number of children (n) varies from 230 to 380 for different variables: Insulin: n = 380, 194 females and 186 males; Free Leptin Index, n = 376, 190 females and 186 males; Adiponectin; n = 377, 191 females and 186 males; DHEAS: n = 374, 190 females and 184 males; Testosterone; n = 231, 113 females and 118 males; Estradiol, n = 112, 112 females and 0 males.

Table 5.5 Four-way decomposition of the association between fat mass and TBLH BMC

	Females			Males		
	ß	95% CI	p-value	ß	95% CI	p-value
Insulin						
Total association	**0.035**	**0.025 to 0.046**	**< 0.001**	**0.039**	**0.031 to 0.048**	**< 0.001**
Controlled direct association	**0.034**	**0.023 to 0.044**	**< 0.001**	**0.041**	**0.030 to 0.051**	**< 0.001**
Reference interaction	0.000	-0.001 to 0.001	0.831	-0.000	- 0.001 to 0.000	0.304
Mediated interaction	0.001	-0.000 to 0.003	0.085	-0.002	-0.005 to 0.000	0.101
Pure indirect association	0.001	-0.002 to 0.003	0.710	0.001	-0.004 to 0.007	0.630
Free Leptin Index [a]						
Total association	**0.041**	**0.029 to 0.052**	**< 0.001**	**0.042**	**0.034 to 0.050**	**< 0.001**
Controlled direct association	**0.068**	**0.047 to 0.089**	**< 0.001**	**0.064**	**0.047 to 0.081**	**< 0.001**
Reference interaction	0.000	-0.000 to 0.001	0.407	0.000	-0.000 to 0.001	0.320
Mediated interaction	-0.003	-0.009 to 0.002	0.268	**-0.009**	**-0.013 to -0.004**	**< 0.001**
Pure indirect association	**-0.025**	**-0.039 to -0.010**	**0.001**	**-0.014**	**-0.027 to -0.001**	**0.032**
Adiponectin						
Total association	**0.033**	**0.022 to 0.044**	**< 0.001**	**0.038**	**0.029 to 0.046**	**< 0.001**
Controlled direct association	**0.034**	**0.024 to 0.044**	**< 0.001**	**0.037**	**0.029 to 0.045**	**< 0.001**
Reference interaction	**-0.003**	**-0.006 to -0.000**	**0.031**	0.000	-0.002 to 0.003	0.759
Mediated interaction	0.002	-0.001 to 0.004	0.173	-0.000	-0.001 to 0.001	0.765
Pure indirect association	0.001	-0.001 to 0.002	0.434	0.001	-0.001 to 0.002	0.355
DHEAS						
Total association	**0.036**	**0.026 to 0.047**	**< 0 001**	**0.037**	**0.028 to 0.045**	**< 0.001**
Controlled direct association	**0.036**	**0.026 to 0.047**	**<0.001**	**0.036**	**0.027 to 0.045**	**< 0.001**
Reference interaction	0.001	-0.001 to 0.002	0.268	0.001	-0.001 to 0.002	0.396
Mediated interaction	-0.001	-0.002 to 0.001	0.278	-0.000	-0.001 to 0.001	0.932
Pure indirect association	-0.000	-0.002 to 0.001	0.786	-0.000	-0.001 to 0.000	0.932
Testosterone						
Total association	**0.044**	**0.030 to 0.058**	**< 0.001**	**0.035**	**0.024 to 0.045**	**< 0.001**
Controlled direct association	**0.043**	**0.029 to 0.057**	**< 0.001**	**0.034**	**0.023 to 0.044**	**< 0.001**
Reference interaction	0.000	-0.001 to 0.002	0.828	0.000	-0.002 to 0.003	0.796
Mediated interaction	-0.000	-0.002 to 0.002	0.828	0.001	-0.003 to 0.004	0.785
Pure indirect association	0.001	-0.001 to 0.003	0.473	0.000	-0.002 to 0.003	0.760
Estradiol						
Total association	**0.040**	**0.026 to 0.054**	**< 0.001**	.	.	.
Controlled direct association	**0.039**	**0.026 to 0.054**	**< 0.001**	.	.	.
Reference interaction	0.003	-0.001 to 0.006	0.128	.	.	.
Mediated interaction	-0.002	-0.005 to 0.001	0.184	.	.	.
Pure indirect association	-0.001	-0.003 to 0.002	0.607	.	.	.

All models adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC. Bold emphasis indicates statistical significance at $p < 0.05$.

The values are effect estimates (ß), 95% confidence intervals (CI) and p-values from the 4-way decomposition model, as outlined in Figure 5.2.

TBLH BMC, total-body-less-head bone mineral content; DHEAS, dehydroepiandrosterone sulphate.

[a] Free Leptin Index log-transformed using the natural log.

The values at which the 4-way decomposition was computed are presented in Table 5.1.

Number of children (n) varies from 230 to 380 for different variables:

Insulin: n = 380, 194 females and 186 males; Free Leptin Index, n = 376, 190 females and 186 males; Adiponectin; n = 377, 191 females and 186 males; DHEAS: n = 374, 190 females and 184 males; Testosterone; n = 231, 113 females and 118 males; Estradiol, n = 112, 112 females and 0 males.

Table 5.6 Associations between fat mass and endocrine factors (mediator model)

Exposure	Outcome	Females		Males	
		ß (95% CI)	p-value	ß (95% CI)	p-value
Fat Mass	Insulin	**0.260 (0.120 to 0.400)**	**< 0.001**	**0.406 (0.317 to 0.496)**	**< 0.001**
	Free Leptin Index [a]	**0.186 (0.167 to 0.205)**	**< 0.001**	**0.177 (0.162 to 0.193)**	**< 0.001**
	Adiponectin	-0.117 (-0.274 to 0.040)	0.144	-0.102 (-0.263 to 0.060)	0.216
	DHEAS	-0.022 (-0.048 to 0.005)	0.107	-0.001 (-0.031 to 0.029)	0.932
	Testosterone	-5.239 (-12.195 to 1.717)	0.140	**10.310 (0.089 to 20.531)**	**0.048**
	Estradiol	-1.548 (-3.174 to 0.078)	0.062	.	.

All models adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC. Bold emphasis indicates statistical significance at $p < 0.05$.

TBLH BMC, total-body-less-head bone mineral content; DHEAS, dehydroepiandrosterone sulphate.

[a] Free Leptin Index log-transformed using the natural log.

Number of children (n) varies from 230 to 380 for different variables: Insulin: n = 380, 194 females and 186 males; Free Leptin Index, n = 376, 190 females and 186 males; Adiponectin; n = 377, 191 females and 186 males; DHEAS: n = 374, 190 females and 184 males; Testosterone; n = 231, 113 females and 118 males; Estradiol, n = 112, 112 females and 0 males.

Table 5.7 Alternative analysis strategy for 4-way decomposition of the association between fat mass and TBLH BMC excluding outliers

	Females			**Males**		
	ß	95% CI	p-value	ß	95% CI	p-value
Free Leptin Index[a]						
Total association	**0.036**	**0.026 to 0.046**	**< 0.001**	**0.033**	**0.025 to 0.040**	**< 0.001**
Controlled direct association	**0.052**	**0.032 to 0.072**	**< 0.001**	**0.044**	**0.027 to 0.061**	**< 0.001**
Reference interaction	0.000	-0.000 to 0.001	0.530	0.001	-0.000 to 0.001	0.240
Mediated interaction	-0.003	-0.010 to 0.004	0.335	**-0.007**	**-0.013 to -0.002**	**0.007**
Pure indirect association	-0.013	-0.027 to 0.001	0.063	-0.004	-0.018 to 0.010	0.539

All models adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC. Bold emphasis indicates statistical significance at $p < 0.05$.

TBLH BMC, total-body-less-head bone mineral content.

[a] Free Leptin Index log-transformed using the natural log.

The values at which the 4-way decomposition was computed are presented in Table 5.1.

n = 368, 187 females and 181 males.

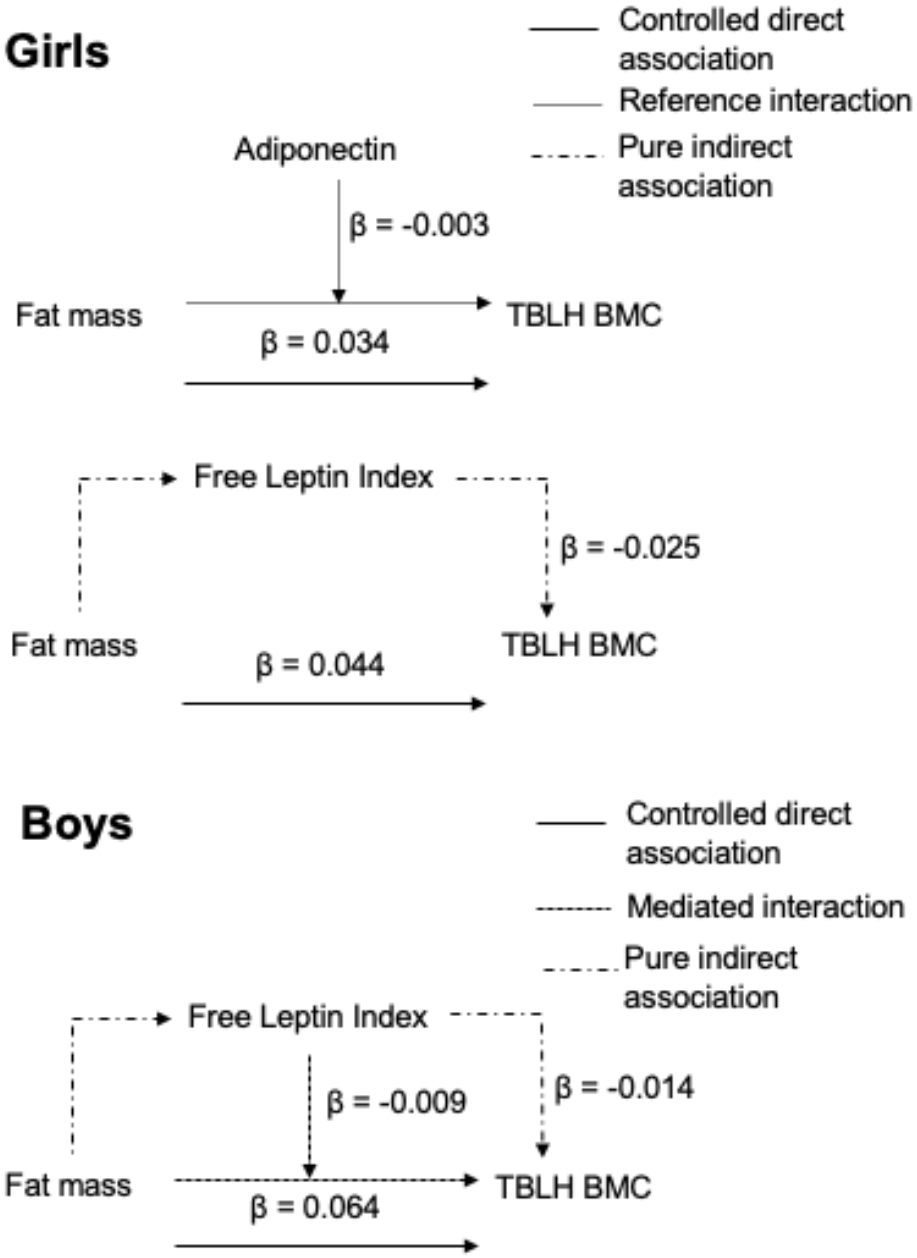

Figure 5.3 Summary of significant mediation and moderation effects from the 4-way decomposition.

TBLH BMC, total-body-less-head bone mineral content.

All models adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC.

Females: n = 191 for adiponectin, n = 190 for free leptin index, Males: n = 181.

5.5 Discussion

Our study is the first to formally test the mediation and interaction of insulin, free leptin index, adiponectin, DHEAS, testosterone and estradiol in the relationship between fat mass and TBLH BMC in a population sample of predominantly normal weight pre- and early-pubertal children. The positive relationship between fat mass and TBLH BMC was suppressed by mediation through free leptin index in females and males, and moderated by adiponectin and free leptin index in females and males respectively. Our findings indicate that the association between fat mass with TBLH BMC was largely not explained by the endocrine factors we assessed. Greater fat mass provides additional loading on bones, which may explain the positive association between fat mass with TBLH BMC. This proposed mechanism of mechanical loading due to increased fat mass is further supported by associations of a greater magnitude observed between lower-limb fat mass and BMC compared to upper-limb fat and BMC (unpublished observations), indicating the relationship between fat mass and BMC is more pronounced in load-bearing bones. However, as highlighted in a 2020 review article, the complex relationship between mechanical loading, due to lean mass, fat mass or weight-bearing activity, with bone metabolism, and the moderating role of associated endocrine and paracrine factors in this association is not yet fully understood (Kirk *et al.*, 2020).

The positive association between fat mass and TBLH BMC is in line with previous research in pre- and early-pubertal children (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006). The ALSPAC study demonstrated positive associations between fat mass and TBLH BMC in pre- and early-pubertal females and males aged 9.9 years, after accounting for lean mass (Clark, Ness

and Tobias, 2006). However, studies in adolescents and young adults have found negative (Gracia-Marco *et al.*, 2012) or null (Janicka *et al.*, 2007) associations between fat mass and BMC. This may reflect an interaction between fat mass and pubertal status, whereby the positive association between fat mass and TBLH BMC disappears or inverses during puberty (Dimitri, 2018). It may therefore be reasonable to suggest that pubertal status contributes to the differences observed between studies (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006; Janicka *et al.*, 2007; Gracia-Marco *et al.*, 2012).

5.5.1 Insulin

In vivo evidence indicates that insulin acts as an anabolic agent for bone (Thrailkill *et al.*, 2005). However, similar to our findings, in children aged 7 to 15 years, HOMA-IR did not meet the criteria for mediation with fat mass and TBLH BMC (Kindler *et al.*, 2019). Likewise, in females and males aged 15 to 16 years, although insulin was negatively associated with TBLH BMC, insulin did not mediate the fat mass and TBLH BMC relationship (Lawlor *et al.*, 2012). The median levels of fasting insulin we observed were consistent with those observed in previous studies in the same age group (Peplies *et al.*, 2014), suggesting our sample was representative of children aged 9 to 11 years. Our observations replicate those of other studies, demonstrating that insulin does not mediate the association between fat mass and TBLH BMC (Lawlor *et al.*, 2012; Kindler *et al.*, 2019).

5.5.2 Leptin and Free Leptin Index

Previous studies of the association between leptin and BMC have been inconclusive. In vitro studies indicate that leptin may stimulate peripherally-

mediated osteogenic and hypothalamically-mediated antiosteogenic effects on bone (Dimitri, Wales and Bishop, 2011). In children aged 7 to 8 years, plasma leptin was not associated with TBLH BMC (Garnett *et al.*, 2004). However, in children and adolescents aged 5 to 16 years, free leptin index was inversely associated with osteoprotegerin (Dimitri, Wales and Bishop, 2011). Reduced osteoprotegerin may lead to increased bone resorption, potentially resulting in decreased bone mass. However, as bone mass was not measured in the aforementioned study, it is unclear whether the association between free leptin index and osteoprotegerin resulted in decreased bone mass (Dimitri, Wales and Bishop, 2011). The pure indirect association we observed indicates that although the total association between fat mass with TBLH BMC was positive, the pathway through free leptin index acted as a suppressor in this relationship. The mediated interaction we observed indicates that free leptin index was positively associated with fat mass, and as free leptin index increased, the relationship between fat mass with TBLH BMC became less positive in males. It is therefore possible that in males with greater levels of fat mass, the net association between fat mass with TBLH BMC may become negative (Dimitri, 2019). As around 80% of the participants in our study were a healthy weight, research in populations with a greater proportion of children and adolescents with overweight and obese weight status is needed to test this hypothesis.

The differences in the associations between fat mass, free leptin index and BMC between females and males may be due to the sex-specific patterns of leptin levels through childhood and adolescence, with greater absolute leptin concentrations observed in pre- and early-pubertal females compared to males (Blum *et al.*, 1997; Ellis and Nicolson, 1997), which we also observed. However,

as the free leptin index by sex by fat mass interaction was not statistically significant, the sex difference we observed may be superficial, due to reduced statistical power when stratifying the sample by sex. Differences in body composition, endocrine factors, as well as differences in pubertal status between females and males (Blum *et al.*, 1997; Ellis and Nicolson, 1997; Nagy *et al.*, 1997) may further explain the different associations in females and males between fat mass, free leptin index and TBLH BMC we observed.

5.5.3 Adiponectin

Adiponectin has been inversely associated with fat mass in children aged 9.9 years (Sayers *et al.*, 2010). Further, adiponectin has been inversely associated with TBLH BMC at age 9.9 years and with the change in TBLH BMC from age 9.9 years to 15.5 years in the ALSPAC study cohort (Sayers *et al.*, 2010) independently of lean mass and fat mass. Similar to our findings, in adults adiponectin was inversely associated with BMC in females but not in males, potentially reflecting an influence of sex hormones on the action of adiponectin on bone (Bi, Loo and Henry, 2020). It is also possible that the sex difference we observed may be related to differences in fat mass between females and males, as females had greater fat mass than males in our sample. However, the reasons for the sex difference have not been formally tested, and it remains possible that our findings may be due to chance.

5.5.4 DHEAS

Central adiposity has been positively associated with DHEAS, and children with obesity were more likely to have high DHEAS than normal weight children in a population sample of prepubertal children aged 7 years (Corvalán, Uauy and

Mericq, 2013). Despite the positive association between obesity and premature adrenarche with advanced bone age (Sopher *et al.*, 2011), in children aged 7-8 years, DHEAS did not explain any additional variance in TBLH BMC beyond that of lean mass and fat mass (Garnett *et al.*, 2004). The median levels of DHEAS we observed were within the reference ranges for pre- and early-pubertal children (Elmlinger, Kühnel and Ranke, 2002), indicating our sample is representative of children aged 9 to 11 years. Therefore, our findings confirm previous evidence indicating that DHEAS does not have an independent association with bone when controlling for body composition.

5.5.5 Testosterone and Estradiol

In children aged 7-8 years, fat mass was positively associated with testosterone and estradiol, though neither were associated with TBLH BMC, as was the case in our study (Garnett *et al.*, 2004). As three-quarters of our sample did not have signs of clinical puberty, it is possible that serum levels of sex steroids were too low for us to detect an effect. Although females had higher levels of testosterone than males, this is likely explained by the significantly greater proportion of pubertal females compared to males (Sizonenko and Paunier, 1975). We were also limited by the sensitivity of the estradiol measure, as 27% of females and 96% of males fell below the lower limit of quantitation in our sample. Previous studies have hypothesised that sex steroids may play a role in the positive associations between fat mass and bone mass in pre- and early-pubertal children (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006). Our findings do not support this hypothesis in pre- and early-pubertal children, though more sensitive measures of estradiol would be valuable in examining this further. The serum levels of testosterone and estradiol we observed are

typical for pre- and early-pubertal children aged 9 to 11 years (Sizonenko and Paunier, 1975; Elmlinger, Kühnel and Ranke, 2002). However, as sex steroid levels rise during puberty, further research into these relationships in adolescents is needed to understand the mediating role of sex steroids in the relationship between fat mass and BMC in adolescence.

5.5.6 Translation of Findings

We found across our outcome models that 1 kg greater fat mass was associated with around 0.01 kg greater TBLH BMC, after accounting for the effects of age, stature, pubertal status, lean mass, and baseline BMC. This increase seems to be modest, reflecting ~ 5 to 10% of the annual BMC accretion for children aged 9 to 11 years (Molgaard, Thomsen and Michaelsen, 2001). The pure indirect association between free leptin index with TBLH BMC explained -0.025 kg difference in TBLH BMC in females and -0.015 kg difference in TBLH BMC in males, when fat mass increased from the median to the 75th percentile. The mediated interaction between fat mass and free leptin index with TBLH BMC explained a -0.008 kg difference in TBLH BMC in males only, when fat mass increased from the median to the 75th percentile. The reference interaction between fat mass and adiponectin with TBLH BMC explained a -0.003 kg difference in females, when fat mass increased from the median to the 75th percentile, with adiponectin fixed at the median level. Although statistically significant, these associations represent a relatively small difference in absolute values. However, the negative interactions we observed may suggest that the observed estimates would be greater in a population with a greater proportion of children with overweight and obese weight status, though this requires further exploration.

5.5.7 Strengths and Limitations

Strengths of this study included the population-based sample of children, the measurement of fat mass and bone outcomes by DXA, and the analysis strategy of controlling for baseline TBLH BMC at age 6 to 8. There are several limitations that should be considered when interpreting the results. The assessment of BMC is not simple, and the interpretation of results is challenging in growing children. The determinants of vBMD remain unclear, as it is not possible to obtain true measures for it from DXA. Therefore, the International Society for Clinical Densitometry recommends adjusting TBLH BMC using height z-score (International Society for Clinical Densitometry, 2019b). We used TBLH BMC, as recommended by the International Society for Clinical Densitometry, and adjusted the data for age and stature, components of the height z-score, to account for body size (International Society for Clinical Densitometry, 2019b). Future research with high-resolution peripheral quantitative computed tomography (pQCT) measures of bone micro-architecture would be valuable in further understanding the relationship between fat mass with bone in healthy children. The endocrine measures we used were from a fasted sample, which may not adequately reflect endocrine status across the day, due to diurnal changes in endocrine factors (Goji, 1993; Ankarberg-Lindgren *et al.*, 2001; Bachran *et al.*, 2012). This may lead to an under- or over-estimation of the association between endocrine factors and bone, though previous studies have also used fasted samples (Garnett *et al.*, 2004; Lawlor *et al.*, 2012). Further, although stature, age, pubertal status, lean mass, and baseline TBLH BMC were controlled for, residual confounding remains a potential limitation in our study and in all other observational studies.

5.6 Conclusion

Our findings indicate that fat mass was positively associated with BMC in pre- and early-pubertal children. Although adiponectin and free leptin index acted as mediators and moderators in the relationship between fat mass with BMC, these associations account for a relatively small contribution in terms of absolute values. These observations highlight that fat mass retains its positive relationship with BMC independently of the endocrine markers we assessed, and strategies to maintain bone mass, such as PA and nutrition, should be considered when recommending fat loss (Tobias, 2010). As the relationship between fat mass and endocrine factors with BMC is likely moderated by weight status and pubertal stage (Dimitri, 2018), further research is needed to assess whether these observations extend to children and adolescents with overweight and obese weight status and with more advanced pubertal status.

Chapter 6 Physical Activity Volume and Intensity Distribution in Relation to Bone, Lean and Fat Mass in Children Aged 9 to 11 Years

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6.1 Abstract

Introduction: Considering PA volume and intensity may provide novel insights into the relationships of PA with bone, lean, and fat mass. This study aimed to assess the associations of PA volume, PA intensity distribution, and MVPA with TBLH BMC, lean mass, and fat mass in children.

Methods: A population sample of 290 Finnish children (158 females) aged 9 to 11 years from the PANIC Study was studied. PA, including MVPA, was assessed with a combined heart rate and movement sensor, and the uniaxial acceleration was used to calculate average-acceleration (a proxy metric for PA volume) and intensity-gradient (reflective of PA intensity distribution). Linear regression analysed the associations of PA volume, PA intensity and MVPA with BMC, lean mass, and fat mass assessed by DXA.

Results: PA volume was positively associated with BMC in females (unstandardised regression coefficient (ß) = 0.26) and males (ß = 0.47), and positively associated with lean (ß = 7.33) and negatively associated with fat mass in males (ß = -20.62). PA intensity was negatively associated with BMC in

males (ß = -0.13). MVPA was positively associated with lean mass in females and males (ß = 0.007 to 0.012), negatively associated with fat mass in females and males (ß = -0.030 to -0.029).

Conclusion: PA volume may be important for improving BMC in females and males, and increasing lean and reducing fat mass in males, whereas MVPA may be important for favourable lean and fat outcomes in both sexes.

6.2 Introduction

PA is positively associated with BMC and lean mass and inversely associated with fat mass in children and adolescents (Riddoch *et al.*, 2009; Zymbal *et al.*, 2019). PA increases BMC by increasing mechanical loads on bones, and the skeleton adapts in response to these strains (Hart *et al.*, 2017). PA may also indirectly influence BMC via lean and fat mass, as greater lean and fat mass increase mechanical loading on bones (Hart *et al.*, 2017). During childhood and adolescence, BMC, lean mass, and fat mass increase with linear growth, with the amount of bone accrued during growth potentially determining the risk of osteoporosis in later life (Baxter-Jones *et al.*, 2003; Weaver *et al.*, 2016). BMC tracks throughout childhood and adolescence, and as such, the interrelationships between BMC, body composition and PA in pre- and early-puberty are of particular interest (Baxter-Jones *et al.*, 2003; Weaver *et al.*, 2016).

Few studies have considered bone, muscle, and fat outcomes together when investigating the association between PA and BMC. In children aged 11 years, device-measured MVPA was positively associated with TBLH BMC, controlling for lean and fat mass, though this association was not significant when only

controlling for lean mass (Tobias *et al.*, 2007). Females and males with greater levels of MVPA from age 5 to 17 years had greater leg lean mass and greater proximal femur aBMD at age 17, with 29 to 49% of the relationship between MVPA and aBMD mediated via lean mass, though fat mass was not considered (Zymbal *et al.*, 2019). Although these studies support the importance of considering body composition when investigating the association between PA and bone mass, previous research has focused on MVPA as a summary measure of PA intensity, which is related to energy expenditure (Tobias *et al.*, 2007; Zymbal *et al.*, 2019). Applying cut-points to categorize PA intensity condenses the PA intensity continuum into broad categories, validated against oxygen consumption, which may not be relevant for muscle and bone strengthening activities (Corder *et al.*, 2007; Zymbal *et al.*, 2019; Rowlands *et al.*, 2020). Further, as movement is accumulated across an intensity continuum, rather than focusing on specific intensities of activity, the whole intensity spectrum should be considered when examining the relationships of PA with BMC, lean mass, and fat mass (Rowlands *et al.*, 2020).

To address the limitations of applying cut-points to PA data, Rowlands and colleagues (Rowlands *et al.*, 2018) proposed using two accelerometer metrics to capture the volume and intensity distribution of the PA profile. PA volume is reflected in the average-acceleration and intensity distribution and can be characterised by the gradient of the relationship between intensity and time accumulated at that intensity (Rowlands *et al.*, 2018). In adolescents and young adults, PA volume and intensity distribution were positively associated with TBLH BMC, indicating that accumulating PA volume at any intensity, or increasing intensity without increasing volume, could be beneficial for BMC

(Rowlands *et al.*, 2020). However, the associations of PA volume and intensity distribution with BMC, lean mass, and fat mass, compared with the traditional approach of summarising MVPA based on energy expenditure, in pre- and early-pubertal children remain unknown.

This study aimed to: 1) assess the associations of PA volume (average-acceleration) and intensity distribution (intensity-gradient) with TBLH BMC, lean mass, and fat mass in a population sample of pre- and early-pubertal children aged 9 to 11 years; 2) repeat the analysis with MVPA as the PA exposure variable, to check whether the findings differ based on accelerometer metrics used; and 3) apply translational metrics to illustrate the profile of the PA volume and intensity distribution associated with improved BMC and lean mass, and reduced fat mass in this cohort.

6.3 Methods

6.3.1 Study Design and Participants

This study used cross-sectional data from the 2-year follow-up of the PANIC Study, as described in Section 3.1. Given the importance of the pre- and early-pubertal period for bone development, the 2-year follow-up data were used in these analyses, when children were aged 9 to 11 years, in order to capture this potentially critical period for bone accrual (Baxter-Jones *et al.*, 2003; Weaver *et al.*, 2016). For the present analyses, we excluded children who used oral corticosteroids, as they could influence BMC (Weaver *et al.*, 2016), and children with musculoskeletal injuries and diseases. Complete and valid data on the main variables used in the present analyses were available for 290 children (158 females). Of these children, 99% were Caucasian. The children included in

these analyses did not differ in age, stature, pubertal status, weight status, TBLH BMC, lean mass, or fat mass to the children who did not have complete data (Table 6.1). Inclusion and exclusion criteria are displayed in Figure 6.1.

Table 6.1 Differences in characteristics of children included and children excluded from analyses

	Included children (n = 290)	**Excluded children (n = 127 to 147)**	***p* value for difference**
Age (years)	9.8 (0.4)	9.7 (0.4)	0.28
Stature (cm)	140.8 (6.1)	139.8 (6.6)	0.12
Weight (kg)	32.9 (29.5 to 38.7)	33.2 (28.9 to 38.7)	0.84
Pubertal Status (n, %)			
prepubertal	218 (75.1)	104 (80.0)	0.28
pubertal	72 (24.9)	26 (20.0)	
IOTF Definition (n, %)			
thin	31 (10.7)	13 (8.9)	0.09
normal weight	216 (74.5)	100 (68.0)	
overweight	33 (11.4)	30 (20.4)	
obese	10 (3.4)	4 (2.7)	
TBLH BMC (kg)	0.9 (0.2)	0.9 (0.2)	0.46
TBLH lean mass (kg)	21.8 (2.9)	21.8 (3.1)	0.90
TBLH fat mass (kg)	6.7 (4.4 to 10.3)	7.2 (4.0 to 12.0)	0.49

The values are means (SD), medians (IQR) or numbers (percentages) of children, and *p*-values are for the differences between females and males. Differences between included and excluded children were tested with independent samples *t* test for continuous variables with normal distributions, with Mann-Whitney U test for continuous variables with skewed distributions, and with Fishers exact test for categorical variables. Bold emphasis indicates statistical significance at $p < 0.05$.

Number of included and excluded children: 290 and 147 for age, stature, body weight, and IOTF weight status; 290 and 130 for pubertal status; 290 and 127 for TBLH BMC, TBLH lean mass, and TBLH fat mass

BMI-SDS, body mass index standard deviation score; IOTF, International Obesity Task Force; TBLH, total-body-less-head; BMC, bone mineral content.

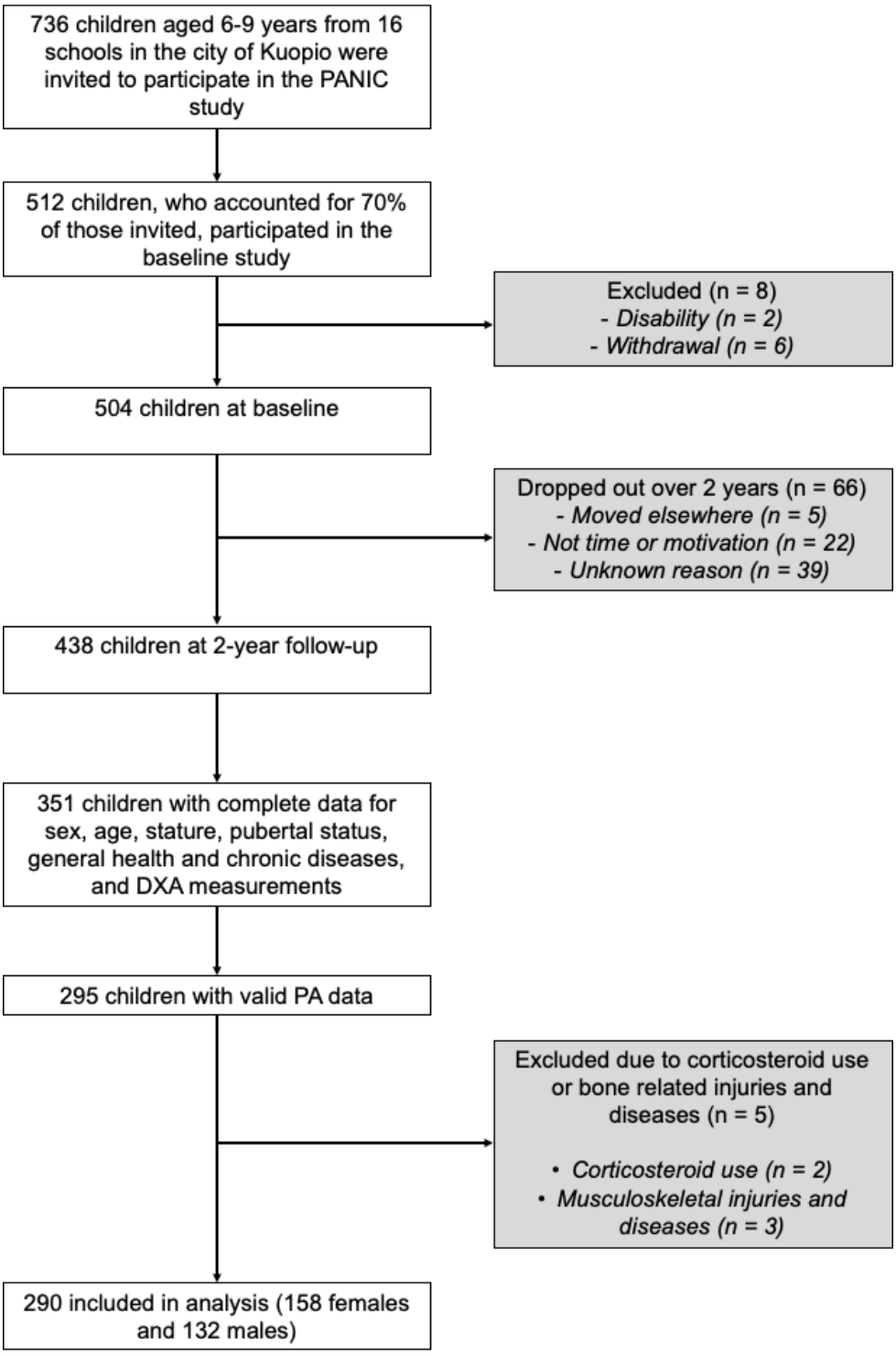


Figure 6.1 Participant flow chart.

PANIC, Physical Activity and Nutrition in Children; DXA, Dual-energy X-ray absorptiometry; PA, Physical activity.

6.3.2 Assessment of General Health and Pubertal Status

General health and pubertal status were assessed as described in Section 3.3 and Section 3.4.

6.3.3 Anthropometry

Stature and body weight were assessed as described in Section 3.5. BMI (kg/m2) was calculated, and the BMI cut-offs were applied to classify children as thin, normal weight, or living with overweight or obesity as it related to their weight status (Cole and Lobstein, 2012).

6.3.4 Assessment of Bone Mineral Content and Body Composition

TBLH, lower-limb and upper-limb BMC (kg), lean mass (kg), and fat mass (kg) were measured as described in Section 3.6. TBLH BMC, lower-limb BMC, and upper-limb BMC were used as the bone outcomes of interest, as evidence indicates that for pre- and early-pubertal children BMC is a more accurate and reliable measure than aBMD (Wren *et al.*, 2005).

6.3.5 Assessment of Physical Activity

PA was assessed using Actiheart (CamNtech Ltd, Papworth, UK), as described in Section 3.7.1. MVPA was modelled from the combined sensing signal, as described in Section 3.7.1.3, using a branched equation framework (Brage *et al.*, 2004; Collings *et al.*, 2017). The acceleration data were summarised in counts, and converted to the International System of Units unit of m/s^2 by multiplying Actiheart counts by 0.003 (Brage *et al.*, 2005). PA data were expressed as the fraction of time spent at a given intensity to account for differences in wear time (Brage *et al.*, 2013; Collings *et al.*, 2017). For the

present analysis, we primarily used the uniaxial acceleration signal as the PA exposure, as mechanical loading is more relevant to bone than PA energy expenditure (Janz *et al.*, 2003).

6.3.5.1 Intensity Distribution

The process for calculating the intensity-gradient is based on the method previously described by Rowlands and colleagues (Rowlands *et al.*, 2018). To calculate the intensity-gradient, the acceleration signal was summarised as a fraction of wear time spent in 25 acceleration thresholds (m/s^2) (0.075, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 6, 7, 8, 9) across the movement intensity continuum. To generate the variable, the natural log of intensity (the midpoint of each intensity bin) and the fraction of wear time (adjusted for diurnal imbalance in non-wear) accumulated in each intensity bin was calculated. These variables were entered into a regression model, with the natural log of fraction of time accumulated as the outcome and the natural log of the midpoint of the intensity bin as the predictor. The regression equation is as follows:

$$\log(fraction\ of\ wear\ time\ accumulated\ at\ each\ intensity) = b_0 + b_1(\log(midpoint\ of\ each\ intensity\ bin))$$

This provided a measure of a participant's PA intensity distribution, with a more negative slope indicating relatively more time at low intensities and less time at high intensities, and a less negative slope indicating relatively less time at low intensities and more time at high intensities (Figure 6.2).

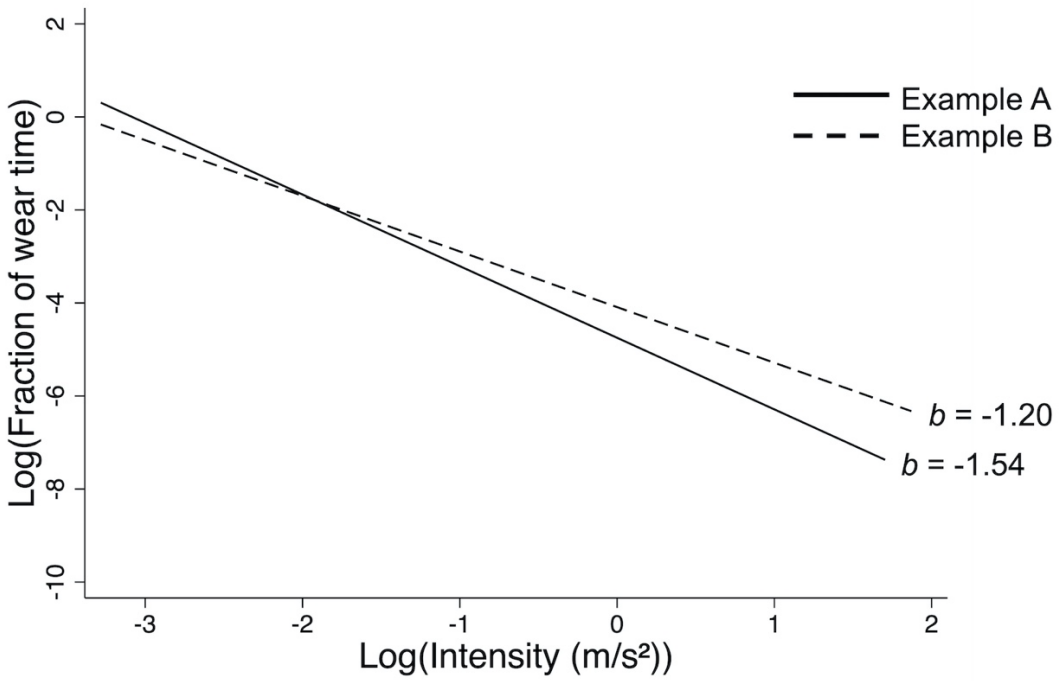


Figure 6.2. Example of the fitted regression line of log-transformed intensity and fraction of wear time.

These examples are from a 24-hour protocol and adjusted for diurnal imbalance in non-wear, based on two participant's intensity distributions. Participant A (solid line) spent less of their total wear time at a high-intensity of PA, resulting in a lower intensity-gradient (b = -1.54) compared to participant B (dotted line) who has a higher intensity-gradient (b = -1.20).

The R^2 (indicative of goodness of fit of the linear model), gradient (b_1), 95% CI for the gradient, and intercept (b_0) of the regression equation were recorded for each participant (Rowlands *et al.*, 2018).

The process for calculating the intensity-gradient is shown in Figure 6.3.

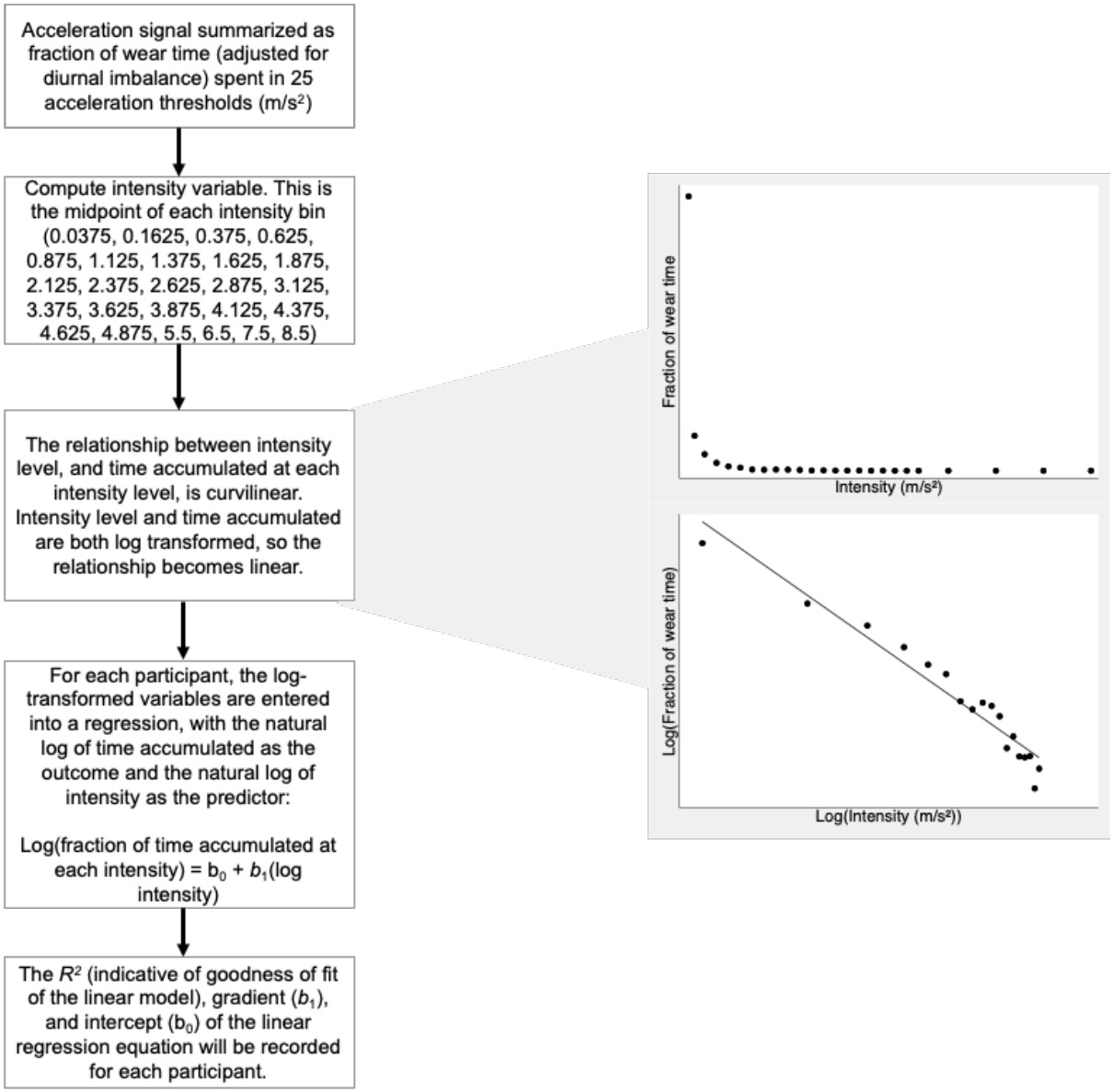


Figure 6.3. Flow chart demonstrating the process of calculating the intensity gradient.

Example plots are from the raw and log-transformed intensity distribution from one participant, with the fitted linear regression line for the log-transformed data.

The mean R^2 of the log-log regression, reflective of the fit of the regression line, was 0.89 (SD = 0.03, range = 0.77 to 0.96). The cases with a lower R^2, around 0.80, indicating a poorer linear fit (Figure 6.4), tended to have relatively more very light intensity activity, and relatively less sedentary time and high-intensity activity compared to cases with a higher R^2 (Figure 6.5). In these cases, the

intensity-gradient was mid-range in the sample, indicating that although the fit was worse, the intensity-gradient was still capturing the distribution of intensity across the spectrum.

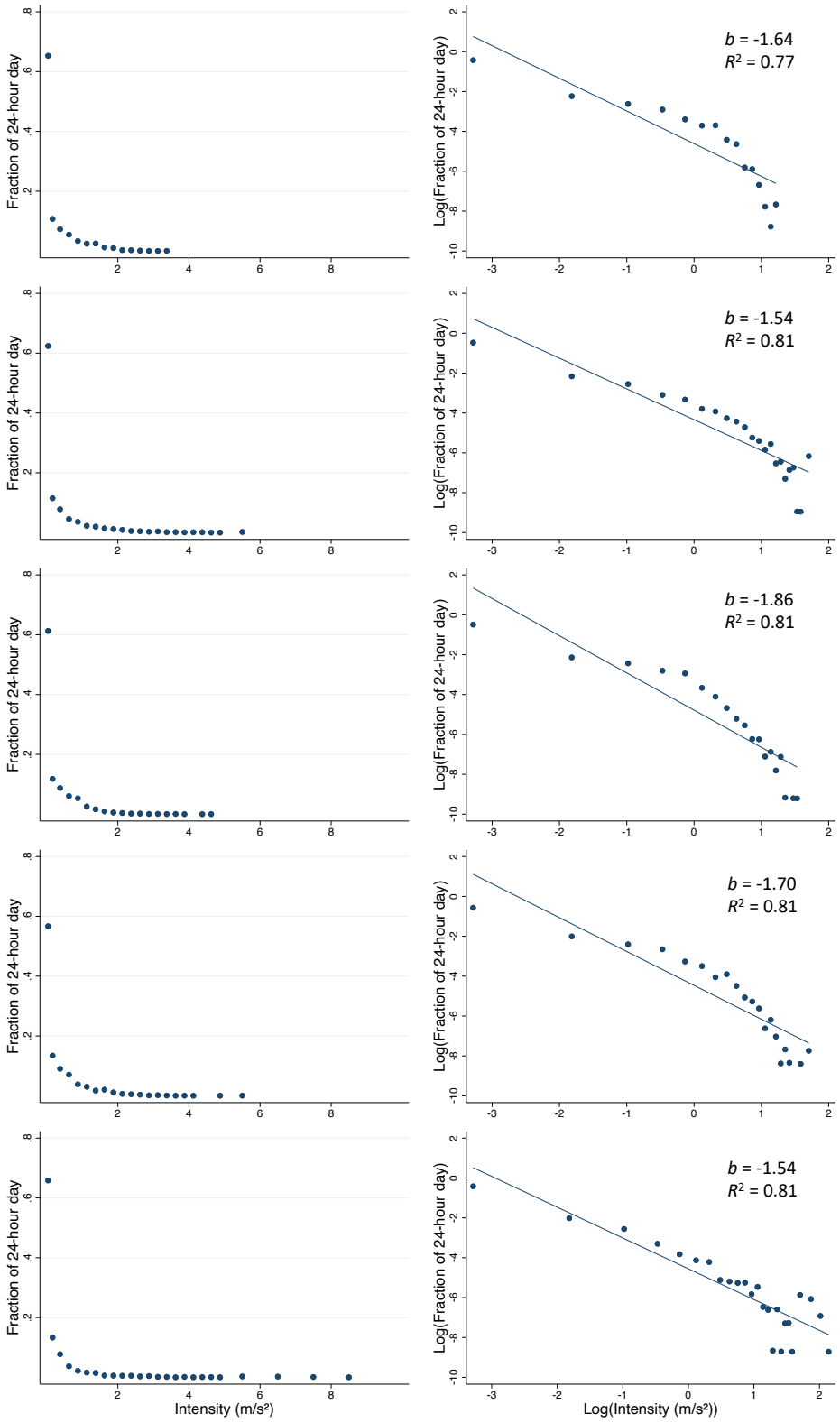


Figure 6.4. Examples of intensity gradient plots with poorer linear fits.

Example plots are from the raw and log-transformed intensity distribution from five participants, with the fitted linear regression line for the log-transformed data. The slope (*b*) and R^2, indicative of the model fit, are shown.

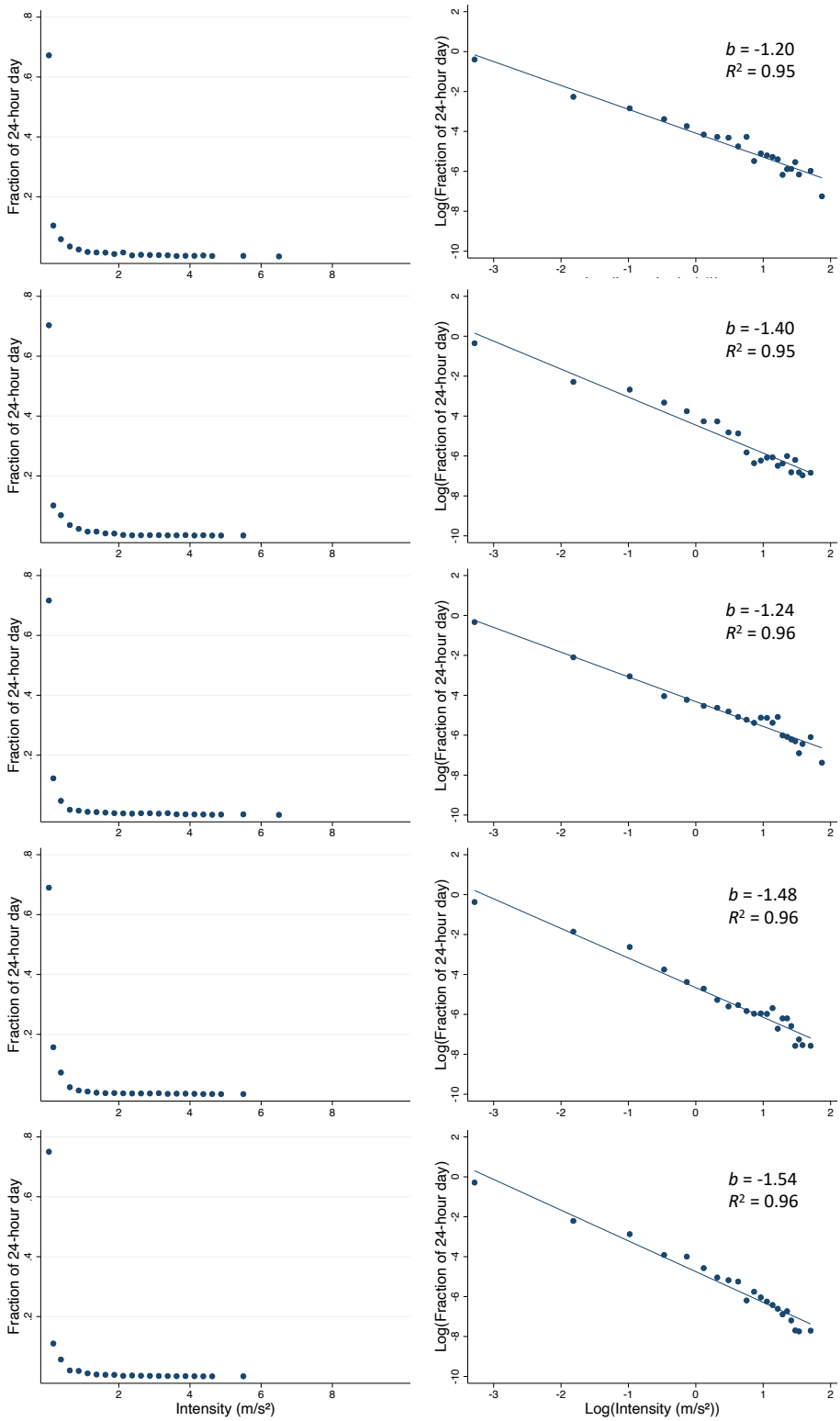


Figure 6.5 Examples of intensity gradient plots with better linear fits.

Example plots are from the raw and log-transformed intensity distribution from five participants, with the fitted linear regression line for the log-transformed data. The slope (b) and R^2, indicative of the model fit, are shown.

6.3.5.2 Average-Acceleration

The average-acceleration is calculated as the mean acceleration across wear time, adjusted for diurnal imbalance in non-wear, providing a proxy for the daily volume of PA (Rowlands *et al.*, 2018).

6.3.5.3 Translational Metrics

Translational metrics were calculated as the intensity above which a child's most active 2, 5, 10, 15, 30, 60, 120, 240, and 480 minutes (MX metrics, whereby X = time in minutes) were accumulated. These metrics provide an illustration of how PA is accumulated (Rowlands *et al.*, 2019a). Levels of acceleration associated with different activities were applied to allow interpretation of MX metrics. Activities such as skipping, running, and soccer were characterised as acceleration > 4 m/s^2, based on data in children aged 8 years collected at the hip (Janz *et al.*, 2003), 13 years collected at the trunk (Corder *et al.*, 2005), and 6 to 16 years collected at the hip (Puyau *et al.*, 2002). Brisk walking (5.2 km/hour) was characterised as acceleration ~1.5 m/s^2, and slow walking (3.2 km/hour) was characterised as acceleration ~0.75 m/s^2, based on data in children aged 13 years collected at the trunk (Corder *et al.*, 2005). As some of the previous studies used Actigraph accelerometers, Actigraph counts were converted to Actiheart counts by dividing by 5, and then converted to m/s^2, as the International System of Units, by multiplying by 0.003 (Brage *et al.*, 2005; Harvey *et al.*, 2012).

6.3.6 Statistical Analysis

Analyses were performed with Stata/SE for Mac software, Version 16.1 (StataCorp LLC, College Station, TX, USA), and radar plots created in R software (R Core Team, 2021). As there were no differences between the included and excluded children in terms of age, stature, body weight, BMI categories, pubertal status, and TBLH BMC, lean, and fat mass, we proceeded with a complete-case analysis (Table 6.1). The means and SDs or the medians and IQR were calculated for the total study sample and stratified by sex. Independent samples t-tests, Mann-Whitney U tests, and Fisher's exact tests were used to test for sex differences in participant characteristics. We stratified all further analyses by sex, based on the biological differences between females and males in the studied age group (Baxter-Jones *et al.*, 2003).

Linear regression was used to assess the associations of average-acceleration and intensity-gradient with TBLH BMC, lean mass, and fat mass. Activity variables were mean-centred for analysis. Model 1 included the activity variable (average-acceleration or intensity-gradient) adjusted for age, stature, pubertal status, and accelerometer wear time. Adjusting for age, stature and pubertal status is recommended when examining BMC in paediatric populations (International Society for Clinical Densitometry, 2019b). Model 2 included additional adjustment for the alternate activity metric (average-acceleration or intensity-gradient), and Model 3 additionally included the interaction term for average-acceleration by intensity-gradient (International Society for Clinical Densitometry, 2019b). For BMC as the outcome, Models 4 and 5 included additional adjustment for lean and fat mass, respectively, and Model 6 included adjustment for lean and fat mass (Tobias *et al.*, 2007). For lean mass as the

outcome, the final model (Model 5) included adjustment for fat mass, and for fat mass at the outcome, the final model (Model 4) included adjustment for lean mass. Analysis was repeated with MVPA as the PA exposure variable. Although the PANIC study included a lifestyle intervention, there were no differences between the intervention and control group in terms of TBLH BMC, lower-limb BMC, upper-limb BMC, lean mass, fat mass, average-acceleration, intensity-gradient, and MVPA. We therefore did not adjust for the intervention in our analyses. Unstandardised regression coefficients (ß), their 95% CIs and p-values were reported. The alpha was set as 0.05.

To present descriptive MX metrics, the females and males were stratified into four groups based on the sex-specific means for average-acceleration and intensity-gradient. Children were split into 1) low-volume (average-acceleration < mean) and low-intensity (intensity-gradient < mean), 2) low-volume (average-acceleration < mean) and high-intensity (intensity-gradient ≥ mean), 3) high-volume (average-acceleration ≥ mean) and low-intensity (intensity-gradient < mean), and 4) high-volume (average-acceleration ≥ mean) and high-intensity (intensity-gradient ≥ mean). This allows the description of PA patterns in children with a similar volume but varying intensity distribution, and vice versa. The mean and standard error of the group MX metrics based on the raw data and standardised based on the sex-specific mean were visualised on radar plots, using an openly accessible R script (Rowlands *et al.*, 2019a; Maylor *et al.*, 2021). To demonstrate how PA could be accumulated for a 1 SD greater average-acceleration, we applied the calculation by Rowlands and colleagues, assuming that the introduced activity would replace time spent at the average-acceleration: 1440 x (SD of average-acceleration)/(acceleration associated with

a specific activity – average-acceleration) (Rowlands *et al.*, 2018). In our analysis, the SD of average-acceleration was 0.0579 m/s^2 in females, and 0.0696 m/s^2 in males.

We used the following calculation to demonstrate how this increase could be achieved through high-intensity activities equivalent to 4 m/s^2 in females:

$$1440 \times (0.0579) \div (4 - 0.1914) = 21.9\ minutes$$

We used the following calculation to demonstrate how this increase could be achieved through activities equivalent to brisk walking (1.5 m/s^2) in females:

$$1440 \times (0.0579) \div (1.5 - 0.1914) = 63.7\ minutes$$

We used the following calculation to demonstrate how this increase could be achieved through light-intensity activities equivalent to 0.75 m/s^2 in females:

$$1440 \times (0.0579) \div (0.75 - 0.1914) = 149.3\ minutes$$

We used the following calculation to demonstrate how this increase could be achieved through high-intensity activities equivalent to 4 m/s^2 in males:

$$1440 \times (0.0696) \div (4 - 0.2151) = 26.5\ minutes$$

We used the following calculation to demonstrate how this increase could be achieved through activities equivalent to brisk walking (1.5 m/s^2) in males:

$$1440 \times (0.0696) \div (1.5 - 0.2151) = 78.0\ minutes$$

We used the following calculation to demonstrate how this increase could be achieved through light-intensity activities equivalent to 0.75 m/s^2 in males:

$$1440 \times (0.0696) \div (0.75 - 0.2151) = 187.4\, minutes$$

6.4 Results

6.4.1 Descriptive Characteristics

In this study, females were younger, shorter, and lighter, with lower levels of BMC, lean mass, MVPA, and lower average-acceleration compared to males (Table 6.2). The proportion of pubertal children was greater in females than males, whereas more males were prepubertal. The correlations between the average-acceleration and intensity-gradient was 0.66 (p < 0.001), between the average-acceleration and MVPA was 0.62 (p < 0.001), and between the intensity-gradient with MVPA was 0.39 (p < 0.001).

Table 6.2 Descriptive characteristics of children

	Total (n = 290)	Females (n = 158)	Males (n = 132)	p-value for sex difference
Age (years)	9.8 (0.4)	9.7 (0.4)	9.8 (0.5)	**0.02**
Stature (cm)	140.8 (6.1)	140.1 (6.3)	141.7 (5.7)	**0.03**
Body weight (kg)	32.9 (29.5 to 38.7)	32.2 (28.8 to 35.9)	34.3 (30.1 to 39.5)	**0.01**
BMI-SDS	-0.2 (1)	-0.2 (1)	-0.1 (1.1)	0.34
Pubertal status (n, %)				
prepubertal	218 (75.2)	103 (65.2)	115 (87.1)	**< 0.001**
pubertal	72 (24.8)	55 (34.8)	17 (12.9)	
IOTF definition (n, %)				
thin	31 (10.7)	15 (9.5)	16 (12.1)	0.52
normal weight	216 (74.5)	123 (77.9)	93 (70.5)	
overweight	33 (11.4)	16 (10.1)	17 (12.9)	
obese	10 (3.4)	4 (2.5)	6 (4.5)	
TBLH BMC (kg)	0.9 (0.2)	0.9 (0.2)	1 (0.2)	**0.002**
TBLH lean mass (kg)	21.8 (2.9)	20.8 (2.7)	23.0 (2.7)	**< 0.001**
TBLH fat mass (kg)	6.7 (4.4 to 10.3)	6.7 (5 to 10.9)	6.7 (3.8 to 10)	0.16
MVPA (mins/day)	89.7 (57.1 to 127.3)	71.5 (51.3 to 99.2)	115.2 (78.9 to 161.9)	**< 0.001**
PA guidelines* (n, %)				
< mean 60 mins MVPA/day	77 (26.7)	54 (34.4)	23 (17.6)	**0.001**
≥ mean 60 mins MVPA/day	211 (73.3)	103 (65.6)	108 (82.4)	
Average-acceleration (m/s^2)	0.20 (0.06)	0.19 (0.06)	0.22 (0.07)	**0.002**
Intensity-gradient	-1.67 (0.16)	-1.69 (0.14)	-1.65 (0.18)	0.07
Wear time (hours)	103.2 (14.8)	103.0 (13.5)	103.5 (16.3)	0.79
MX metrics (m/s^2)				
M2	4.13 (3.50 to 4.75)	4.00 (3.50 to 4.75)	4.25 (3.50 to 5.00)	0.53
M5	3.50 (2.75 to 4.00)	3.25 (2.75 to 4.00)	3.50 (3.00 to 4.13)	0.21
M10	2.75 (2.25 to 3.25)	2.75 (2.25 to 3.25)	2.75 (2.50 to 3.50)	0.09
M15	2.50 (2.00 to 3.00)	2.50 (2.00 to 2.75)	2.50 (2.25 to 3.00)	0.06
M30	2.00 (1.50 to 2.25)	1.75 (1.50 to 2.25)	2.00 (1.75 to 2.50)	**0.005**
M60	1.25 (1.00 to 1.50)	1.25 (1.00 to 1.50)	1.50 (1.25 to 1.75)	**< 0.001**
M120	0.75 (0.75 to 1.00)	0.75 (0.50 to 1.00)	1.00 (0.75 to 1.00)	**< 0.001**
M1/3	0.08 (0.08 to 0.25)	0.08 (0.08 to 0.25)	0.08 (0.08 to 0.25)	0.66

The values are means (standard deviations), medians (interquartile ranges) or numbers (percentages) of children, and *p*-values are for the differences between females and males. Differences between females and males were tested with independent samples *t* test for continuous variables with normal distributions, with Mann-Whitney U test for continuous variables with skewed distributions, and with Fishers exact test for categorical variables. Bold emphasis indicates statistical significance at $p < 0.05$.

* For PA guidelines, n = 288 (157 females) due to poor heart rate recordings for two participants preventing the application of the branched equation modelling.

BMI-SDS, Body mass index standard deviation score; IOTF, International Obesity Task Force; TBLH, Total-body-less-head; BMC, bone mineral content; MVPA, moderate-to-vigorous physical activity, MX, the intensity above which a child's most active X minutes are accumulated; M2, the intensity above which a child's most active 2 minutes are accumulated; M5, the intensity above which a child's most active 5 minutes are accumulated; M10, the intensity above which a child's most active 10 minutes are accumulated; M15, the intensity above which a child's most active 15 minutes are accumulated; M30, the intensity above which a child's most active 30 minutes are accumulated; M60, the intensity above which a child's most active 60 minutes are accumulated; M120, the intensity above which a child's most active 120 minutes are accumulated; M1/3, the intensity above which a child's most active 480 minutes are accumulated.

6.4.2 Associations Between Physical Activity Volume (Average-Acceleration), Physical Activity Intensity Distribution (Intensity-Gradient) and Bone Mineral Content

In females, average-acceleration was positively associated with TBLH BMC (Table 6.3, Model 1), though this association became non-significant after adjustment for intensity-gradient (Model 2), the product term of intensity-gradient by average-acceleration (Model 3), and lean mass (Model 4). When adjusting for fat mass (Model 5), and lean and fat mass (Model 6), average-acceleration was positively associated with TBLH BMC. Intensity-gradient, and the product of intensity-gradient by average-acceleration, were not associated with TBLH BMC in females. Associations in the fully-adjusted model (Model 6) were non-significant in lower-limb BMC and in upper-limb BMC in females (Table 6.4).

In males, average-acceleration was not associated with TBLH BMC (Table 6.3) in the minimally-adjusted model (Model 1), though this association became significant after adjustment for intensity-gradient (Model 2). After adjustment for the product term of intensity-gradient by average-acceleration (Model 3), and for lean mass (Model 4) this association was non-significant. When adjusting for fat

mass (Model 5), and for lean and fat mass (Model 6), average-acceleration was positively associated with TBLH BMC. Intensity-gradient was not associated with TBLH BMC (Table 6.3) in the minimally-adjusted model (Model 1), or after adjustment for average-acceleration (Model 2). When adjusting for the product of intensity-gradient by average-acceleration (Model 3), lean mass (Model 4), fat mass (Model 5), and lean and fat mass (Model 6), intensity-gradient was negatively associated with TBLH BMC. The product of intensity-gradient by average-acceleration was not associated with TBLH BMC in any model in males. Site-specific analyses showed that in the fully-adjusted model (Model 6), average-acceleration was positively associated with lower-limb BMC, and the association between intensity-gradient with lower-limb BMC was non-significant. In the fully-adjusted model (Model 6), average-acceleration was not associated with upper-limb BMC, and intensity-gradient was negatively associated with upper-limb BMC (Table 6.5).

Table 6.3 Associations of physical activity volume (average-acceleration) and intensity distribution (intensity-gradient) with total-body-less-head bone mineral content, lean mass, and fat mass (n = 290)

	Model 1 (adjusted for age, stature, pubertal status, and wear time)		**Model 2 (Model 1 + alternate activity metric)**		**Model 3 (Model 2 + Intensity X Volume interaction)**		**Model 4 (Model 3 + lean mass)**		**Model 5 (Model 3 + fat mass)**		**Model 6 (Model 3 + lean mass and fat mass)**	
	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*
TBLH BMC												
Females												
Intensity[a]	0.092 (-0.032 to 0.216)	0.145	0.009 (-0.144 to 0.163)	0.905	0.019 (-0.137 to 0.175)	0.809	0.034 (-0.099 to 0.167)	0.614	0.041 (-0.076 to 0.157)	0.490	0.050 (-0.048 to 0.149)	0.317
Volume[b]	**0.354 (0.053 to 0.654)**	**0.021**	0.340 (-0.035 to 0.716)	0.075	0.372 (-0.013 to 0.757)	0.058	0.201 (-0.131 to 0.533)	0.233	**0.402 (0.114 to 0.689)**	**0.007**	**0.264 (0.019 to 0.510)**	**0.035**
Intensity X Volume	.	.	.	.	-0.810 (-2.878 to 1.258)	0.440	-1.560 (-3.338 to 0.219)	0.085	-0.597 (-2.144 to 0.951)	0.447	-1.209 (-2.525 to 0.106)	0.071
Males												
Intensity[a]	-0.043 (-0.167 to 0.080)	0.487	-0.167 (-0.336 to 0.003)	0.054	**-0.171 (-0.340 to -0.001)**	**0.048**	**-0.164 (-0.306 to -0.022)**	**0.024**	**-0.133 (-0.259 to -0.008)**	**0.037**	**-0.134 (-0.241 to -0.027)**	**0.015**
Volume[b]	0.160 (-0.162 to 0.483)	0.327	**0.464 (0.020 to 0.908)**	**0.041**	0.389 (-0.069 to 0.848)	0.095	0.143 (-0.248 to 0.534)	0.471	**0.698 (0.354 to 1.041)**	**0.000**	**0.471 (0.170 to 0.773)**	**0.002**
Intensity X Volume	.	.	.	.	0.857 (-0.491 to 2.204)	0.211	0.771 (-0.360 to 1.903)	0.180	-0.037 (-1.046 to 0.973)	0.943	0.039 (-0.825 to 0.903)	0.928
TBLH lean mass												
Females												
Intensity[a]	1.104 (-0.582 to 2.790)	0.198	-0.153 (-2.232 to 1.926)	0.884	-0.386 (-2.487 to 1.714)	0.717	.	.	-0.302 (-2.371 to 1.767)	0.774	.	.
Volume[b]	**4.935 (0.867 to 9.003)**	**0.018**	**5.158 (0.076 to 10.241)**	**0.047**	4.398 (-0.788 to 9.584)	0.096	.	.	4.512 (-0.595 to 9.619)	0.083	.	.
Intensity X Volume	.	.	.	.	19.270 (-8.596 to 47.137)	0.174	.	.	20.103 (-7.342 to 47.549)	0.150	.	.
Males												
Intensity[a]	1.435 (-0.148 to 3.018)	0.075	-0.151 (-2.326 to 2.025)	0.891	-0.160 (-2.346 to 2.025)	0.885	.	.	0.027 (-2.094 to 2.149)	0.980	.	.
Volume[b]	**5.694 (1.618 to 9.770)**	**0.007**	**5.969 (0.273 to 11.664)**	**0.040**	5.794 (-0.122 to 11.710)	0.055	.	.	**7.333 (1.513 to 13.153)**	**0.014**	.	.
Intensity X Volume	.	.	.	.	2.004 (-15.377 to 19.386)	0.820	.	.	-2.457 (-19.551 to 14.637)	0.776	.	.
TBLH fat mass												
Females												
Intensity[a]	-1.828 (-6.286 to 2.630)	0.419	-1.326 (-6.894 to 4.242)	0.639	-1.185 (-6.844 to 4.473)	0.680	-0.984 (-6.558 to 4.589)	0.728	.	.	.	.
Volume[b]	-3.990 (-14.894 to 6.913)	0.471	-2.059 (-15.672 to 11.554)	0.765	-1.599 (-15.572 to 12.373)	0.821	-3.884 (-17.769 to 10.001)	0.581	.	.	.	.
Intensity X Volume	.	.	.	.	-11.640 (-86.720 to 63.440)	0.760	-21.651 (-96.032 to 52.729)	0.566	.	.	.	.
Males												
Intensity[a]	**-5.017 (-9.469 to -0.566)**	**0.027**	-1.768 (-7.933 to 4.397)	0.571	-1.997 (-8.101 to 4.107)	0.518	-1.880 (-7.794 to 4.035)	0.530	.	.	.	.
Volume[b]	**-15.450 (-27.015 to -3.885)**	**0.009**	-12.231 (-28.371 to 3.910)	0.136	-16.376 (-32.897 to 0.145)	0.052	**-20.624 (-36.872 to -4.375)**	**0.013**	.	.	.	.
Intensity X Volume	.	.	.	.	47.458 (-1.082 to 95.999)	0.055	45.989 (-1.053 to 93.030)	0.055	.	.	.	.

The values are unstandardised regression coefficients (ß), their 95% confidence intervals (CI), and *p* values from linear regression models.

Model 1 included the activity variable (average-acceleration or intensity-gradient) adjusted for age, stature, pubertal status, and accelerometer wear time, Model 2 included additional adjustment for the alternate activity metric (average-acceleration or intensity-gradient), Model 3 additionally included the interaction term for average-acceleration by intensity-gradient. For BMC as the outcome, Model 4 included additional adjustment for lean mass, Model 5 included additional adjustment for fat mass, and Model 6 included adjustment for lean and fat mass. For lean mass as the outcome, the final model (Model 5) included adjustment for fat mass, and for fat mass at the outcome, the final model (Model 4) included adjustment for lean mass.

Bold emphasis indicates statistical significance at $p < 0.05$.

[a]Intensity is reflected in the intensity-gradient, calculated from data collected with a 24-hour protocol, adjusted for diurnal imbalance in non-wear, as the regression line from log-log plot of intensity (x) and fraction of wear time accumulated (y).

[b]Volume is reflected in the average-acceleration across data, collected with a 24-hour protocol, adjusted for diurnal imbalance in non-wear.

Activity variables were mean-centred before entry into analysis, with interaction terms computed from the centred scores.

TBLH, Total-body-less-head; BMC, bone mineral content.

Table 6.4 Associations of physical activity volume (average-acceleration) and intensity distribution (intensity-gradient) with lower-limb and upper-limb bone mineral content, lean mass, and fat mass in 158 females

	Model 1 (adjusted for age, stature, pubertal status, and wear time)		**Model 2 (Model 1 + alternate activity metric)**		**Model 3 (Model 2 + Intensity X Volume interaction)**		**Model 4 (Model 3 + limb-specific lean mass)**		**Model 5 (Model 3 + limb-specific fat mass)**		**Model 6 (Model 3 + limb-specific lean mass and fat mass)**	
	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*
Lower-limb BMC												
Intensity[a]	46.052 (-12.802 to 104.905)	0.124	15.354 (-57.709 to 88.416)	0.679	18.929 (-55.258 to 93.115)	0.615	25.829 (-37.916 to 89.574)	0.425	23.684 (-31.224 to 78.593)	0.395	28.480 (-18.166 to 75.127)	0.230
Volume[b]	**148.325 (5.268 to 291.383)**	**0.042**	125.957 (-52.674 to 304.589)	0.166	137.616 (-45.576 to 320.807)	0.140	49.130 (-109.991 to 208.250)	0.543	**158.560 (22.937 to 294.183)**	**0.022**	87.583 (-29.041 to 204.207)	0.140
Intensity X Volume					-295.433 (-1,279.780 to 688.914)	0.554	-661.329 (-1,512.454 to 189.796)	0.127	-155.748 (-884.640 to 573.145)	0.673	-455.382 (-1,079.197 to 168.433)	0.151
Upper-limb BMC												
Intensity[a]	9.286 (-6.958 to 25.531)	0.260	1.391 (-18.792 to 21.575)	0.892	2.514 (-17.973 to 23.001)	0.809	9.808 (-5.677 to 25.292)	0.213	4.209 (-14.806 to 23.224)	0.662	10.403 (-4.201 to 25.008)	0.161
Volume[b]	34.421 (-5.078 to 73.921)	0.087	32.394 (-16.952 to 81.741)	0.197	36.055 (-14.534 to 86.645)	0.161	3.820 (-34.732 to 42.372)	0.845	37.090 (-9.836 to 84.017)	0.120	7.037 (-29.345 to 43.420)	0.703
Intensity X Volume					-92.768 (-364.601 to 179.064)	0.501	-194.592 (-400.136 to 10.952)	0.063	-67.590 (-319.925 to 184.746)	0.597	-169.401 (-363.555 to 24.752)	0.087
Lower-limb lean mass												
Intensity[a]	536.556 (-196.191 to 1,269.303)	0.150	-58.680 (-959.962 to 842.602)	0.898	-163.741 (-1,073.855 to 746.373)	0.723	.	.	-146.893 (-1,042.679 to 748.893)	0.746	.	.
Volume[b]	**2,356.843 (593.029 to 4,120.657)**	**0.009**	**2,442.331 (238.772 to 4,645.889)**	**0.030**	2,099.707 (-147.682 to 4,347.095)	0.067	.	.	2,173.905 (-38.669 to 4,386.479)	0.054	.	.
Intensity X Volume					8,682.442 (-3,393.505 to 20,758.389)	0.157	.	.	9,177.293 (-2,713.979 to 21,068.565)	0.129	.	.
Upper-limb lean mass												
Intensity[a]	65.731 (-191.948 to 323.411)	0.615	-143.638 (-460.583 to 173.306)	0.372	-172.835 (-493.693 to 148.023)	0.289	.	.	-159.155 (-475.021 to 156.711)	0.321	.	.
Volume[b]	**649.813 (27.940 to 1,271.686)**	**0.041**	**859.071 (84.168 to 1,633.975)**	**0.030**	763.857 (-28.454 to 1,556.167)	0.059	.	.	772.210 (-7.325 to 1,551.745)	0.052	.	.
Intensity X Volume					2,412.845 (-1,844.496 to 6,670.186)	0.265	.	.	2,616.037 (-1,575.714 to 6,807.788)	0.219	.	.
Lower-limb fat mass												
Intensity[a]	-582.718 (-2,367.452 to 1,202.015)	0.520	-291.318 (-2,519.748 to 1,937.112)	0.797	-214.931 (-2,478.985 to 2,049.124)	0.851	-135.499 (-2,364.588 to 2,093.590)	0.905	.	.	.	.
Volume[b]	-1,620.059 (-5,981.833 to 2,741.716)	0.464	-1,195.654 (-6,643.979 to 4,252.671)	0.665	-946.541 (-6,537.281 to 4,644.199)	0.738	-1,965.119 (-7,529.352 to 3,599.113)	0.486	.	.	.	.
Intensity X Volume					-6,312.780 (-36,353.640 to 23,728.080)	0.679	-10,524.677 (-40,287.374 to 19,238.020)	0.486	.	.	.	.
Upper-limb fat mass												
Intensity[a]	-181.354 (-629.054 to 266.346)	0.425	-145.406 (-704.670 to 413.859)	0.608	-123.253 (-691.349 to 444.842)	0.669	-63.117 (-624.138 to 497.905)	0.824	.	.	.	.
Volume[b]	-359.334 (-1,454.714 to 736.045)	0.518	-147.501 (-1,514.856 to 1,219.854)	0.832	-75.259 (-1,478.085 to 1,327.568)	0.916	-341.038 (-1,737.788 to 1,055.712)	0.630	.	.	.	.
Intensity X Volume					-1,830.692 (-9,368.533 to 5,707.148)	0.632	-2,670.226 (-10,117.183 to 4,776.730)	0.480	.	.	.	.

The values are unstandardised regression coefficients (ß), 95% confidence intervals (CI), and *p* values from linear regression models. Model 1 included the activity variable (average-acceleration or intensity-gradient) adjusted for age, stature, pubertal status, and accelerometer wear time, Model 2 included additional adjustment for the alternate activity metric (average-acceleration or intensity-gradient), Model 3 additionally included the interaction term for average-acceleration by intensity-gradient. For BMC as the outcome, Model 4 included additional adjustment for lean mass, Model 5 included additional adjustment for fat mass, and Model 6 included adjustment for lean and fat mass. For lean mass as the outcome, the final model (Model 5) included adjustment for fat mass, and for fat mass at the outcome, the final model (Model 4) included adjustment for lean mass.

Bold emphasis indicates statistical significance at $p < 0.05$.

[a]Intensity is reflected in the intensity-gradient, calculated from data collected with a 24-hour protocol adjusted for diurnal imbalance in non-wear, as the regression line from log-log plot of intensity (x) and fraction of wear time accumulated (y).

[b]Volume is reflected in the average-acceleration across data, collected with a 24-hour protocol adjusted for diurnal imbalance in non-wear.

Activity variables were mean-centred before entry into analysis, with interaction terms computed from the centred scores.

BMC, bone mineral content.

Table 6.5 Associations of physical activity volume (average-acceleration) and intensity distribution (intensity-gradient) with lower-limb and upper-limb bone mineral content, lean mass, and fat mass in 112 males

	Model 1 (adjusted for age, stature, pubertal status, and wear time)		**Model 2 (Model 1 + alternate activity metric)**		**Model 3 (Model 2 + Intensity X Volume interaction)**		**Model 4 (Model 3 + limb-specific lean mass)**		**Model 5 (Model 3 + limb-specific fat mass)**		**Model 6 (Model 3 + limb-specific lean mass and fat mass)**	
	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*
Lower-limb BMC												
Intensity[a]	-6.010 (-67.683 to 55.662)	0.847	-45.045 (-130.657 to 40.566)	0.300	-47.195 (-132.645 to 38.255)	0.276	-58.342 (-127.263 to 10.579)	0.096	-29.400 (-88.947 to 30.148)	0.330	-40.262 (-89.720 to 9.195)	0.110
Volume[b]	64.901 (-96.174 to 225.976)	0.427	146.924 (-77.205 to 371.054)	0.197	108.059 (-123.228 to 339.347)	0.357	38.808 (-148.341 to 225.957)	0.682	**285.590 (121.767 to 449.413)**	**0.001**	**206.170 (68.744 to 343.596)**	**0.004**
Intensity X Volume					444.936 (-234.608 to 1,124.480)	0.197	317.243 (-231.301 to 865.788)	0.255	-17.883 (-497.437 to 461.671)	0.941	-23.494 (-421.129 to 374.141)	0.907
Upper-limb BMC												
Intensity[a]	-15.001 (-30.813 to 0.812)	0.063	**-32.703 (-54.336 to -11.069)**	**0.003**	**-33.132 (-54.780 to -11.485)**	**0.003**	-11.868 (-27.338 to 3.601)	0.131	**-31.278 (-49.498 to -13.057)**	**0.001**	**-14.347 (-28.457 to -0.238)**	**0.046**
Volume[b]	7.079 (-34.874 to 49.033)	0.739	**66.628 (9.991 to 123.265)**	**0.022**	**58.861 (0.268 to 117.454)**	**0.049**	5.325 (-36.390 to 47.041)	0.801	**92.858 (42.688 to 143.029)**	**0.000**	33.980 (-5.545 to 73.505)	0.091
Intensity X Volume					88.919 (-83.234 to 261.072)	0.309	86.303 (-33.276 to 205.883)	0.156	-0.949 (-147.869 to 145.972)	0.990	34.648 (-75.964 to 145.259)	0.536
Lower-limb lean mass												
Intensity[a]	632.376 (-65.539 to 1,330.291)	0.075	224.579 (-745.208 to 1,194.366)	0.648	212.803 (-760.159 to 1,185.764)	0.666	.	.	300.559 (-628.816 to 1,229.934)	0.523	.	.
Volume[b]	**1,943.862 (125.583 to 3,762.140)**	**0.036**	1,534.921 (-1,003.961 to 4,073.803)	0.234	1,321.995 (-1,311.523 to 3,955.513)	0.322	.	.	2,197.483 (-359.349 to 4,754.315)	0.091	.	.
Intensity X Volume					2,437.628 (-5,299.903 to 10,175.159)	0.534	.	.	155.247 (-7,329.294 to 7,639.788)	0.967	.	.
Upper-limb lean mass												
Intensity[a]	-149.902 (-395.872 to 96.067)	0.230	**-457.228 (-791.949 to -122.506)**	**0.008**	**-457.500 (-793.841 to -121.159)**	**0.008**	.	.	**-437.362 (-749.677 to -125.048)**	**0.006**	.	.
Volume[b]	324.179 (-320.944 to 969.302)	0.322	**1,156.753 (280.460 to 2,033.047)**	**0.010**	**1,151.838 (241.463 to 2,062.213)**	**0.014**	.	.	**1,521.000 (661.035 to 2,380.965)**	**0.001**	.	.
Intensity X Volume					56.273 (-2,618.497 to 2,731.043)	0.967	.	.	-919.561 (-3,437.910 to 1,598.788)	0.471	.	.
Lower-limb fat mass												
Intensity[a]	**-2,042.183 (-3,808.822 to -275.543)**	**0.024**	-611.230 (-3,053.071 to 1,830.611)	0.621	-699.067 (-3,118.855 to 1,720.720)	0.568	-864.301 (-3,174.390 to 1,445.788)	0.460	.	.	.	.
Volume[b]	**-6,499.014 (-11,077.933 to -1,920.096)**	**0.006**	-5,386.016 (-11,778.705 to 1,006.674)	0.098	**-6,974.167 (-13,523.815 to -424.520)**	**0.037**	**-8,000.648 (-14,273.475 to -1,727.821)**	**0.013**	.	.	.	.
Intensity X Volume					18,181.533 (-1,061.964 to 37,425.030)	0.064	16,288.805 (-2,097.196 to 34,674.805)	0.082	.	.	.	.
Upper-limb fat mass												
Intensity[a]	-454.477 (-923.142 to 14.187)	0.057	-77.149 (-725.022 to 570.724)	0.814	-100.732 (-742.537 to 541.073)	0.757	232.294 (-380.596 to 845.184)	0.455	.	.	.	.
Volume[b]	**-1,560.722 (-2,774.686 to -346.757)**	**0.012**	-1,420.239 (-3,116.357 to 275.879)	0.100	**-1,846.620 (-3,583.796 to -109.444)**	**0.037**	**-2,685.072 (-4,337.798 to -1,032.345)**	**0.002**	.	.	.	.
Intensity X Volume					4,881.308 (-222.683 to 9,985.298)	0.061	**4,840.345 (102.765 to 9,577.925)**	**0.045**	.	.	.	.

The values are regression coefficients (ß), 95% confidence intervals (CI), and *p* values from linear regression models. Model 1 included the activity variable (average-acceleration or intensity-gradient) adjusted for age, stature, pubertal status, and accelerometer wear time, Model 2 included additional adjustment for the alternate activity metric (average-acceleration or intensity-gradient), Model 3 additionally included the interaction term for average-acceleration by intensity-gradient. For BMC as the outcome, Model 4 included additional adjustment for lean mass, Model 5 included additional adjustment for fat mass, and Model 6 included adjustment for lean and fat mass. For lean mass as the outcome, the final model (Model 5) included adjustment for fat mass, and for fat mass at the outcome, the final model (Model 4) included adjustment for lean mass.

Bold emphasis indicates statistical significance at $p < 0.05$.

[a]Intensity is reflected in the intensity-gradient, calculated from data collected with a 24-hour protocol adjusted for diurnal imbalance in non-wear, as the regression line from log-log plot of intensity (x) and fraction of wear time accumulated (y).

[b]Volume is reflected in the average-acceleration across data, collected with a 24-hour protocol adjusted for diurnal imbalance in non-wear.

Activity variables were mean-centred before entry into analysis, with interaction terms computed from the centred scores.

BMC, bone mineral content.

6.4.3 Associations Between Physical Activity Volume (Average-Acceleration), Physical Activity Intensity Distribution (Intensity-Gradient) and Lean Mass

In females, average-acceleration was positively associated with TBLH lean mass (Table 6.3), and this association persisted after adjustment for intensity-gradient (Model 2), though became non-significant after adjustment for the product term of intensity-gradient by average-acceleration (Model 3), and fat mass (Model 5). Intensity-gradient, and the product of intensity-gradient by average-acceleration were not associated with TBLH lean mass in any model. Associations in the upper-limb and lower-limb were also non-significant in the fully-adjusted model (Model 5) (Table 6.4).

In males, average-acceleration was positively associated with TBLH lean mass (Table 6.3), and this association persisted after adjustment for intensity-gradient (Model 2). The association became non-significant when adjusting for the product term of intensity-gradient by average-acceleration (Model 3) but was significant when adjusting for fat mass (Model 5). Intensity-gradient, and the product of intensity-gradient by average-acceleration were not associated with TBLH lean mass in any model. Associations in the fully-adjusted model (Model 5) were non-significant in lower-limb lean mass (Table 6.5). In the upper-limb, average-acceleration was positively associated with lean mass and intensity-gradient was negatively associated with lean mass in the fully-adjusted model (Model 5) (Table 6.5).

6.4.4 Associations Between Physical Activity Volume (Average-Acceleration), Physical Activity Intensity Distribution (Intensity-Gradient) and Fat Mass

In females, average-acceleration, intensity-gradient, and the product of intensity-gradient by average-acceleration were not associated with TBLH fat mass in any model (Table 6.3). This was also the case with lower-limb and upper-limb fat mass (Table 6.4).

In males, average-acceleration was negatively associated with TBLH fat mass (Model 1) (Table 6.3). Although this association was not significant after adjustment for intensity-gradient (Model 2), after additional adjustment for the product term of intensity-gradient by average-acceleration (Model 3) and for lean mass (Model 4) the negative association became significant again. Intensity-gradient was negatively associated with TBLH fat mass (Model 1), though this association was not independent of average-acceleration and lean mass (Model 2 to 4). The product of intensity-gradient by average-acceleration were not associated with TBLH fat mass in any model. In the lower-limb, the associations between average-acceleration, intensity-gradient, and the product of intensity-gradient by average-acceleration were similar in terms of significance and direction to those with TBLH fat mass. In the upper-limb, average-acceleration was negatively associated with fat mass, and the product of intensity-gradient by average-acceleration was positively associated with fat mass (Table 6.5).

The adjusted main effects of average-acceleration and intensity-gradient with TBLH BMC, lean, and fat mass are illustrated in Figure 6.6.

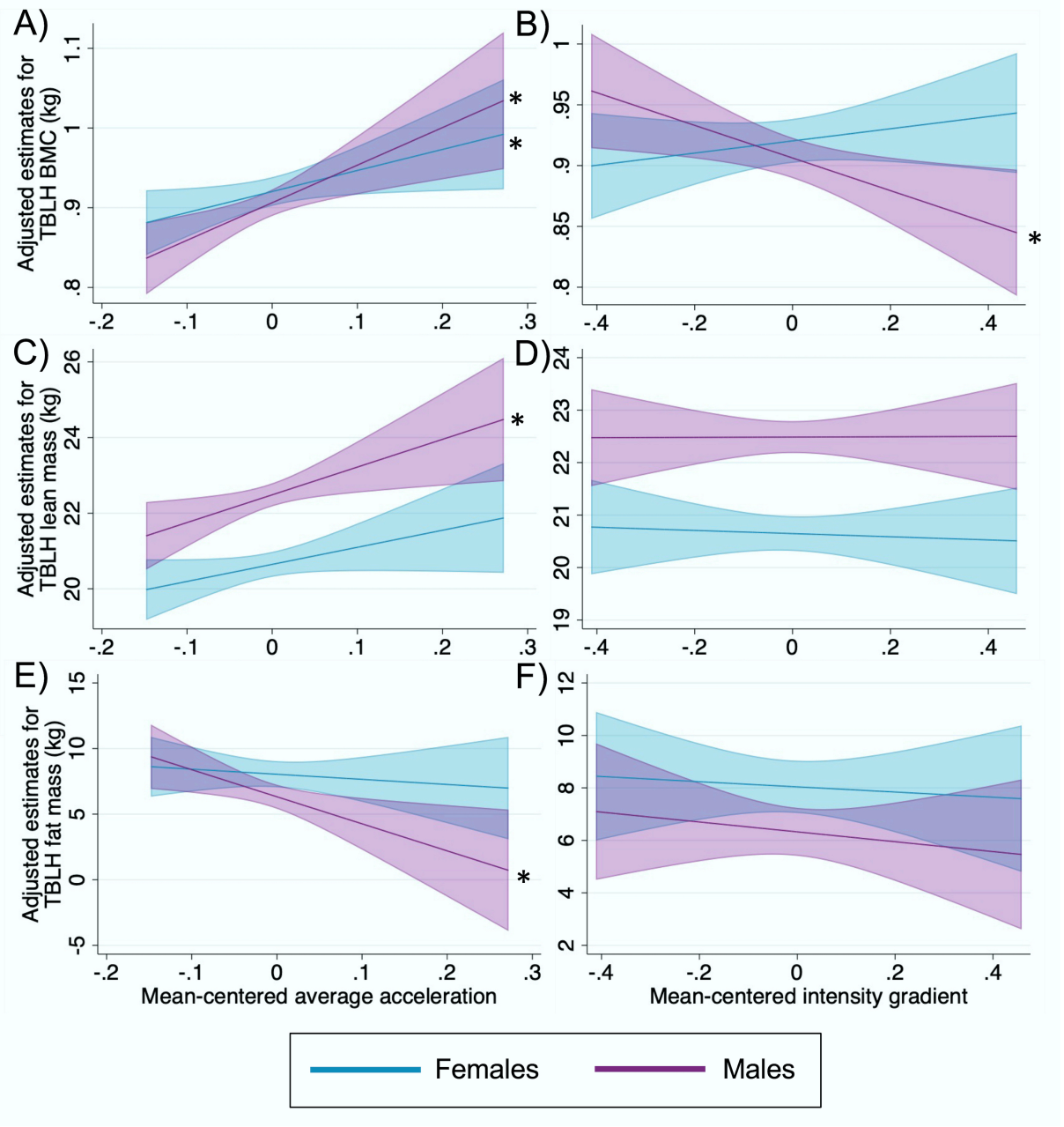


Figure 6.6 Main associations between average-acceleration and intensity-gradient with TBLH BMC, lean mass and fat mass.

*indicates statistical significance ($p < 0.05$)

Values are predicted for a female and a male with mean levels of covariates from the fully adjusted models. All models are adjusted for age, stature, pubertal status, wear time, alternate activity metric and the product term for average acceleration by intensity gradient. A and B are additionally adjusted for lean mass and fat mass (model 6), C and D are additionally adjusted for fat mass (model 5), and E and F are additionally adjusted for lean mass (model 4).

TBLH, Total-body-less-head; *BMC*, bone mineral content.

6.4.5 Associations Between MVPA with Bone Mineral Content, Lean, and Fat Mass

When repeating our analysis with MVPA as the exposure variable, there was no association with TBLH BMC in females or males when adjusting for lean and fat mass. MVPA was positively associated with TBLH lean mass, adjusted for fat mass, and negatively associated with TBLH fat mass, adjusted for lean mass, in females and males (Table 6.6).

6.4.6 Translation to Physical Activity Patterns

The raw and standardised MX metrics for low and high volume and intensity profiles are presented in Figure 6.7 for females and in Figure 6.8 for males. On average, all profiles accumulated 60 minutes at an intensity equivalent to slow to brisk walking. In the high-volume and high-intensity group this included 5 minutes of high-intensity activity, and in the high-volume and low-intensity and the low-volume and high-intensity groups this included 2 minutes of high-intensity activity. The high-volume groups, with either low-intensity or high-intensity, accumulated more light activity compared to the low-volume groups, with 2 hours at an intensity equivalent to slow walking in females and males. The most active third of the day was accumulated at a high-intensity by the high-volume groups compared to the low-volume groups in females and males.

Table 6.6 Associations of moderate-to-vigorous physical activity with total-body-less-head bone mineral content, lean mass, and fat mass (n = 288, 157 females and 131 males)

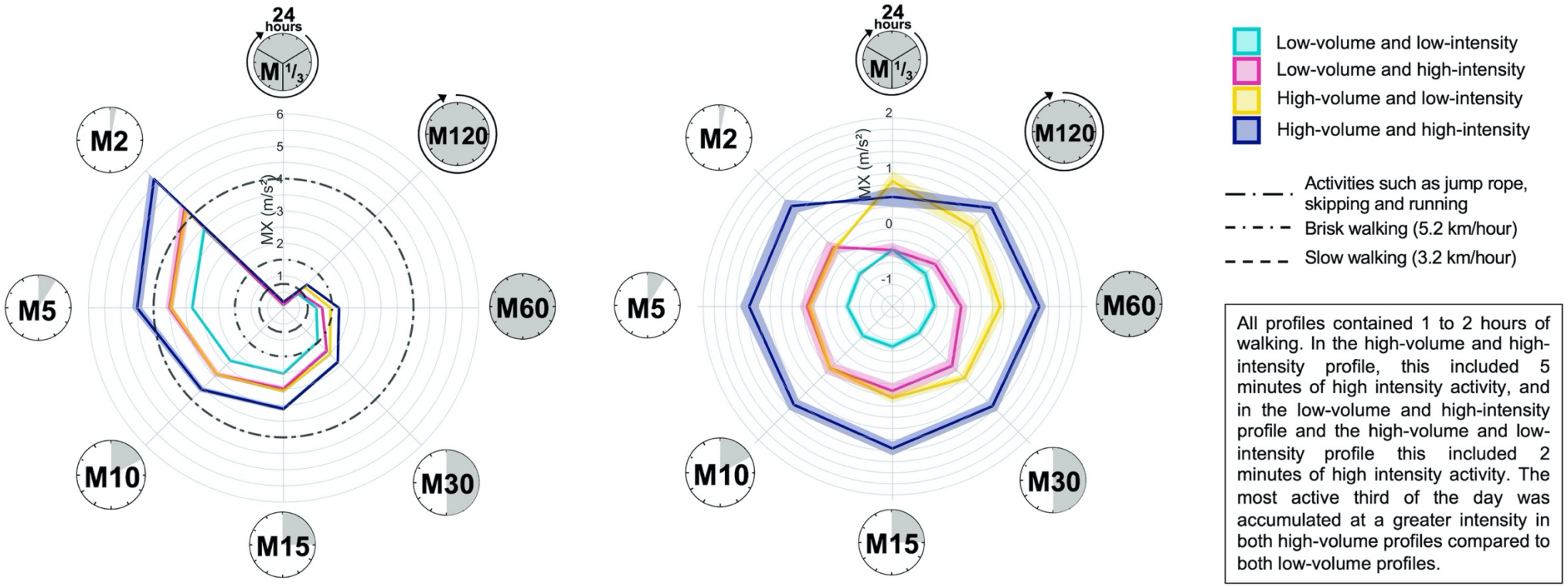
	Model 1 (adjusted for age, sex, stature, and pubertal status)		**Model 2 (Model 1 + lean mass)**		**Model 3 (Model 1 + fat mass)**		**Model 4 (Model 1 + lean mass and fat mass)**	
	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*	ß (95% CI)	*p*
TBLH BMC								
Females	-0.000 (-0.001 to 0.000)	0.194	**0.001 (0.000 to 0.001)**	**0.001**	0.000 (-0.000 to 0.001)	0.137	0.000 (-0.000 to 0.001)	0.545
Males	-0.000 (-0.000 to 0.000)	0.647	-0.000 (-0.001 to 0.000)	0.084	**0.000 (0.000 to 0.001)**	**0.005**	0.000 (-0.000 to 0.000)	0.120
TBLH lean mass								
Females	**0.010 (0.004 to 0.015)**	**0.001**	**.**	**.**	**0.012 (0.006 to 0.018)**	**0.000**	.	.
Males	0.004 (-0.000 to 0.009)	0.066	.	.	**0.007 (0.002 to 0.012)**	**0.006**	.	.
TBLH fat mass								
Females	**-0.023 (-0.039 to -0.008)**	**0.003**	**-0.030 (-0.045 to -0.014)**	**0.000**	.	.	.	.
Males	**-0.026 (-0.038 to -0.014)**	**0.000**	**-0.029 (-0.041 to -0.017)**	**0.000**	.	.	.	.

The values are regression coefficients (ß), 95% confidence intervals (CI), and *p* values from linear regression models. Model 1 included moderate-to-vigorous physical activity adjusted for age, stature, pubertal status, and accelerometer wear time. For bone mineral content as the outcome, Model 2 included additional adjustment for lean mass, Model 3 included additional adjustment for fat mass, and Model 4 included adjustment for lean and fat mass. For lean mass as the outcome, the final model (Model 3) included adjustment for fat mass, and for fat mass at the outcome, the final model (Model 2) included adjustment for lean mass.

Bold emphasis indicates statistical significance at $p < 0.05$.

TBLH, Total-body-less-head; BMC, bone mineral content.

Figure 6.7 Illustration of the physical activity profile for raw (left) and standardised (right) MX metrics in females.



Profiles are presented for four groups, 1) high-volume and high-intensity, 2) high-volume and low-intensity, 3) low-volume and high-intensity, and 4) low-volume and low-intensity. High-volume was defined as average-acceleration ≥ mean, low-volume was defined as average-acceleration < mean, high-intensity was defined as intensity-gradient ≥ mean, low-intensity was defined as intensity-gradient < mean.

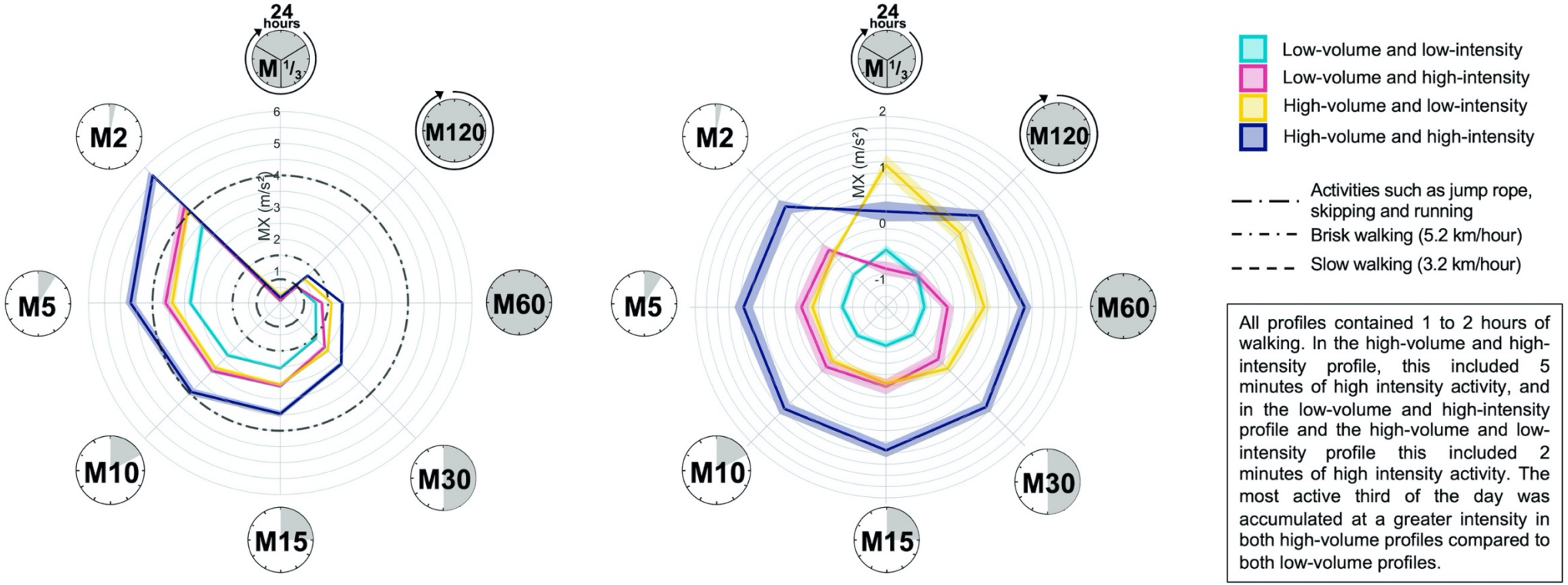
Values are the mean; error ribbons are the standard error of the mean. Standardised metrics were standardised based on the sex-specific mean.

High-volume was defined as average-acceleration ≥ mean, low-volume was defined as average-acceleration < mean, high-intensity was defined as intensity-gradient ≥ mean, low-intensity was defined as intensity-gradient < mean,

The MX metrics show the acceleration above which a child's most active X minutes are accumulated. Each plot shows (clockwise) *M1/3,* the intensity above which a child's most active 480 minutes are accumulated; *M120,* the intensity above which a child's most active 120 minutes are

accumulated; *M60,* the intensity above which a child's most active 60 minutes are accumulated; *M30,* the intensity above which a child's most active 30 minutes are accumulated; *M15,* the intensity above which a child's most active 15 minutes are accumulated; *M10,* the intensity above which a child's most active 10 minutes are accumulated; *M5,* the intensity above which a child's most active 5 minutes are accumulated; *M2,* the intensity above which a child's most active 2 minutes are accumulated.

Figure 6.8 Illustration of the physical activity profile for raw (left) and standardised (right) MX metrics in males.



Profiles are presented for four groups, 1) high-volume and high-intensity, 2) high-volume and low-intensity, 3) low-volume and high-intensity, and 4) low-volume and low-intensity. High-volume was defined as average-acceleration ≥ mean, low-volume was defined as average-acceleration < mean, high-intensity was defined as intensity-gradient ≥ mean, low-intensity was defined as intensity-gradient < mean.

Values are the mean; error ribbons are the standard error of the mean.

High-volume was defined as average-acceleration ≥ mean, low-volume was defined as average-acceleration < mean, high-intensity was defined as intensity-gradient ≥ mean, low-intensity was defined as intensity-gradient < mean.

The MX metrics show the acceleration above which a child's most active X minutes are accumulated. Each plot shows (clockwise) *M1/3,* the intensity above which a child's most active 480 minutes are accumulated; *M120,* the intensity above which a child's most active 120 minutes are accumulated; *M60,* the intensity above which a child's most active 60 minutes are accumulated; *M30,* the intensity above which a child's most

active 30 minutes are accumulated; *M15,* the intensity above which a child's most active 15 minutes are accumulated; *M10,* the intensity above which a child's most active 10 minutes are accumulated; *M5,* the intensity above which a child's most active 5 minutes are accumulated; *M2,* the intensity above which a child's most active 5 minutes are accumulated; *M2,* the intensity above which a child's most active 2 minutes are accumulated.

6.5 Discussion

This study is the first to examine the associations of average-acceleration, as a measure of PA volume, and intensity-gradient, as a measure of PA intensity distribution, with TBLH BMC, lean mass, and fat mass derived from DXA in a population sample of pre- and early-pubertal children, alongside assessing the associations between MVPA and these outcomes. Greater PA volume was associated with increased BMC in females, and with increased BMC and lean mass, and reduced fat mass, in males. A higher intensity distribution was associated with reduced BMC in males. Although the novel approach revealed unique relationships for bone outcomes, MVPA was positively associated with lean mass and negatively associated with fat mass in females and males and was potentially better than our new approach at capturing activity relevant to lean and fat mass in females. Even so, it remains unclear whether it was volume or intensity of MVPA driving these associations, highlighting the utility of independent metrics which capture both the volume and intensity of PA. Adjusting for lean and fat mass altered the relationships between PA with BMC, emphasising the importance of considering lean and fat mass alongside bone. The high-volume PA profiles included short periods of high-intensity activity, with several hours of light activity, suggesting that increasing PA at any intensity may improve BMC and lean mass and reduce fat mass in children.

Compared to reference values for children aged 9 years, the children in our study had typical median levels of TBLH aBMD and lean mass (Zemel *et al.*, 2011; Shepherd *et al.*, 2012; Weber *et al.*, 2013), though our study had a lower proportion of children living with obesity than the ISCOLE study (Roman-Vinas *et al.*, 2016). It is difficult to compare PA levels between study samples as the

amount of PA reported is dependent on accelerometer protocol and placement, epoch lengths, wear time algorithms, and intensity cut-points (Banda *et al.*, 2016). Even so, the children in our sample may be more active than children aged 9 to 11 years in ISCOLE study (Roman-Vinas *et al.*, 2016).

6.5.1 Bone Mineral Content

We did not observe an association between MVPA and TBLH BMC, adjusting for lean and fat mass, which highlights the importance of considering PA exposures relating to mechanical loading rather than energy expenditure when examining the relationship between PA and bone mass. Our findings indicated that a greater volume of PA is associated with favourable bone outcomes in pre- and early-pubertal children, and this association is partially explained by lean mass. However, for a given volume of PA, males accumulating the volume at a lower intensity had greater TBLH BMC, though this was likely driven by the association at the upper-limb only. The inverse relationship between intensity distribution and BMC in males we observed is unexpected, given that bone responds positively to strains brought about by high impact loading cycles (Hart *et al.*, 2017). This observation may be because the types of osteogenic activities that males were engaging in were not captured in the high end of the intensity spectrum (Hart *et al.*, 2017). In children ages 6 to 11 years, accelerometer counts during jumping, captured in 15 second epochs, were lower than those observed during running, despite GRFs being greater (Janz *et al.*, 2003). This disassociation between accelerometer counts and GRFs during jumping may be further magnified with longer epoch lengths, and therefore, the use of the 60-second epoch length may not have captured high impact activity. This may have been more apparent in males, due to potential sex and gender

differences in engagement in PA types, with girls in Finland participating in less high-intensity activity than boys, which is likely to result in differences in osteogenic responses (Lehtonen, Oja and Hakamäki, 2022). Therefore, the types of activities which are positively associated with BMC may not have been reflected within the intensity-gradient, and sex and gender differences in activity participation may explain why the inverse relationship between intensity with BMC was only observed in males.

The IBDS found that in participants age 17 to 23 years, both average-acceleration and intensity-gradient were positively associated with TBLH BMC, after adjustment for similar covariates to those used in our analysis (Rowlands *et al.*, 2020). Although lean and fat mass were not separated as covariates, total mass was adjusted for, meaning our final models are comparable (Rowlands *et al.*, 2020). The IBDS used a 5-second epoch to calculate the intensity-gradient, so may have been more likely to capture osteogenic activity, and therefore more likely to find positive associations between intensity-gradient with bone outcomes. In addition, PA levels may account for the different findings, as we observed higher levels of mean daily MVPA compared to the IBDS sample, and our MVPA levels are likely an underestimation due to the 60-second epoch used to capture the accelerometer data, compared to the 5-second epoch used in the IBDS (Baquet *et al.*, 2007). Further, our participants were younger than those from the IBDS sample, which may contribute to the different observations, as maturation may moderate the skeletal response to mechanical loading (Weaver *et al.*, 2016). Therefore, the differences in maturity status and PA levels may account for the different findings between our study and the IBDS (Rowlands *et al.*, 2020).

In our study and the IBDS, the high-volume activity profiles were characterised by relatively low amounts of high-intensity activity, at least 60 minutes of MVPA, and several hours of being lightly active (Rowlands *et al.*, 2020). Our findings indicate that for active children, greater PA volume may elicit positive bone outcomes, and accruing several hours of light-intensity, weight-bearing activity may also be beneficial for BMC.

6.5.2 Lean Mass

The positive association between average-acceleration and lean mass was evident in males only when controlling for intensity-gradient. However, the association in females was approaching statistical significance, so is likely due to reduced statistical power, lower lean mass and lower average-acceleration in females compared to males. Further, MVPA was positively associated with lean mass in females and males. Previous studies have not examined average-acceleration and intensity-gradient in relation to lean mass, though research has found that more active children and adolescents have greater lean mass, similar to the positive associations we observed between MVPA with lean mass (Deere *et al.*, 2012; Zymbal *et al.*, 2019). However, these studies have been unable to separate the associations of PA volume and intensity, as all summary intensities of PA should not be included in the same model due to issues with multicollinearity (Deere *et al.*, 2012; Zymbal *et al.*, 2019). This further highlights the potential benefits of using the accelerometer metrics proposed by Rowlands and colleagues (Rowlands *et al.*, 2018), as it allows the full intensity spectrum to be characterised and analysed without issues of multicollinearity, though other

methods have been proposed which also account for this (Aadland *et al.*, 2021). Our findings indicate that PA volume and MVPA are important for lean mass.

6.5.3 Fat Mass

Of the previous studies that have examined the associations between average-acceleration and intensity-gradient with measures of adiposity in children, ours is the only one to observe an independent relationship between average-acceleration and a measure of adiposity, albeit only in males. It is unclear what accounts for the null finding we observed in females. Levels of fat mass and the prevalence of overweight and obesity did not differ between females and males, though the females were less active in terms of PA volume and MVPA than the males. It is therefore possible that the females were not accumulating enough weight-bearing PA to observe an association with fat mass, as we did observe a negative association between MVPA with fat mass in females and males.

Intensity-gradient was independently negatively associated with BIA-assessed body fat percentage in adolescent girls (Rowlands *et al.*, 2018), and with waist-to-height ratio in primary school children (Fairclough *et al.*, 2019). Furthermore, multivariate pattern analysis demonstrated that PA at vigorous intensities had the strongest relationship with waist-to-height ratio in children ages 5 to 18 years, emphasising the importance of accruing PA at the high end of the intensity spectrum (Aadland *et al.*, 2020b). Although our sample had a lower prevalence of children living with overweight and obesity compared to these previous studies, differences in epoch length, which are discussed below, likely account for the differences in findings (Rowlands *et al.*, 2018; Fairclough *et al.*, 2019; Aadland *et al.*, 2020b). Our findings suggest that greater PA volume is

important for reducing fat mass in males in this cohort, whilst MVPA may reduce fat mass in females and males in this cohort, with a relatively low prevalence of children living with overweight and obese weight status.

6.5.4 Translation of Findings

The purpose of this section is to translate the findings from the regression models into minutes per day of PA to provide a more easily interpretable result regarding effect size. Given the limitations with 60-second epoch used with the accelerometer data, which will be discussed further in Section 6.5.5, the current section is to aid interpretation rather than to provide specific PA recommendations. A 1 SD greater average-acceleration was independently associated with 0.02 kg greater TBLH BMC in females, and with 0.03 kg greater TBLH BMC and 0.51 kg greater TBLH lean mass in males. This equates to ~2% of the annual growth in TBLH BMC in females and males, and ~18 % of the annual growth in TBLH lean mass in males (Zemel *et al.*, 2011; Cossio Bolaños *et al.*, 2019). Given that ~40% of adult total body BMC is accrued in the four years surrounding PHV, accruing more bone during this period is important for increasing PBM in young adulthood (Baxter-Jones *et al.*, 2011). A 1 SD greater average-acceleration was associated with 1.4 kg lower TBLH fat mass in males, which equates to ~4% lower body fat percentage. In order to translate a 1 SD difference in average-acceleration into minutes per day of PA, the equations provided by Rowlands *et al.* (2018) were applied, as described in Section 6.3.6.

In females, a 1 SD (0.0579 m/s^2) increase in average-acceleration could be achieved by replacing time per day spent at the average-acceleration level with an accumulation of:

1) 22 minutes of high-intensity activities such as running, jumping, and skipping OR
2) 64 minutes of brisk walking OR
3) 2.5 hours of slow walking/light activity OR
4) Any combination of the above such that the sum of the increases is equal to 0.0579 m/s^2, as described in Section 6.3.6.

In males, a 1 SD (0.0696 m/s^2) increase in average-acceleration could be achieved by replacing time per day spent at the average-acceleration level with:

1) 27 minutes of high-intensity activities such as running, jumping, and skipping OR
2) 78 minutes of brisk walking OR
3) 3 hours of slow walking/light activity OR
4) Any combination of the above such that the sum of the increases is equal to 0.0705 m/s^2, as described in Section 6.3.6.

These increases in activity are unlikely to be achievable for most children, with a meta-analysis of PA intervention studies indicating that PA interventions in children tend to only increase PA by around 4 minutes per day (Metcalf, Henley and Wilkin, 2012). However, the flexibility in how PA can be accumulated is advantageous, as self-selecting physical activity may increase enjoyment and consequentially, adherence (Hamlyn-Williams, Freeman and Parfitt, 2014). Even so, the translation of these findings must be considered in light of the limitations with the 60-second epoch used, and as such should not be interpreted as specific recommendations. Whilst the average-acceleration metric is not influenced by epoch length, the intensity-gradient metric likely is,

and therefore the associations between average-acceleration with bone, lean, and fat outcomes may be exaggerated when adjusting for intensity-gradient calculated from 60-second epoch data, overstating the importance the volume over intensity. The limitations with the 60-second epoch are expanded on below in Section 6.5.5.

6.5.5 Strengths and Limitations

The accelerometry methodology may explain the differences between our findings and those which have found PA intensity to be equally or more important than volume for both BMC and fat mass (Rowlands *et al.*, 2018; Fairclough *et al.*, 2019; Rowlands *et al.*, 2020). In our study, accelerometer data was averaged over 60-seconds, whereas data in more recent studies examining intensity-gradient has been averaged over 5-seconds (Rowlands *et al.*, 2018; Fairclough *et al.*, 2019; Rowlands *et al.*, 2020). As high-intensity activity is accrued in short bursts in children (typically in bouts < 10-seconds), it is impossible to capture these short bursts of high-intensity activity with a 60-second epoch (Baquet *et al.*, 2007). As such, our intensity-gradient metric may be blunted, limiting our ability to detect an effect. This is supported by multivariate pattern analysis of the associations between PA intensity signature of varying epoch lengths with metabolic health, which has shown that the intensity most strongly associated with metabolic health is lower the longer the epoch, reflecting the dilution over longer epoch lengths (Aadland *et al.*, 2020a). Given that many large longitudinal studies (Riddoch *et al.*, 2009; Zymbal *et al.*, 2019), as well as the International Children's Accelerometry Database (Aadland *et al.*, 2020b), have accelerometer data in 60-second epochs, investigating the effect of epoch length on intensity-gradient should be a priority before this

metric is applied in research using longer epochs. In addition, the dynamic range of the Actiheart, ±2.5 *g*, in our study may lead to an underestimation of high-intensity activity, compared with the dynamic range of ±8 *g* of the Actigraph and GENEActiv used in previous studies (Rowlands *et al.*, 2018; Fairclough *et al.*, 2019; Rowlands *et al.*, 2020). As peak acceleration during everyday PA, such as running and jumping, may exceed 5 *g* at the hip and the wrist, high-intensity activities, which may be meaningful for bone outcomes, could be missed when the dynamic range is limited to ±2.5 *g* (Rowlands and Stiles, 2012). Further, given the high levels of PA and low levels of fat mass in our sample, it is unknown whether these findings extend to less active children and to children living with overweight and obesity. Although we adjusted for important covariates, residual confounding remains a potential limitation in all observational studies, and causality cannot be assumed as bidirectional relationships are possible.

The strengths of our study include the population-based sample of children, the comparison of novel accelerometer metrics with MVPA estimates based on energy expenditure, and the measurement of BMC, lean mass, and fat mass by DXA. The application of novel accelerometer metrics to characterise volume and intensity allowed the independent contributions of each of these aspects of PA to be examined. The translation of the findings with MX metrics makes the findings easy to understand and apply to TBLH BMC, lean mass, and fat mass, and we have been able to provide clinically relevant recommendations.

6.6 Conclusion

A higher volume of PA was associated with greater BMC in females, and with greater BMC and lean mass, and reduced fat mass in males, whilst more MVPA was associated with greater lean mass and lower fat mass in females and males, in a population sample of children aged 9 to 11 years. The relationship between PA volume and BMC was influenced by lean and fat mass, and body composition should be considered in future research to better understand the association between PA and BMC. The high-volume PA profiles were characterised by at least 2 minutes of high-intensity activity, as well as several hours of light activity. These findings support the current WHO recommendations to increase total activity, though our analyses need replicating with accelerometer data averaged over shorter epochs (Chaput *et al.*, 2020). These intricacies would have been missed if only MVPA was explored as the exposure of interest. This highlights the importance of using methods that account for the whole PA profile and which align PA with mechanical loading rather than energy expenditure when considering bone as an outcome.

Chapter 7 Cross-Sectional and Longitudinal Associations Between the 24-hour Movement Behaviours, Including Muscle and Bone Strengthening Activity, with Bone and Lean Mass from Childhood to Adolescence

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7.1 Abstract

Background: This study aimed to assess whether MVPA, sport and exercise as a proxy measure of muscle and bone strengthening activity, sedentary behaviour, and sleep were associated with TBLH BMC and TBLH lean mass cross-sectionally and longitudinally from age 6 to 9 years and age 9 to 11 years to age 15 to 17 years.

Methods: We used longitudinal data from a population sample of Finnish children from the PANIC study (age 6 to 9 years: n = 478, 229 females; age 9 to 11 years: n = 384, 197 females; age 15 to 17 years: n = 222, 103 females). Linear regression analysed the cross-sectional and longitudinal associations between accelerometer-assessed MVPA, sedentary time and sleep, and questionnaire-assessed sport and exercise participation and screen time with DXA-assessed TBLH BMC and lean mass.

Results: In females, MVPA at age 6 to 9 years was positively associated with TBLH BMC at age 15 to 17 years (β = 0.008, p = 0.010). Sport and exercise at age 9 to 11

years was positively associated with TBLH BMC (β = 0.020, *p* = 0.002) and lean mass (β = 0.343, *p* = 0.040) at age 15 to 17 years. MVPA at age 9 to 11 years was positively associated with TBLH lean mass (β = 0.272, *p* = 0.004) at age 15 to 17 years. In males, sleep at age 6 to 9 years was positively associated with TBLH lean mass (β = 0.382, *p* = 0.003) at age 15 to 17 years. Sport and exercise at age 9 to 11 years was positively associated with TBLH BMC (β = 0.027, *p* = 0.012) and lean mass (β = 0.721, *p* < 0.001) at age 15 to 17 years.

Conclusions: Promoting engagement in the 24-hour movement behaviours in childhood, particularly sport and exercise to strengthen muscle and bone, is important in supporting bone and lean mass development in adolescence.

7.2 Introduction

PA during childhood and adolescence is a well-established and important factor in determining PBM, achieved by young adulthood (Baxter-Jones *et al.*, 2008b; Janz *et al.*, 2010; Elhakeem *et al.*, 2020). Around 40% of young adult BMC is accrued in the 4 years surrounding PHV (Baxter-Jones *et al.*, 2011), so understanding the association between PA and BMC during childhood and adolescence is particularly important in enhancing bone accrual, and consequentially PBM. The relationship between PA and BMC is mediated by skeletal muscle, as muscle contractions transfer forces to the bones, and the skeleton adapts in response to this loading (Schoenau and Frost, 2002). During childhood and adolescence, lean mass increases substantially (Baxter-Jones *et al.*, 2003). There are critical synergies between the trajectories of bone and lean tissue accrual, whereby PHV is observed, followed by peak lean mass velocity, and then by peak BMC velocity, and lean mass is consistently positively related to BMC in children and adolescents (Iuliano-Burns,

Mirwald and Bailey, 2001; Baxter-Jones *et al.*, 2003; Sioen *et al.*, 2016). Therefore, considering muscle outcomes alongside BMC is important in understanding the relationship between PA and bone in childhood and adolescence.

There is increasing interest in how the 24-hour day is distributed across the movement continuum, between sleep, sedentary behaviour, and PA, and how each of these components may influence health outcomes (Tremblay *et al.*, 2016). This has led to the development of the Canadian 24-Hour Movement Guidelines for Children and Youth, published in 2016 (Tremblay, Carson and Chaput, 2016), followed by similar guidelines from Australia (Okely *et al.*, 2022) and the WHO (Chaput *et al.*, 2020). The guidelines promote engaging in MVPA, muscle and bone strengthening activities, and LPA, sufficient sleep, and limiting screen time and sitting time (Tremblay *et al.*, 2016; Okely *et al.*, 2022), with the WHO highlighting that any increase in PA and reduction in sedentary behaviour may be beneficial for health (Chaput *et al.*, 2020). These recommendations are to improve physical and psychosocial health across childhood, adolescence and into adulthood, and to promote healthy habits into young adulthood. This is particularly relevant as PA tends to decrease and sedentary behaviours tend to increase across puberty (Moore *et al.*, 2020).

MVPA has been positively associated with BMC in children and adolescents, with those who do more MVPA during childhood having improved muscle and bone outcomes in late adolescence and young adulthood, even after declines in MVPA during adolescence (Zymbal *et al.*, 2019; Elhakeem *et al.*, 2020). However, the WHO recommendation regarding participation in MVPA is largely based on evidence on

improving adiposity and other cardiometabolic risk factors and is not necessarily as relevant for muscle and bone health (Chaput *et al.*, 2020). The importance of specific muscle and bone strengthening activities is acknowledged in the WHO guidelines, as well as national guidelines across the globe (Tremblay *et al.*, 2016; Piercy *et al.*, 2018; Department of Health and Social Care, 2019; Chaput *et al.*, 2020). However, aerobic activities remain the primary focus of the guidelines, though the guidelines do also describe some recommendations of muscle and bone strengthening activities (Faigenbaum *et al.*, 2020). This is reflected in surveillance of adherence to the PA guidelines, which tends to focus solely on the MVPA component, as highlighted by a 2020 review which found that in the UK, none of the childhood PA surveys assessed muscle and bone strengthening activity (Strain *et al.*, 2020). Given that it is during childhood and adolescence when bone is most responsive to PA (Gunter, Almstedt and Janz, 2012), it is crucial to target muscle and bone strengthening activity in childhood and adolescence. A 2020 rapid review of evidence found that weight-bearing activities, including weight-bearing sports participation, do improve muscle and bone outcomes in children and adolescents (Public Health England, 2021). However, as much of this evidence is based on studies in athletes, whose level of training may be far above that of the general population, are unique in terms of physique and body composition, and are likely genetically predisposed to be an athlete, further research is needed to investigate the relationship between muscle and bone strengthening activity and muscle and bone health in the general population. The current evidence is also based on intervention studies, in which the types of activities programmed may not reflect every day PA choices of children and adolescents (Gunter, Almstedt and Janz, 2012). As such, it is unclear whether participation in self-selected muscle and bone

strengthening activities, such as sport and exercise, is associated with better muscle and bone outcomes in the general population, and if so, whether these associations persist from childhood into adolescence and young adulthood. This is particularly important to consider, given that organised sport and exercise account for a large part of children's PA (Sport England, 2022).

Investigating movement behaviours individually, such as MVPA, has its limitations as it does not account for the role of the other important movement behaviours, such as sedentary time and sleep, which can have a reverse or additional effect on musculoskeletal phenotypes (Pereira *et al.*, 2019). Although being physically active is considered important for muscle and bone health (particularly when it is weight-bearing and dynamic in nature), PA may only be one part of the equation, and reducing sedentary behaviour and sleeping optimally may also contribute to better muscle and bone health by increasing PA levels during the day. Meanwhile, in sedentary behaviour, the musculoskeletal system in unloaded, which may have deleterious effects on muscle and bone (Koedijk *et al.*, 2017). However, a systematic review found strong evidence to suggest no association between accelerometer-measured sedentary behaviour after adjustment for MVPA and insufficient evidence for an association between self- or proxy-reported sedentary behaviour with total body bone outcomes in children and adolescents, though the authors highlight a lack of high-quality evidence with limited longitudinal studies (Koedijk *et al.*, 2017). Evidence for the relationship between sedentary behaviour and lean mass in children and adolescents is limited, and the studies that have investigated these relationships have reported conflicting findings between sedentary behaviours with fat-free mass based on skinfold measures or BIA (Fulton *et al.*, 2009; Riso *et al.*,

2018). It has been hypothesized that sleep may also influence bone health, as bone turnover markers peak overnight (Swanson *et al.*, 2018), and sleep restriction has been shown to lead to lower levels of bone formation markers and unchanged or higher levels of bone resorption markers in adult males, which could ultimately lead to bone loss (Swanson *et al.*, 2021). However, there is limited research into the relationships between sleep and bone health in children, and the current evidence has shown conflicting results and is limited to cross-sectional studies (Casazza, Hanks and Fernandez, 2011; Cheng *et al.*, 2021). Sleep deficit downregulates hormones important in protein synthesis, and therefore in muscle mass maintenance (Buchmann *et al.*, 2016). However, current evidence supports sleep has either no (Carter *et al.*, 2011) or negative (Baird *et al.*, 2016) association with fat-free mass in children. Therefore, it remains unclear whether sedentary behaviour and sleep are important for muscle and bone health in childhood and adolescence.

Although the 24-hour movement behaviours have been investigated in relation to other health outcomes, to the best of our knowledge, the associations of the 24-hour movement behaviours of MVPA, muscle and bone strengthening activity, sedentary behaviour, and sleep have yet to be investigated in relation to muscle and bone health longitudinally from childhood to adolescence. Therefore, the aim of this study was to assess whether MVPA, sport and exercise (as a proxy measure of muscle and bone strengthening activity), sedentary behaviour (i.e., sedentary time, screen time), and sleep are associated with TBLH BMC and TBLH lean mass by DXA cross-sectionally (at age 6 to 9 years, age 9 to 11 years, age 15 to 17 years), and longitudinally (from age 6 to 9 years and age 9 to 11 years to age 15 to 17 years).

7.3 Methods

7.3.1 Study Design and Participants

This study used longitudinal data from baseline (at age 6 to 9 years), 2-year follow-up (at age 9 to 11 years) and 8-year follow-up (at age 15 to 17 years), as described in Section 3.1. For the present analyses, we excluded participants who used oral corticosteroids at any timepoint, as this could influence BMC (Weaver *et al.*, 2016), and participants with musculoskeletal injuries and diseases. Complete and valid data for age, stature, general health, pubertal status, DXA outcomes, and at least one movement behaviour were available for 478 children (229 females, 249 males) at age 6 to 9 years, 384 children (197 females, 187 males) at age 9 to 11 years, and 222 adolescents (103 females, 119 males) at age 15 to 17 years. The participants included in these analyses did not differ in age, stature, pubertal status, weight status, TBLH BMC, lean mass, or fat mass to the participants who did not have complete data. Inclusion and exclusion criteria are displayed in Figure 7.1

7.3.2 Assessment of General Health and Pubertal Status

General health and pubertal status were assessed as described in Section 3.3 and Section 3.4

7.3.3 Anthropometry

Stature and body weight were assessed as described in Section 3.5. BMI (kg/m^2) was calculated, BMI-SDS was calculated using the Finnish reference values, and the BMI cut-offs were applied to classify children and adolescents as thin, normal weight, or living with overweight or obesity as it related to their weight status (Saari *et al.*, 2011; Cole and Lobstein, 2012).

7.3.4 Assessment of Bone Mineral Content and Body Composition

TBLH BMC (kg), aBMD (g/cm^2), lean mass (kg), and fat mass (kg) were measured as described in Section 3.6. TBLH BMC was used as the bone outcome of interest, as this is more appropriate than TBLH aBMD in longitudinal studies of bone accrual (Leonard *et al.*, 2004a; Wren *et al.*, 2005; Baxter-Jones *et al.*, 2011). TBLH lean mass was considered as a secondary outcome, as lean mass is a key determinant of BMC, and the relationship between PA and BMC is mediated by lean mass (Zymbal *et al.*, 2019).

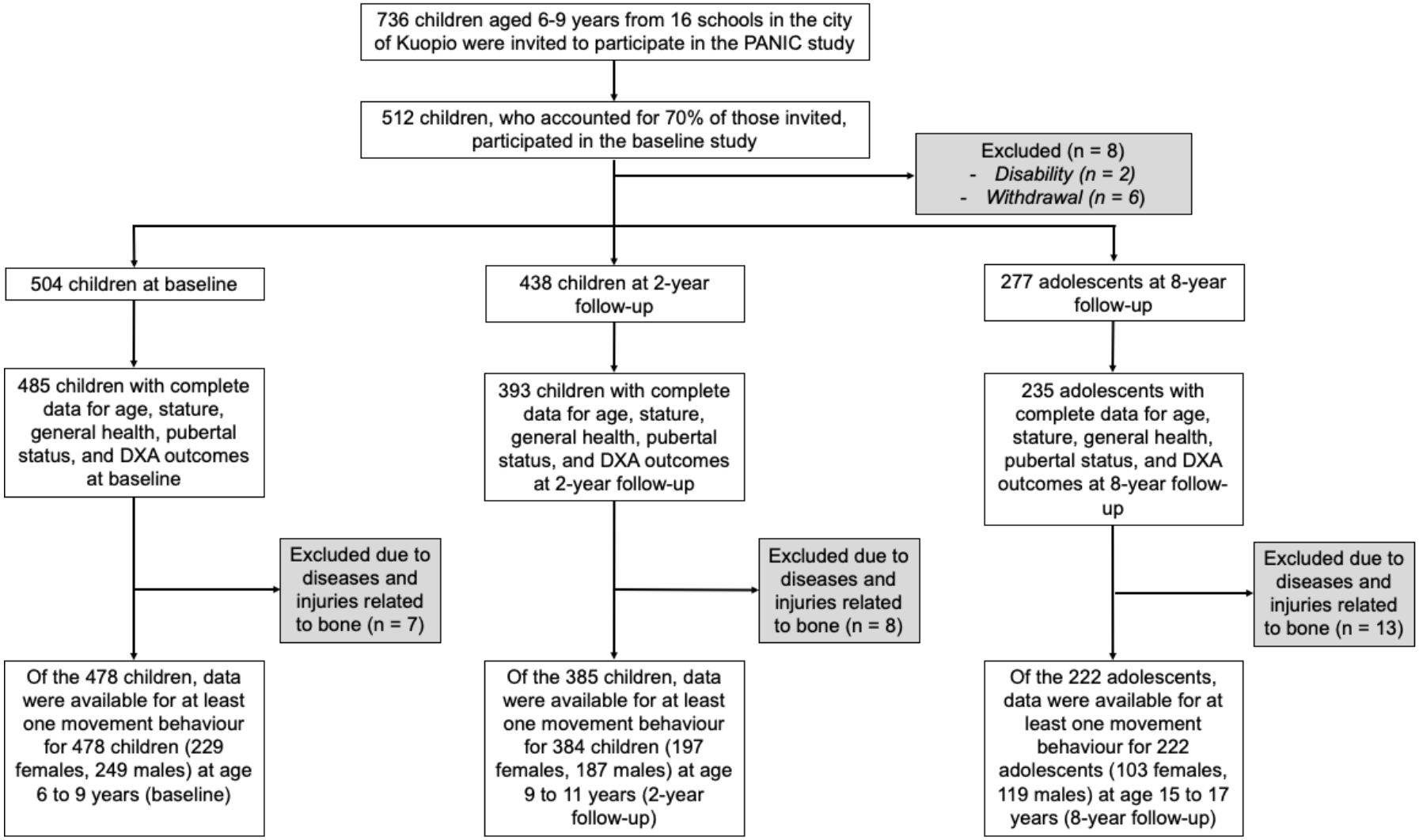

Figure 7.1 Participant flow chart.

DXA, dual-energy X-ray absorptiometry; MVPA, moderate-to-vigorous physical activity; PANIC, Physical Activity and Nutrition in Children.

7.3.5 Assessment of 24-hour Movement Behaviours

MVPA, muscle and bone strengthening activity, sedentary behaviour, and sleep were assessed, as these behaviours are outlined in the Canadian 24-hour Movement Guidelines for Children and Youth (Tremblay, Carson and Chaput, 2016), the Australian 24-hour Movement Guidelines for Children and Young People (Okely *et al.*, 2022) and, with the exception of sleep, the WHO Guidelines on Physical Activity and Sedentary Behaviour for Children and Adolescents (Chaput *et al.*, 2020).

7.3.5.1 Moderate-to-Vigorous Physical Activity

PA was assessed using Actiheart (CamNtech Ltd, Papworth, UK), as described in Section 3.7.1. MVPA was modelled from the combined sensing signal, as described in Section 3.7.1.3, using a branched equation framework (Brage *et al.*, 2004; Collings *et al.*, 2017). The children and adolescents were defined as meeting the PA guidelines if they had at least an average of 60 minutes of MVPA per day, as described in the WHO guidelines (Chaput *et al.*, 2020).

7.3.5.2 Muscle and Bone Strengthening Activity

Sport and exercise participation were assessed as described in Section 3.7.2.1. Sports and organised exercise participation was used as a proxy measure for muscle and bone strengthening activity. Previous studies which have assessed adherence to the muscle and bone strengthening guidelines have used frequency questionnaires, with activities such as push-ups, sit-ups and weight-lifting classed as muscle strengthening, and a wide variety of activities classed as bone strengthening, including basketball, cheerleading, dance, hockey, gymnastics, soccer, racket

sports, and weight lifting (Hyde *et al.*, 2021). In addition, the Finnish Physical Activity Guidelines for children and adolescents suggest body weight training, gym training, group PA, and stair walking as muscle strengthening activity, and gymnastics, athletics and ball games as bone strengthening activity examples (Ministry of Education and Culture, 2021). The types of activities classed as muscle and bone strengthening therefore tend to be captured within 'sport and organised exercise participation'. However, there is no recommended session duration defined in the muscle and bone strengthening activity guidelines (Chaput *et al.*, 2020). Hence, children and adolescents who participated in sport and organised exercise at least three times a week were defined as meeting the guidelines (Chaput *et al.*, 2020).

7.3.5.3 Sedentary Behaviour

As limiting total sedentary time and recreational screen time is recommended by the WHO (Chaput *et al.*, 2020), measures of both sedentary time and screen time were included in this study. Sedentary time was modelled from the combined heart rate and movement sensor data as described in Section 3.7.1. To further describe each child's sedentary behaviours, screen time was assessed with the PANIC Physical Activity Questionnaire, as described in Section 3.7.2.

7.3.5.4 Sleep

Sleep duration was assessed from the combined heart rate and movement sensor data as described in Section 3.7.1.4. The children at age 6 to 9 years and age 9 to 11 years were defined as meeting the guidelines if their average daily sleep was between 9 and 11 hours, and the adolescents at age 15 to 17 years if their average daily sleep was between 8 and 10 hours (Tremblay, Carson and Chaput, 2016;

Janssen, Roberts and Thompson, 2017; Roberts *et al.*, 2017; Leppänen *et al.*, 2022).

7.3.6 Statistical Analysis

Analyses were performed with Stata/SE for Mac software, Version 17.0 (StataCorp LLC, College Station, TX, USA). There were no differences between the children who did meet the inclusion criteria and those who did not meet the inclusion criteria in terms of age, stature, BMI categories, and pubertal status at age 6 to 9 years, age 9 to 11 years, and age 15 to 17 years. Further, the adolescents at age 15 to 17 years who met the inclusion criteria for the longitudinal analysis did not differ in terms of age, stature, BMI categories and pubertal status at age 6 to 9 years, age 9 to 11 years and age 15 to 17 years from those who did not. Therefore, we proceeded with a complete-case analysis.

The means and SDs or the medians and IQRs were calculated stratified by sex, for each timepoint. We stratified all further analyses by sex, based on the biological differences in stature, TBLH BMC, lean mass and fat mass between females and males in the studied age group (Riddoch and Boreham, 1995; Baxter-Jones *et al.*, 2003; Klitsie *et al.*, 2013).

Cross-sectional analyses were carried out at age 6 to 9 years, age 9 to 11 years, and age 15 to 17 years. Linear regression was used to assess the associations of minutes per day of MVPA, sport and exercise, sedentary time, screen time, and sleep with TBLH BMC and TBLH lean mass. Analyses were adjusted for age, stature, pubertal status, study group (control or intervention), and TBLH fat mass.

When considering TBLH BMC as the outcome, we adjusted additionally for lean mass. Further, estimates for MVPA and sedentary time were mutually adjusted for each other, as correlation analyses for females and males separately at age 6 to 9 years, age 9 to 11 years, and age 15 to 17 years showed moderate to strong inverse relationships between these two variables (age 6 to 9 years, females: r = -0.58, p < 0.001, males: r = -0.59, p < 0.001; age 9 to 11 years, females: r = -0.60, p < 0.001, males: r = -0.72, p < 0.001; age 15 to 17 years, females: r = -0.63, p < 0.001, males: -0.66, p < 0.001).

Longitudinal analyses were carried out between age 6 to 9 years and age 15 to 17 years, and between age 9 to 11 years and age 15 to 17 years, as it is possible that the longitudinal associations may differ between these age groups. Linear regression was used to assess the associations of minutes per day of MVPA, sport and exercise, sedentary time, screen time, and sleep at age 6 to 9 years with TBLH BMC and TBLH lean mass at age 15 to 17 years. For all longitudinal analyses, models included adjustment for age, stature, pubertal status, study group, and TBLH fat mass at age 15 to 17 years, with additional adjustment for TBLH lean mass at age 15 to 17 years and TBLH BMC at age 6 to 9 years when considering TBLH BMC as the outcome, and with TBLH lean mass at age 6 to 9 years when considering TBLH lean mass as the outcome. As with the cross-sectional analyses, MVPA and sedentary time at age 6 to 9 years were mutually adjusted for each other. The movement behaviour of interest at age 15 to 17 years was not adjusted for, as this greatly reduced the sample size for the device-measured variables of MVPA, sedentary time, and sleep (i.e., when analysing the relationship between MVPA at age 6 to 9 years with TBLH BMC at age 15 to 17 years, MVPA at age 15 to 17 years

was not adjusted for). All analyses were repeated with data from age 9 to 11 years instead of age 6 to 9 years. For both the cross-sectional and longitudinal analysis, models were computed with robust standard errors and results were expressed as regression coefficients (β) representing the changes in the outcomes per 10-minute change in the movement behaviour, their 95% CIs, and *p*-values.

Further longitudinal analyses were conducted by grouping the participants based on their activity status at age 6 to 9 years and age 15 to 17 years. This was only done for sport and exercise participation, as the sample size of participants with valid measurements at age 6 to 9 years and age 15 to 17 years was greatly limited for the device-measured variables (MVPA, sedentary time, sleep). A median split of average time spent in sport and exercise per day was used to categorise the participants. Participants were categorised as 1) persistently inactive (below median at age 6 to 9 years and age 9 to 11 years), 2) decreasingly active (above median at age 9 to 11 years and below median at age 15 to 17 years), 3) increasingly active (below median at age 6 to 9 years and above median at age 15 to 17 years), and 4) persistently active (above median at age 6 to 9 years and age 15 to 17 years).

Linear regression with robust standard errors was used to assess differences in TBLH BMC and lean mass at age 15 to 17 years between the persistently inactive, decreasingly active, increasingly active, and persistently active groups, adjusted for age, stature, pubertal status, study group, and fat mass at age 15 to 17 years, with additional adjustment for TBLH lean mass at age 15 to 17 years and TBLH BMC at age 6 to 9 years when considering TBLH BMC as the outcome, and with TBLH lean mass at age 6 to 9 years when considering TBLH lean mass as the outcome.

Estimated marginal means for each group were computed and pairwise comparisons between each pairing were performed. All analyses were then repeated, replacing the age 6 to 9 years data with the age 9 to 11 years data. For all analyses, statistical significance was set at alpha level 0.05.

7.4 Results

7.4.1 Descriptive Statistics

At age 6 to 9 years, males were taller, and had greater TBLH BMC and lean mass, and lower fat mass than females (Table 7.1). Males had greater MVPA, greater sport and exercise participation, no difference in sedentary time, greater screen time, and no difference in sleep duration compared to females (Table 7.2). A greater proportion of males met the guidelines for MVPA and muscle and bone strengthening activity than females, a greater proportion of females met the guidelines for screen time, and there was no difference in the proportion of females and males meeting the sleep guidelines (Table 7.2).

At age 9 to 11 years, males had greater TBLH BMC and lean mass, and lower fat mass than females. A greater proportion of females were pubertal compared to males (Table 7.1). Males had greater MVPA, greater sport and exercise participation, no difference in sedentary time, greater screen time, and less sleep compared to females (Table 7.2). A greater proportion of males met the guidelines for MVPA, there were no differences in the proportion of females and males meeting the muscle and bone strengthening guidelines, a greater proportion of females met the guidelines for screen time, and there were no differences in the proportion of females and males meeting the sleep guidelines.

At age 15 to 17 years, males were taller, heavier, and slightly older than females, with greater TBLH BMC and lean mass, and lower fat mass than females (Table 7.1). More females had achieved pubertal stage 5, as described by Tanner, compared to males. Males had greater MVPA, no difference in sport and exercise participation, no difference in sedentary time, greater screen time and less sleep compared to females (Table 7.2). There were no differences in the proportion of females and males meeting the MVPA guidelines, the muscle and bone strengthening guidelines, and the screen time guidelines, and a greater proportion of females met the sleep guidelines compared to males.

Table 7.1 Characteristics of females and males at age 6-9, at age 9-11, and age 15-17 years

	Age 6 to 9 years			**Age 9 to 11 years**			**Age 15 to 17 years**		
	Females	**Males**	***p* value for group difference**	**Females**	**Males**	***p* value for group difference**	**Females**	**Males**	***p* value for group difference**
	Mean/Median (SD/IQR)	**Mean/Median (SD/IQR)**		**Mean/Median (SD/IQR)**	**Mean/Median (SD/IQR)**		**Mean/Median (SD/IQR)**	**Mean/Median (SD/IQR)**	
Age (years)	7.62 (0.38)	7.66 (0.4)	0.24	9.73 (0.43)	9.80 (0.43)	0.079	**15.73 (0.40)**	**15.85 (0.45)**	**0.036**
Stature (cm)	**127.88 (5.65)**	**129.66 (5.6)**	**0.001**	140.06 (6.44)	141.32 (6.1)	0.051	**166.18 (5.61)**	**176.51 (7.39)**	**<0.001**
Weight (kg)	26.54 (5.08)	27.32 (5.01)	0.089	33.85 (7.36)	35.23 (7.49)	0.070	**57.79 (8.72)**	**65.13 (13.23)**	**<0.001**
BMI-SDS	-0.17 (1.05)	-0.18 (1.1)	0.94	-0.14 (1.01)	-0.09 (1.1)	0.64	0.02 (0.85)	-0.16 (1.07)	0.18
Pubertal Status									
% (cases) pubertal stage 1	96.5 (221)	98.8 (246)	0.13	**65.0 (128)**	**86.6 (162)**	**<0.001**	**0 (0)**	**0 (0)**	**0.003**
% (cases) pubertal stage 2	3.5 (8)	1.2 (3)		**35.0 (69)**	**13.4 (25)**		**0 (0)**	**0 (0)**	
% (cases) pubertal stage 3	0 (0)	0 (0)		**0 (0)**	**0 (0)**		**3.9 (4)**	**13.5 (16)**	
% (cases) pubertal stage 4	0 (0)	0 (0)		**0 (0)**	**0 (0)**		**53.4 (55)**	**61.3 (73)**	
% (cases) pubertal stage 5	0 (0)	0 (0)		**0 (0)**	**0 (0)**		**42.7 (44)**	**25.2 (30)**	
IOTF Definition									
% (cases) thin	9.2 (21)	10.8 (27)	0.50	9.6 (19)	10.2 (19)	0.81	6.8 (7)	11.8 (14)	0.29
% (cases) normal weight	76 (174)	77.1 (192)		74.6 (147)	70.6 (132)		82.5 (85)	75.6 (90)	
% (cases) overweight	10.9 (25)	7.2 (18)		12.7 (25)	15 (28)		6.8 (7)	10.9 (13)	
% (cases) obese	3.9 (9)	4.8 (12)		3 (6)	4.3 (8)		3.9 (4)	1.7 (2)	
TBLH BMC (kg)	**0.66 (0.14)**	**0.69 (0.14)**	**0.010**	**0.91 (0.21)**	**0.97 (0.21)**	**0.011**	**1.91 (0.27)**	**2.27 (0.45)**	**<0.001**
TBLH lean mass (kg)	**16.87 (1.99)**	**18.69 (2.13)**	**<0.001**	**20.83 (2.79)**	**22.93 (2.80)**	**<0.001**	**35.13 (3.83)**	**47.58 (6.82)**	**<0.001**
TBLH fat mass (kg)	**4.90 (3.67 to 7.25)**	**3.56 (2.38 to 5.85)**	**<0.001**	**7 (5.06 to 11.18)**	**6.48 (3.77 to 10.85)**	**0.014**	**15.55 (12.14 to 17.99)**	**8.18 (5.85 to 13.67)**	**<0.001**

For continuous variables, values are mean/median and standard deviation/interquartile range, and p-values from independent samples t-tests or Mann-Whitney U tests to test for the sex difference. For categorical variables, values are % (n), with Fisher's exact p-values to test for the sex difference.

Bold emphasis indicates statistical significance at $p < 0.05$.

At age 6 to 9 years, n = 478 (229 females, 249 males). At age 9 to 11 years, n = 384 (197 females, 187 males). At age 15 to 17 years, n = 222 (103 females, 119 males).

BMC, bone mineral content; BMI, body mass index; IOTF, International Obesity Task Force; IQR, interquartile range; SD, standard deviation; SDS, standard deviation score; TBLH, total-body-less-head.

Table 7.2 Movement behaviours of females and males at age 6-9, at age 9-11, and age 15-17 years

	Age 6 to 9 years			**Age 9 to 11 years**			**Age 15 to 17 years**		
	Females	**Males**	***p* value for group difference**	**Females**	**Males**	***p* value for group difference**	**Females**	**Males**	***p* value for group difference**
	Mean/Median (SD/IQR)	**Mean/Median (SD/IQR)**		**Mean/Median (SD/IQR)**	**Mean/Median (SD/IQR)**		**Mean/Median (SD/IQR)**	**Mean/Median (SD/IQR)**	
MVPA (min/day)	**85 (55 to 127)**	**126 (81 to 178)**	**<0.001**	**72 (51 to 99)**	**115 (79 to 162)**	**<0.001**	**32 (18 to 48)**	**47 (26 to 71)**	**0.033**
MVPA guidelines									
% (cases) < 60 mins/day	**28.3 (52)**	**14.8 (27)**	**0.002**	**34.4 (54)**	**17.6 (23)**	**0.001**	80.0 (32)	65.2 (43)	0.13
% (cases) ≥ 60 mins/day	**71.7 (132)**	**85.2 (155)**		**65.6 (103)**	**82.4 (108)**		20.0 (8)	34.8 (23)	
Sport and exercise participation (min/day)	**9 (0 to 17)**	**13 (9 to 21)**	**0.008**	**9 (0 to 26)**	**17 (9 to 34)**	**0.028**	14 (0 to 40)	18 (0 to 68)	0.37
Muscle and bone strengthening guidelines									
% (cases) < 3 times/week	**87.8 (201)**	**79.4 (197)**	**0.019**	76.5 (150)	67.9 (127)	0.068	70.9 (73)	61.3 (73)	0.16
% (cases) ≥ 3 times/week	**12.2 (28)**	**20.6 (51)**		23.5 (46)	32.1 (60)		29.1 (30)	38.7 (46)	
Sedentary time (min/day)	242 (131)	232 (129)	0.47	393 (108)	386 (100)	0.54	598 (143)	587 (140)	0.72
Screen time (min/day)	**92 (49)**	**113 (55)**	**<0.001**	**108 (53)**	**135 (59)**	**<0.001**	**308 (166)**	**367 (199)**	**0.017**
Screen time guidelines									
% (cases) > 2 hours/day	**20.5 (47)**	**35.9 (89)**	**< 0.001**	**30.6 (60)**	**53.5 (100)**	**< 0.001**	95.1 (98)	96.6 (114)	0.74
% (cases) ≤ 2 hours/day	**79.5 (182)**	**64.1 (159)**		**69.4 (136)**	**46.5 (87)**		4.9 (5)	3.4 (4)	
Sleep (hour/night)	9.69 (0.46)	9.65 (0.55)	0.46	**9.23 (0.55)**	**9.08 (0.48)**	**0.013**	**7.73 (0.75)**	**7.46 (0.64)**	**0.012**
Sleep guidelines									
% (cases) not meeting age-specific guidelines	9.1 (17)	11.6 (22)	0.50	31 (49)	40.5 (53)	0.108	**65.4 (53)**	**81.3 (78)**	**0.025**
% (cases) meeting age-specific guidelines	90.9 (169)	88.4 (168)		69 (109)	59.5 (78)		**34.6 (28)**	**18.7 (18)**	

For continuous variables, values are mean/median and standard deviation/interquartile range, and p-values from independent samples t-tests or Mann-Whitney U tests to test for the sex difference. For categorical variables, values are % (n), with Fisher's exact p-values to test for the sex difference.

Bold emphasis indicates statistical significance at $p < 0.05$.

At age 6 to 9 years, for MVPA, n = 366 (184 females, 182 males). For sport and exercise and screen time, n = 477 (229 females, 248 males). For sedentary time, n = 365 (184 females, 181 males). For sleep, n = 376 (186 females, 190 males).

At age 9 to 11 years, for MVPA, n = 288 (157 females, 131 males). For sport and exercise and screen time, n = 383 (196 females, 187 males). For sedentary time, n = 287 (157 females, 130 males). For sleep, n = 289 (158 females, 131 males).

At age 15 to 17 years, for MVPA and sedentary time, n = 106 (40 females, 66 males). For sport and exercise, n = 222 (103 females, 119 males). For screen time, n = 221 (103 females, 118 males). For sleep, n = 177 (81 females, 96 males).

Meeting the MVPA guidelines was defined as at least 60-minutes average daily MVPA. Meeting the muscle and bone strengthening guidelines was defined as participating in organised sport and exercise at least 3 times per week. Meeting the screen time guidelines was defined as an average of no more than 2 hours of recreational screen time per day. Meeting the sleep guidelines was defined as achieving between 9- and 11-hours average per night for children aged 6 to 11, and as achieving between 8- and 10-hours average per night for adolescents aged 15 to 17 years.

IQR, interquartile range; MVPA, moderate-to-vigorous physical activity; SD, standard deviation.

7.4.2 Cross-sectional Associations of Movement Behaviours with TBLH BMC and Lean Mass in Females

At age 6 to 9 years, sedentary time was negatively associated with TBLH lean mass (Table 7.3). There were no other associations of the movement behaviours with TBLH BMC or TBLH lean mass at age 6 to 9 years (Table 7.3). At age 9 to 11 years, sport and exercise participation was positively associated with TBLH BMC (Table 7.3). MVPA was positively associated with TBLH lean mass, and sleep was negatively associated with TBLH lean mass at age 9 to 11 years (Table 7.3). At age 15 to 17 years, sleep was positively associated with TBLH BMC (Table 7.3). MVPA and sport and exercise participation were positively associated with TBLH lean mass at age 15 to 17 years (Table 7.3).

7.4.3 Cross-sectional Associations of Movement Behaviours with TBLH BMC and Lean Mass in Males

At age 6 to 9 years, sport and exercise participation was positively associated with TBLH BMC and lean mass and sedentary time was positively associated with TBLH lean mass (Table 7.3). There were no other associations between the movement behaviours with TBLH BMC or TBLH lean mass at age 6 to 9 years. At age 9 to 11 years, MVPA, sport and exercise participation, and sedentary time were positively associated with TBLH lean mass. At age 15 to 17 years, screen time was negatively associated with TBLH BMC, and sport and exercise participation was positively associated with TBLH lean mass (Table 7.3).

Table 7.3 Cross-sectional associations of movement behaviours with TBLH BMC and lean mass in females and males

	Age 6 to 9 years[a]		Age 9 to 11 years[b]		Age 15 to 17 years[c]	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Females						
TBLH BMC						
MVPA	0.001 (-0.001 to 0.002)	0.331	0.001 (-0.003 to 0.005)	0.629	0.005 (-0.016 to 0.025)	0.652
Sport and exercise	0.003 (-0.002 to 0.008)	0.270	**0.008 (0.003 to 0.013)**	**0.004**	0.008 (-0.001 to 0.018)	0.081
Sedentary time	-0.000 (-0.001 to 0.000)	0.218	-0.001 (-0.002 to 0.001)	0.219	-0.002 (-0.007 to 0.002)	0.287
Screen time	0.000 (-0.001 to 0.001)	0.929	-0.001 (-0.003 to 0.001)	0.167	-0.001 (-0.003 to 0.001)	0.460
Sleep	0.001 (-0.002 to 0.003)	0.653	0.001 (-0.003 to 0.005)	0.794	**0.008 (0.000 to 0.015)**	**0.043**
TBLH lean mass						
MVPA	0.004 (-0.036 to 0.043)	0.851	**0.142 (0.081 to 0.204)**	**<0.001**	**0.430 (0.115 to 0.744)**	**0.009**
Sport and exercise	0.013 (-0.099 to 0.125)	0.817	0.101 (-0.020 to 0.223)	0.101	**0.358 (0.247 to 0.470)**	**<0.001**
Sedentary time	**-0.017 (-0.033 to -0.001)**	**0.033**	0.013 (-0.015 to 0.041)	0.344	-0.005 (-0.084 to 0.074)	0.895
Screen time	0.019 (-0.008 to 0.045)	0.163	0.030 (-0.009 to 0.068)	0.127	-0.008 (-0.045 to 0.029)	0.667
Sleep	0.018 (-0.046 to 0.081)	0.582	**-0.108 (-0.173 to -0.043)**	**0.001**	-0.096 (-0.218 to 0.026)	0.122
Males						
TBLH BMC						
MVPA	0.001 (-0.001 to 0.002)	0.233	0.000 (-0.004 to 0.004)	0.985	-0.008 (-0.024 to 0.009)	0.357
Sport and exercise	**0.004 (0.001 to 0.007)**	**0.016**	0.005 (-0.001 to 0.011)	0.100	0.011 (-0.001 to 0.022)	0.066
Sedentary time	0.000 (-0.000 to 0.001)	0.264	-0.002 (-0.003 to 0.000)	0.093	-0.003 (-0.008 to 0.001)	0.146
Screen time	-0.000 (-0.001 to 0.001)	0.984	-0.001 (-0.003 to 0.001)	0.338	**-0.002 (-0.004 to -0.000)**	**0.020**
Sleep	0.001 (-0.001 to 0.003)	0.292	0.001 (-0.003 to 0.006)	0.532	-0.005 (-0.018 to 0.008)	0.430
TBLH lean mass						
MVPA	0.035 (-0.003 to 0.073)	0.070	**0.116 (0.053 to 0.178)**	**<0.001**	0.126 (-0.240 to 0.491)	0.494
Sport and exercise	**0.091 (0.022 to 0.161)**	**0.011**	**0.221 (0.092 to 0.350)**	**0.001**	**0.337 (0.142 to 0.532)**	**0.001**
Sedentary time	**0.017 (0.000 to 0.034)**	**0.045**	**0.037 (0.006 to 0.069)**	**0.020**	-0.017 (-0.113 to 0.079)	0.726
Screen time	-0.008 (-0.034 to 0.018)	0.544	-0.037 (-0.080 to 0.006)	0.094	-0.037 (-0.080 to 0.006)	0.092
Sleep	0.028 (-0.023 to 0.078)	0.278	-0.003 (-0.098 to 0.092)	0.945	0.104 (-0.106 to 0.314)	0.328

Values are unstandardised regression coefficients, their 95% confidence intervals, and p-values. The regression coefficient represents the difference in TBLH BMC, and lean mass associated with a 10-minute average daily difference in each movement behaviour.
Bold emphasis indicates statistical significance at $p < 0.05$.

[a] Estimates adjusted for age, stature, pubertal status, and fat mass, measured at age 6 to 9 years, and study group. Estimates for TBLH BMC additionally adjusted for lean mass measured at age 6 to 9 years. MVPA and sedentary time are mutually adjusted for each other.
[b] Estimates adjusted for age, stature, pubertal status, and fat mass, measured at age 9 to 11 years, and study group. Estimates for TBLH BMC additionally adjusted for lean mass measured at age 9 to 11 years. MVPA and sedentary time are mutually adjusted for each other.
[c] Estimates adjusted for age, stature, pubertal status, and fat mass, measured at age 15 to 17 years, and study group. Estimates for TBLH BMC additionally adjusted for lean mass measured at age 15 to 17 years. MVPA and sedentary time are mutually adjusted for each other.

At age 6 to 9 years, n = 184 females and 181 males for MVPA and sedentary time, n = 229 females and 248 males for sport and exercise participation, n = 229 females and 248 males for screen time, n = 186 females and 190 males for sleep.

At age 9 to 11 years, n = 157 females and 130 males for MVPA and sedentary time, n = 196 females and 187 males for sport and exercise participation, n = 196 females and 187 males for screen time, n = 158 females and 131 males for sleep.

At age 15 to 17 years, n = 40 females and 66 males for MVPA and sedentary time, n = 103 females and 119 males for sport and exercise participation, n = 103 females and 118 males for screen time, n = 81 females and 96 males for sleep.

BMC, bone mineral content; MVPA, moderate-to-vigorous physical activity; TBLH, total-body-less-head.

7.4.4 Longitudinal Associations of Movement Behaviours with TBLH BMC and Lean Mass in Females

MVPA at age 6 to 9 years was positively associated with TBLH BMC at age 15 to 17 years (Table 7.4). Sport and exercise participation at age 9 to 11 years was positively associated with TBLH BMC and lean mass at age 15 to 17 years (Table 7.4). MVPA at age 9 to 11 years was positively associated with TBLH lean mass at age 15 to 17 years (Table 7.4).

In terms of sport and exercise participation, from age 6 to 9 years to age 15 to 17 years, 38 females were persistently inactive, 13 were decreasingly active, 26 were increasingly active and 25 were persistently active. Increasingly active females from age 6 to 9 years to age 15 to 17 years had greater TBLH BMC at age 15 to 17 years than persistently inactive females (Figure 7.2). Persistently active females from age 6 to 9 years to age 15 to 17 years had greater TBLH lean mass at age 15 to 17 years than persistently inactive and decreasingly active females, and increasingly active females had greater TBLH lean mass than persistently inactive females. From age 9 to 11 years to age 15 to 17 years, 36 females were persistently inactive, 16 were decreasingly active, 20 were increasingly active, and 31 were persistently active. Persistently active females from age 9 to 11 years to age 15 to 17 years had greater TBLH BMC at age 15 to 17 years than persistently inactive females. Persistently active females from age 9 to 11 years to age 15 to 17 years had greater TBLH lean mass at age 15 to 17 years than persistently inactive, decreasingly active, and increasingly active females.

7.4.5 Longitudinal Associations of Movement Behaviours with TBLH BMC and Lean Mass in Males

Sleep at age 6 to 9 years was positively associated with TBLH lean mass at age 15 to 17 years (Table 7.4). Sport and exercise participation at age 9 to 11 years was positively associated with TBLH BMC and lean mass at age 15 to 17 years (Table 7.4).

In terms of sport and exercise participation, from age 6 to 9 years to age 15 to 17 years, 36 males were persistently inactive, 20 were decreasingly active, 20 were increasingly active, and 36 were persistently active. Increasingly active and persistently active males from age 6 to 9 years to age 15 to 17 years had greater TBLH BMC at age 15 to 17 years than persistently inactive and decreasingly active males (Figure 7.3). Persistently active males from age 6 to 9 years to age 15 to 17 years had greater TBLH lean mass at age 15 to 17 years than persistently inactive and decreasingly active males. From age 9 to 11 years to age 15 to 17 years, 37 males were persistently inactive, 15 were decreasingly active, 15 were increasingly active, and 37 were persistently active. Persistently active males from age 9 to 11 years to age 15 to 17 years had greater TBLH BMC at age 15 to 17 years than persistently inactive, decreasingly active and increasingly active males. Persistently active males from age 9 to 11 years to age 15 to 17 years had greater TBLH lean mass at age 15 to 17 years than persistently inactive and increasingly active males.

Table 7.4 Longitudinal associations of movement behaviours with TBLH BMC and lean mass in females and males

	Age 6 to 9 years → Age 15 to 17 years [a]		Age 9 to 11 years → Age 15 to 17 years [b]	
	β (95% CI)	p	β (95% CI)	p
Females				
TBLH BMC				
MVPA	**0.008 (0.002 to 0.014)**	**0.010**	0.010 (-0.001 to 0.021)	0.082
Sport and exercise	0.014 (-0.006 to 0.034)	0.160	**0.020 (0.008 to 0.032)**	**0.002**
Sedentary time	-0.000 (-0.003 to 0.002)	0.914	0.001 (-0.003 to 0.005)	0.598
Screen time	-0.001 (-0.006 to 0.004)	0.755	-0.003 (-0.009 to 0.003)	0.346
Sleep	-0.003 (-0.015 to 0.008)	0.558	-0.005 (-0.015 to 0.005)	0.306
TBLH lean mass				
MVPA	0.066 (-0.075 to 0.207)	0.354	**0.272 (0.091 to 0.454)**	**0.004**
Sport and exercise	0.202 (-0.215 to 0.620)	0.339	**0.343 (0.016 to 0.670)**	**0.040**
Sedentary time	-0.049 (-0.101 to 0.003)	0.064	0.021 (-0.059 to 0.102)	0.598
Screen time	0.110 (-0.031 to 0.252)	0.125	0.101 (-0.026 to 0.227)	0.118
Sleep	0.126 (-0.135 to 0.388)	0.339	-0.165 (-0.349 to 0.019)	0.077
Males				
TBLH BMC				
MVPA	0.003 (-0.005 to 0.011)	0.476	0.001 (-0.011 to 0.012)	0.887
Sport and exercise	0.012 (-0.000 to 0.025)	0.059	**0.027 (0.006 to 0.048)**	**0.012**
Sedentary time	-0.000 (-0.004 to 0.003)	0.966	-0.001 (-0.007 to 0.005)	0.700
Screen time	-0.002 (-0.007 to 0.004)	0.543	-0.001 (-0.008 to 0.007)	0.852
Sleep	-0.001 (-0.011 to 0.010)	0.918	-0.009 (-0.024 to 0.005)	0.215
TBLH lean mass				
MVPA	0.092 (-0.077 to 0.261)	0.282	0.164 (-0.059 to 0.387)	0.147
Sport and exercise	0.306 (-0.103 to 0.715)	0.141	**0.721 (0.327 to 1.115)**	**<0.001**
Sedentary time	0.016 (-0.068 to 0.099)	0.710	0.042 (-0.087 to 0.171)	0.522
Screen Time	0.063 (-0.056 to 0.181)	0.296	-0.091 (-0.222 to 0.039)	0.167
Sleep	**0.382 (0.138 to 0.626)**	**0.003**	-0.015 (-0.278 to 0.249)	0.913

Values are unstandardised regression coefficients, their 95% confidence intervals, and p-values.
The regression coefficient represents the difference in TBLH BMC and lean mass at age 15 to 17 years associated with a 10-minute average daily difference in each movement behaviour at age 6 to 9 years and age 9 to 11 years.
Bold emphasis indicates statistical significance at $p < 0.05$.

[a] Estimates for TBLH BMC at age 15 to 17 years adjusted for age, stature, pubertal status, lean mass, fat mass and study group measured at age 15 to 17 years, and TBLH BMC measured at age 6 to 9 years. Estimates for TBLH lean mass at age 15 to 17 years adjusted for age, stature, pubertal status, fat mass and study group measured at age 15 to 17 years, and TBLH lean mass measured at age 6 to 9 years. MVPA and sedentary time are mutually adjusted for each other.
[b] Estimates for TBLH BMC at age 15 to 17 years adjusted for age, stature, pubertal status, lean mass, fat mass and study group measured at age 15 to 17 years, and TBLH BMC measured at age 9 to 11 years. Estimates for TBLH lean mass at age 15 to 17 years adjusted for age, stature, pubertal status, fat mass and study group measured at age 15 to 17 years, and TBLH lean mass measured at age 9 to 11 years. MVPA and sedentary time are mutually adjusted for each other.

At age 6 to 9 years to age 15 to 17 years, n = 78 females and 87 males for MVPA and sedentary time, n = 102 females and 112 males for sport and exercise and screen time, n = 79 females and 90 males for sleep.

At age 9 to 11 years to age 15 to 17 years, n = 83 females and 75 males for MVPA and sedentary time, n = 103 females and 104 males for sport and exercise and screen time, n = 84 females and 76 males for sleep.

BMC, bone mineral content; MVPA, moderate-to-vigorous physical activity; TBLH, total-body-less-head.

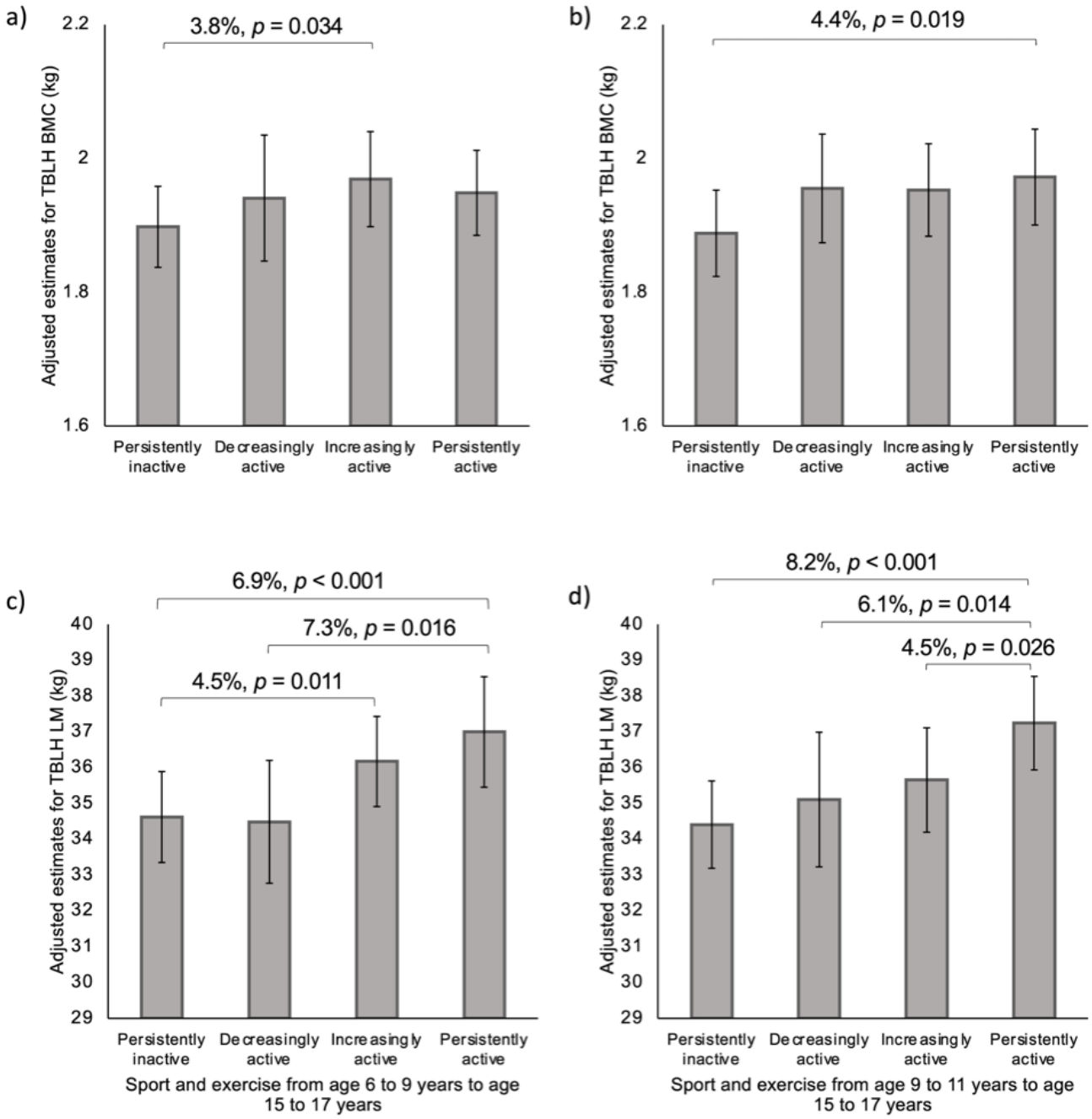

Figure 7.2 Differences in TBLH BMC and lean mass in females aged 15-17 years based on physical activity at age 6-9 years or age 9-11 years and age 15-17 years.

Values are adjusted means (95% CI). Estimates for TBLH BMC are for a female at pubertal stage 4 in the control group with sex-specific mean levels of age, stature, lean mass and fat mass at measured at age 15 to 17 years, and TBLH BMC measured at age 6 to 9 years or age 9 to 11 years. Estimates for TBLH lean mass at age 15 to 17 years are for a female at pubertal stage 4 in the control group with sex-specific mean levels of age, stature, and fat mass at measured at age 15 to 17 years, and TBLH lean mass measured at age 6 to 9 years or age 9 to 11 years.

Figure 2a and 2c are for age 6 to 9 years to age 15 to 17 years. Figure 2b and 2d are for age 9 to 11 years to age 15 to 17 years.

For age 6 to 9 to age 15 to 17 years, the median split is based on 9 minutes at age 6 to 9 years and 15 minutes at age 15 to 17 years. For age 9 to 11 to age 15 to 17 years, the median split is based on 9 minutes at age 9 to 11 years and 14 minutes at age 15 to 17 years.

For age 6 to 9 to age 15 to 17 years, n = 102 (persistently inactive n = 38, decreasingly active n = 13, increasingly active n = 26, persistently active n = 25).

For age 9 to 11 to age 15 to 17 years, n = 103 (persistently inactive n = 36, decreasingly active n = 16, increasingly active n = 20, persistently active n = 31).

BMC, bone mineral content; LM, lean mass; TBLH, total-body-less-head.

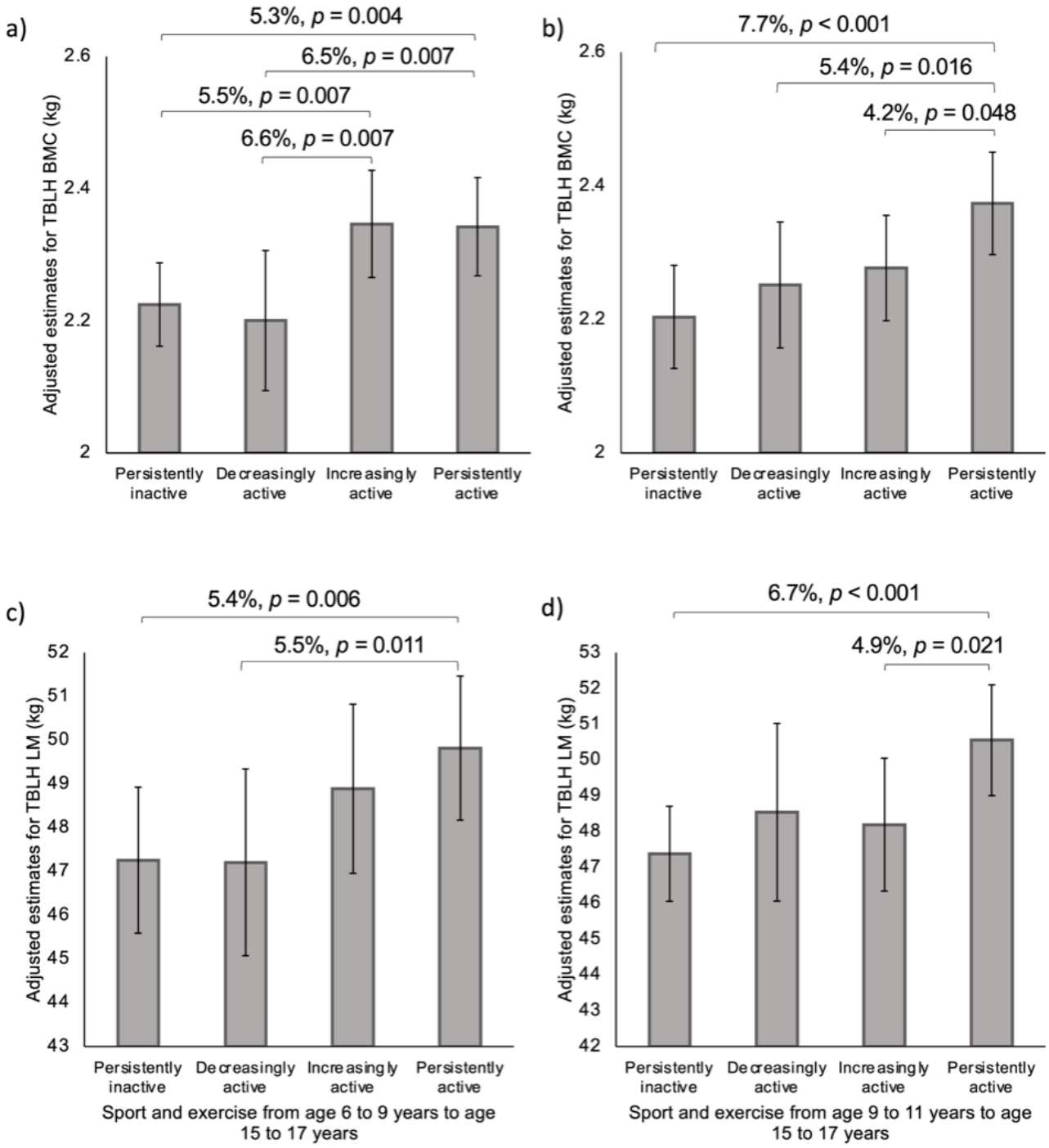

Figure 7.3 Differences in TBLH BMC and lean mass in males aged 15-17 years based on physical activity at age 6-9 years or age 9-11 years and age 15-17 years.

Values are adjusted means (95% CI). Estimates for TBLH BMC are for a male at pubertal stage 4 in the control group with sex-specific mean levels of age, stature, lean mass and fat mass at measured at age 15 to 17 years, and TBLH BMC measured at age 6 to 9 years or age 9 to 11 years. Estimates for TBLH lean mass at age 15 to 17 years are for a male at pubertal stage 4 in the control group with sex-specific mean levels of age, stature, and fat mass at measured at age 15 to 17 years, and TBLH lean mass measured at age 6 to 9 years or age 9 to 11 years.

Figure 3a and 3c are for age 6 to 9 years to age 15 to 17 years. Figure 3b and 3d are for age 9 to 11 years to age 15 to 17 years.

For age 6 to 9 to age 15 to 17 years, the median split is based on 11 minutes at age 6 to 9 years and 18 minutes at age 15 to 17 years. For age 9 to 11 to age 15 to 17 years, the median split is based on 19 minutes at age 9 to 11 years and 19 minutes at age 15 to 17 years.

For age 6 to 9 to age 15 to 17 years, n = 112 (persistently inactive n = 36, decreasingly active n = 20, increasingly active n = 20, persistently active n = 36).

For age 9 to 11 to age 15 to 17 years, n = 104 (persistently inactive n = 37, decreasingly active n = 15, increasingly active n = 15, persistently active n = 37).

BMC, bone mineral content; LM, lean mass; TBLH, total-body-less-head.

7.5 Discussion

To the best of our knowledge, this study is the first to examine the cross-sectional and longitudinal associations of each of the 24-hour movement behaviours with TBLH BMC and lean mass in a general population from childhood to adolescence, including sport and exercise as a proxy measure of muscle and bone strengthening activity. Greater MVPA was associated with improved lean mass cross-sectionally in females and males, and with improved BMC and lean mass longitudinally in females. Higher levels of sport and exercise participation were associated with improved BMC and lean mass cross-sectionally and longitudinally in females and males, though it was important for children to maintain their activity levels into adolescence to maintain their improved TBLH BMC and lean mass. Sedentary time was inversely associated with lean mass in females and positively associated with lean mass in males, though these associations did not persist longitudinally. Screen time was negatively associated with BMC in males cross-sectionally but was not associated with BMC or lean mass longitudinally in females or males. Sleep was positively associated with BMC and inversely associated with lean mass in females cross-sectionally, and positively associated with lean mass in males longitudinally. Our

findings highlight the important role of MVPA, sport and exercise participation, sedentary time, screen time and sleep for muscle and bone health.

The female and male participants of our study had similar median values of aBMD compared to reference values at age 6 to 9 years, lower median values at age 9 to 11 years, though these fell between the reference 10th and 50th percentile, and higher median values at age 15 to 17, though these fell between the reference 50th and 90th percentile, with cross-calibration equations applied to allow comparisons between different DXA systems (Zemel *et al.*, 2011; Shepherd *et al.*, 2012). In terms of lean mass index, in order to account for differences in stature, the female and male participants had similar median values to reference values (Weber *et al.*, 2013). It is difficult to directly compare adherence to the 24-hour movement guidelines across studies due to the fact that that some studies have used self-reports and others devices to measure sleep and PA, and due to different epoch lengths, cut-points, wear sites, wear time protocols, and measurement periods used when measuring sleep and PA using devices (Guinhouya, Samouda and de Beaufort, 2013). Furthermore, the guidelines have been applied differently between studies. However, our sample appeared to have a higher adherence to the MVPA (66% to 85% in our sample compared to 44%), screen time (47% to 80% in our sample compared to 39%), and sleep guidelines (60% to 91% in our sample compared to 42%) at age 6 to 9 and age 9 to 11 years than in a 12-country study of children with mean age 10 years (Roman-Vinas *et al.*, 2016), though there are limited data in adolescents to compare to when the participants were age 15 to 17 years.

7.5.1 Moderate-to-Vigorous Physical Activity

The null cross-sectional associations of MVPA with TBLH BMC in both sexes that we observed are in contrast with the results of some previous studies, as outlined in a 2020 systematic review, which have found consistent positive cross-sectional associations of MVPA with whole-body DXA bone outcomes in males and in both sexes combined, but inconsistent associations in females (Bland *et al.*, 2020). Whereas we observed a longitudinal relationship between MVPA with TBLH BMC in females only, the systematic review found relatively consistent positive longitudinal associations between MVPA and whole body bone outcomes in females and males (Bland *et al.*, 2020). The differences between our findings and those of previous studies may be due to the different devices used to capture PA. In our study, a combined heart rate and movement sensor was used to estimate energy expenditure from acceleration and heart rate, providing a more accurate assessment of energy expenditure than acceleration alone, as was used in previous studies (Corder *et al.*, 2007; Bland *et al.*, 2020). Therefore, our measure of MVPA includes activities which increase heart rate with relatively small increases in acceleration, such as cycling, whereas previous studies have captured MVPA based purely on acceleration. As acceleration is more related to musculoskeletal loading than heart rate, previous studies may have been more likely to capture mechanically loaded MVPA only (Rowlands and Stiles, 2012). Given that mechanical loading is important for bone adaptation (Hart *et al.*, 2017), this may have led to stronger associations between MVPA and bone outcomes in studies which have used acceleration-only estimates of MVPA compared to MVPA based on acceleration and heart rate, as used in our study, though this may also depend on how the accelerometry data is treated (Haapala *et al.*, 2022). However, as the WHO PA guidelines do not stipulate that the

recommended average 60 minutes daily MVPA should be mechanically loaded (Chaput *et al.*, 2020), previous studies may have overestimated the importance of MVPA for bone, as described in the guidelines. Our findings suggest that MVPA is largely not related to BMC in children and adolescents, though females who do more MVPA in childhood may have improved BMC in adolescence.

There is a lack of previous research investigating the associations of accelerometer-assessed MVPA with DXA-assessed lean mass. We observed inconsistent cross-sectional relationships between MVPA and lean mass in females and males, whereas previous studies have reported null associations in females and males (Moliner-Urdiales *et al.*, 2010; Córdoba-Rodríguez *et al.*, 2022), or positive associations in females only, between MVPA and lean mass in children and adolescents (Ness *et al.*, 2007). Whilst we found that the association between MVPA and lean mass persisted longitudinally in females only, Zymbal and colleagues (Zymbal *et al.*, 2019) observed that both females and males who accumulated more MVPA from age 5 to age 17 years had greater leg lean soft tissue at age 17 years (Zymbal *et al.*, 2019). It is unclear what accounts for the different observations across studies. As the longitudinal study by Zymbal and colleagues (Zymbal *et al.*, 2019) used lower-limb measures of lean mass, it is plausible that the observed associations would be stronger than in other studies using whole body measures of lean mass, as the lower-limb is loaded during normal weight-bearing activity, and the accelerometer placement at the hip would be expected to be more sensitive to loading at the hip and lower-limb (Janz *et al.*, 2008). However, given the conflicting findings, further high-quality longitudinal studies are needed to extend our understanding of the relationship between MVPA and lean mass, though our findings

indicate that concurrent MVPA may be important for lean mass in females and males, and that females who do more MVPA in childhood may have improved lean mass in adolescence.

7.5.2 Muscle and Bone Strengthening Activity

Although the positive cross-sectional associations of sport and exercise participation with TBLH BMC and lean mass were inconsistent across timepoints, we did find that sport and exercise participation at age 9 to 11 years was consistently positively associated with TBLH BMC and lean mass at age 15 to 17 years in females and males. Further, females and males who did more sports and exercise from childhood to adolescence had greater TBLH BMC and lean mass at age 15 to 17 years. Studies considering relationships between recreational sport and exercise participation with bone and lean mass in children and adolescents are scarce. In the Western Australia Pregnancy Cohort (Raine) Study, males who consistently participated in sport from age 5 to 17 years had greater whole-body BMC at age 20 than those who dropped out over time (McVeigh *et al.*, 2019). However, there were no differences in whole-body BMC in females based on their sport participation from age 5 to 17 years, and lean mass was not considered as an outcome (McVeigh *et al.*, 2019). The null association in females observed in the Raine cohort differs to our observations in females, potentially due to differences in measurement methods or covariates (McVeigh *et al.*, 2019). Whereas we considered average minutes per day of sport and exercise as our exposure in both cross-sectional and longitudinal analyses, and categorised sport and exercise amounts into 4 groups as our exposure in further longitudinal analyses based on the average minutes per day from age 6 to 9 years to age 15 to 17 years, the Raine study used a binary indicator of

whether the participants took part in any organised sport or not, which may contribute to the differences in findings (McVeigh *et al.*, 2019). A meta-analysis of exercise interventions, including jumping and/or circuit training, found a small, but statistically significant and beneficial effect of exercise on whole body BMC, further supporting our observations (Nogueira, Weeks and Beck, 2014). However, there was no effect of exercise interventions on lean mass (Nogueira, Weeks and Beck, 2014). It is possible that the activities captured within our measure of sport and exercise participation were more diverse than those used in intervention studies, offering a potential explanation for the relationship between sport and exercise participation and lean mass that we observed. Given the scarcity of studies investigating the relationships of sport and exercise participation with BMC and lean mass, further work is needed to better understand these relationships in general populations. Even so, our findings indicate that sport and exercise participation may be important for current BMC and lean mass, and that sport and exercise participation in childhood is important for BMC and lean mass in adolescence. Further, consistently participating in high levels sport and exercise across childhood and adolescence may lead to a greater BMC and lean mass at age 15 to 17 years. Given the current primary focus on aerobic activity in the WHO guidelines (Chaput *et al.*, 2020), our findings emphasise that the unique importance of muscle and bone strengthening activity for muscle and bone health should be promoted alongside MVPA, and included in future surveillance relating to the guidelines.

7.5.3 Sedentary Behaviour

The null cross-sectional and longitudinal associations of sedentary time with BMC that we observed is consistent with the conclusion of a systematic review of previous

studies looking at accelerometer-measured sedentary behaviour with bone outcomes in children and adolescents (Koedijk *et al.*, 2017). However, whilst we found screen time was cross-sectionally negatively associated with TBLH BMC at age 15 to 17 years in males, the systematic review highlighted insufficient evidence for an associations of self- or parental-reported sedentary behaviour, which includes screen time, with total body bone outcomes (Koedijk *et al.*, 2017). In terms of lean mass, we observed inconsistent negative cross-sectional associations between sedentary time and lean mass in females, inconsistent positive cross-sectional associations between sedentary time and lean mass in males, and null longitudinal associations of sedentary time and screen time with lean mass. Previous studies have observed negative cross-sectional associations (Riso *et al.*, 2018) and, similar to our findings, null longitudinal associations (Fulton *et al.*, 2009) between measures of sedentary behaviour and fat-free mass. The positive cross-sectional relationships between sedentary time and lean mass in males are unexpected, as there is no apparent biological reason why being sedentary would increase lean mass once fat mass is controlled for, as we did, though as the relationships do not persist longitudinally, it is possible that unmeasured confounding factors may be influencing the cross-sectional relationships. Although it is important to highlight that evidence supporting the inclusion of sedentary behaviour in the movement guidelines is largely based on adiposity, other cardiometabolic risk factors and psychosocial risk factors, our findings, combined with previous evidence, indicate that largely sedentary behaviour was not detrimental to BMC and lean mass (Koedijk *et al.*, 2017; Chaput *et al.*, 2020).

7.5.4 Sleep

Sleep was largely unrelated to BMC in our study population, except for a positive association at age 15 to 17 years in females. Although there is limited research considering the associations between sleep and bone health, the largely null associations between sleep and TBLH BMC that we observed is consistent with previous research. In a population sample of European children and adolescents, adherence to the sleep guidelines, assessed with questionnaires, was not associated with bone stiffness index, as measured by QUS, though lean mass was not accounted for (Cheng *et al.*, 2021), and similar null findings have been observed in adults (Swanson *et al.*, 2021). Taken together, our findings suggest that sleep duration is largely not related to BMC in children and adolescents, though it may be beneficial in adolescent females. In our sample, sleep was negatively cross-sectionally related to TBLH lean mass in females only at age 9 to 11 years, though this did not persist longitudinally. In males, sleep at age 6 to 9 years was positively associated with TBLH lean mass at age 15 to 17 years. Generally, previous studies have observed null associations between sleep duration and lean mass in children and adolescents (Carter *et al.*, 2011; Diethelm *et al.*, 2011; Butte *et al.*, 2016). However, in girls and boys age 10 to 12 years, sleep duration was positively associated with fat-free mass (Riso *et al.*, 2018). Differences in measures of sleep duration and lean mass may contribute to the different observations between studies. Whereas our study used DXA measures of lean mass, previous studies used DXA-assessed fat-free mass, which includes bone (Carter *et al.*, 2011; Butte *et al.*, 2016), or estimated fat-free mass from skinfold measures (Diethelm *et al.*, 2011; Riso *et al.*, 2018). Further, some studies used device-measured sleep duration (Carter *et al.*, 2011; Butte *et al.*, 2016), as did ours, whereas other studies used a

self or parental report (Diethelm *et al.*, 2011; Riso *et al.*, 2018). Given the conflicting findings, further research would be valuable in better understanding the relationship between device-measured sleep duration and DXA-assessed lean mass, though our findings indicate that a longer sleep duration in childhood may contribute to a greater lean mass in adolescence in males.

7.5.5 Translation of Findings

This section is provided to aid interpretation of the regression model estimates. Given that using a 60-second epoch to capture PA data likely leads to an underestimation of MVPA (further details provided below in Section 7.5.6), this section does not seek to make specific recommendations regarding the dose of MVPA associated with a given change in bone and lean mass (Edwardson and Gorely, 2010). A 10-minute daily increase in MVPA at age 6 to 9 years was associated with 0.008 kg greater TBLH BMC at age 15 to 17 years in females, which equates to around 0.4 % of mean level of BMC at age 15 to 17 years. A 10-minute daily increase in MVPA at age 9 to 11 years was associated with 0.272 kg greater TBLH lean mass in females, which equates to around 0.8 % of the mean level of TBLH lean mass at age 15 to 17 years. A 10-minute daily increase in sport and exercise participation at age 9 to 11 years was associated with 0.020 kg greater TBLH BMC and 0.343 kg greater TBLH lean mass at age 15 to 17 years in females, which equates to around 1.0 % of the mean level of both BMC and lean mass at age 15 to 17 years. A 10-minute daily increase in sleep at age 6 to 9 years was associated with 0.382 kg greater TBLH lean mass at age 15 to 17 years in males, which equates to around 0.8 % of the mean level of lean mass at age 15 to 17 years. A 10-minute daily increase in sport and exercise at age 9 to 11 years was associated

with 0.027 kg greater TBLH BMC and 0.721 greater TBLH lean mass at age 15 to 17 years in males, which equates to around 1.2 % of the mean level of BMC and 1.5 % of the mean level of lean mass at age 15 to 17 years. Whilst these associations were statistically significant, the clinical relevance should be questioned, given the relatively small translation in terms of absolute values. Even so, given that theoretical modelling suggests a 10% increase in PBM may delay the onset of osteoporosis by 13 years in later life (Hernandez, Beaupre and Carter, 2003), and that lean mass in key positive determinant of BMC (Sioen *et al.*, 2016), promoting MVPA, sport and exercise participation and sleep in childhood may contribute to delaying the onset of osteoporosis in later life.

7.5.6 Strengths and Limitations

The strengths of our study include the population-based sample of children with an 8-year follow-up into adolescence and the measurement of BMC and lean mass by DXA. There are several limitations that should be considered when interpreting our findings. As we did not have a direct measure of muscle and bone strengthening activity, we were not able to directly assess the relationship between muscle and bone strengthening activity with BMC and lean mass. We used questionnaire-assessed participation in sport and organised exercise as a proxy measure for muscle and bone strengthening activity. Although previous studies assessing muscle and bone strengthening activities have measured participation in activities which would fall into the sport and organised exercise category, such as push-ups and weight-lifting, there may be other activities, such as swimming, which we have captured and would not be considered bone strengthening (Foster and Armstrong, 2018; Smith *et al.*, 2020; Fraser *et al.*, 2021; Bennie *et al.*, 2022). However, it has

been reported that the most popular types of organised sports and exercise in Finland for boys are football (soccer), ice hockey, and floorball (a type of indoor hockey), and for girls are dance, gymnastics, aerobics, football (soccer), and horseback riding, all of which reflect muscle and bone strengthening activities (Lampinen *et al.*, 2017; Lehtonen, Oja and Hakamäki, 2022). Even so, future studies with a direct assessment of muscle and bone strengthening activity would be valuable in better understanding the relationship between muscle and bone strengthening activity with BMC and lean mass in children and adolescents. The use of the 60-second epoch to capture MVPA may have led to an underestimation of MVPA, particularly when the participants were children (Edwardson and Gorely, 2010), which may have influenced the observed relationships between MVPA with BMC and lean mass (Skinner *et al.*, 2023a). The relationship between MVPA with BMC and lean mass may have been further limited by the dynamic range of the Actiheart (±2.5 *g*), as peak acceleration during running and jumping may exceed 5 *g* at the hip and the wrist (Rowlands and Stiles, 2012). However, as this study used the combined heart rate and movement data, and the calculation of intensity from the combined data is weighted more towards heart rate at high intensities, the dynamic range is likely to have less of an impact on the estimates of MVPA than in the previous studies in Chapter 4 and 6 using acceleration only data. Further, we did not consider the relationships between LPA with BMC and lean mass in this study, so the associations between LPA with BMC and lean mass in children and adolescents remain unknown. Although DXA is the gold-standard for the assessment of BMC and lean mass in children, it is not possible to obtain true vBMD estimates from DXA, and as such the determinants of vBMD in our sample remain unknown. Further, as our sample had relatively high levels of MVPA at age 6 to 9 years and age 9 to 11 years,

it is unknown whether these findings extend to less active children. Finally, although we adjusted for important covariates, residual confounding remains a potential limitation in all observational studies, and causality cannot be assumed.

7.6 Conclusion

MVPA was largely not related to BMC in children and adolescents, though females who do more MVPA in childhood may have improved BMC in adolescence. Concurrent MVPA may be important for lean mass in females and males, and females who do more MVPA in childhood may have improved lean mass in adolescence. Sport and exercise participation may be important for current BMC and lean mass in females and males, and sport and exercise participation in childhood is important for BMC and lean mass in adolescence. Childhood sedentary behaviour was not detrimental to adolescent BMC and lean mass. Sleep duration was largely not related to BMC in children and adolescents, though it may be beneficial in adolescent females, and in males a longer sleep duration in childhood may contribute to a greater lean mass in adolescence. Overall, our findings suggest promoting engagement in the 24-hour movement behaviours in childhood, particularly sport and exercise to strengthen muscle and bone, is important in supporting bone and lean mass development in adolescence. Our findings support the inclusion and promotion of muscle and bone-specific activity in the PA guidelines. Given the particular importance of muscle and bone strengthening activity for bone and lean mass development, future research into the 24-hour movement behaviours should consider muscle and bone strengthening activity in addition to MVPA.

Chapter 8 Summary of Findings, Implications and Future Directions

This purpose of this thesis was to provide novel insights into PA, body composition and associated endocrine factors, and vitamin D status, as determinants of BMC and aBMD in children and adolescents. This chapter aims to summarise, synthesise, discuss, and highlight the implications of this thesis. First, a summary of each chapter and the contribution to the literature will be provided. Following this, a synthesis of the key findings will be presented. In addition, the thesis limitations are highlighted, and details of a further study that was undertaken alongside Chapter 7 to address some of the limitations of this thesis will be presented. Finally, future research directions are provided.

8.1 Summary of Studies and Contribution to the Literature

8.1.1 Chapter 4

Chapter 4 was the first study to examine the sex-specific independent and interactive associations of PA intensity and 25(OH)D levels with aBMD in prepubertal children aged 6 to 8 years. Findings from this study showed that MPA, MVPA and serum 25(OH)D were positively independently associated with TBLH and lower-limb aBMD, though PA intensity did not interact with serum 25(OH)D to influence aBMD. This suggests that in active children (80% meeting the PA guidelines) with largely sufficient vitamin D status (80% with serum 25(OH)D ≥ 50 nmol/L), the positive relationships between MPA and MVPA with aBMD were not dependent on a given level of serum 25(OH)D (Department of Health and Social Care, 2019). Further, the relationships between PA and 25(OH)D with aBMD did not differ based on sex. This study contributes to the literature by adding evidence to support the promotion of MVPA and behaviours

to encourage optimal vitamin D status, such as nutrition and sunlight exposure, to support skeletal health in prepubertal children.

8.1.2 Chapter 5

Chapter 5 explored the relationship between fat mass and TBLH BMC in females and males aged 9 to 11 years, and assessed for the first time the extent to which this relationship is mediated by insulin, leptin (free leptin index), adiponectin, DHEAS, testosterone and estradiol. A novel 4-way decomposition method was used, which is superior to the previously used 'difference method' for mediation analysis, as it allows for interactions between exposures and mediators (VanderWeele, 2016). The results of this study showed that fat mass was positively related to BMC in females and males. This relationship was suppressed by mediation through free leptin index in females and males, and moderated by adiponectin and free leptin index in females and males respectively, though these associations accounted for a relatively small contribution in terms of absolute values. This study highlights the importance of fat mass as an independent determinant of TBLH BMC in pre- and early-pubertal children of largely a healthy weight status.

8.1.3 Chapter 6

Chapter 6 assessed the associations of PA volume, PA intensity distribution, and MVPA, with TBLH BMC, lean mass, and fat mass in females and males aged 9 to 11 years. This was the first study to apply novel PA metrics (average-acceleration and intensity-gradient) to investigate the relationships between PA and BMC in pre- and early-pubertal children. These novel PA metrics offer a

cut-point free approach to summarising PA data, and as such overcome some of the limitations associated with using cut-points, such as the population specific nature of cut-points and the loss of detail when collapsing PA data into three or four broad categories (Rowlands *et al.*, 2020). This study found that PA volume was positively associated with BMC in females and males, and positively associated with lean and negatively associated with fat mass in males. PA intensity distribution was negatively associated with BMC in males. MVPA was positively associated with lean mass in females and males, and negatively associated with fat mass in females and males. These findings contribute to the literature by indicating that PA volume may be important for improving BMC in females and males, and increasing lean and reducing fat mass in males, whereas MVPA may be important for favourable lean and fat outcomes in both sexes. As adjusting for lean and fat mass altered the relationships between PA with BMC, this study further emphasises the importance of considering lean and fat mass alongside bone.

8.1.4 Chapter 7

Chapter 7 examined for the first time whether MVPA, sport and exercise (as a proxy measure of muscle and bone strengthening activity), sedentary behaviour (i.e., sedentary time, screen time), and sleep are associated with TBLH BMC and TBLH lean mass cross-sectionally (at age 6 to 9 years, age 9 to 11 years, age 15 to 17 years), and longitudinally (from age 6 to 9 years and age 9 to 11 years to age 15 to 17 years). This study found that MVPA was positively associated with lean mass cross-sectionally in females and males, and with BMC and lean mass longitudinally in females. Sport and exercise participation were positively associated with BMC and lean mass cross-sectionally and

longitudinally in females and males, though it was important for children to maintain their activity levels into adolescence to maintain their improved TBLH BMC and lean mass. Sedentary time was inversely and positively associated with lean mass in females and males respectively, though these associations did not persist longitudinally. Screen time was negatively associated with BMC in males cross-sectionally but was not associated with BMC or lean mass longitudinally in females or males. Sleep was positively associated with BMC and inversely associated with lean mass in females cross-sectionally, and positively associated with lean mass in males longitudinally. These findings contribute to the literature by suggesting that promoting engagement in the 24-hour movement behaviours in childhood, particularly participating in sport and exercise to strengthen muscle and bone rather than overall MVPA, is important in supporting bone and lean mass development in adolescence.

8.2 Synthesis of Findings

A summary of the interrelationships between PA, vitamin D status, body composition and bone as indicated in this thesis is presented in Figure 8.1. This section will present a synthesis of the overall findings of this thesis.

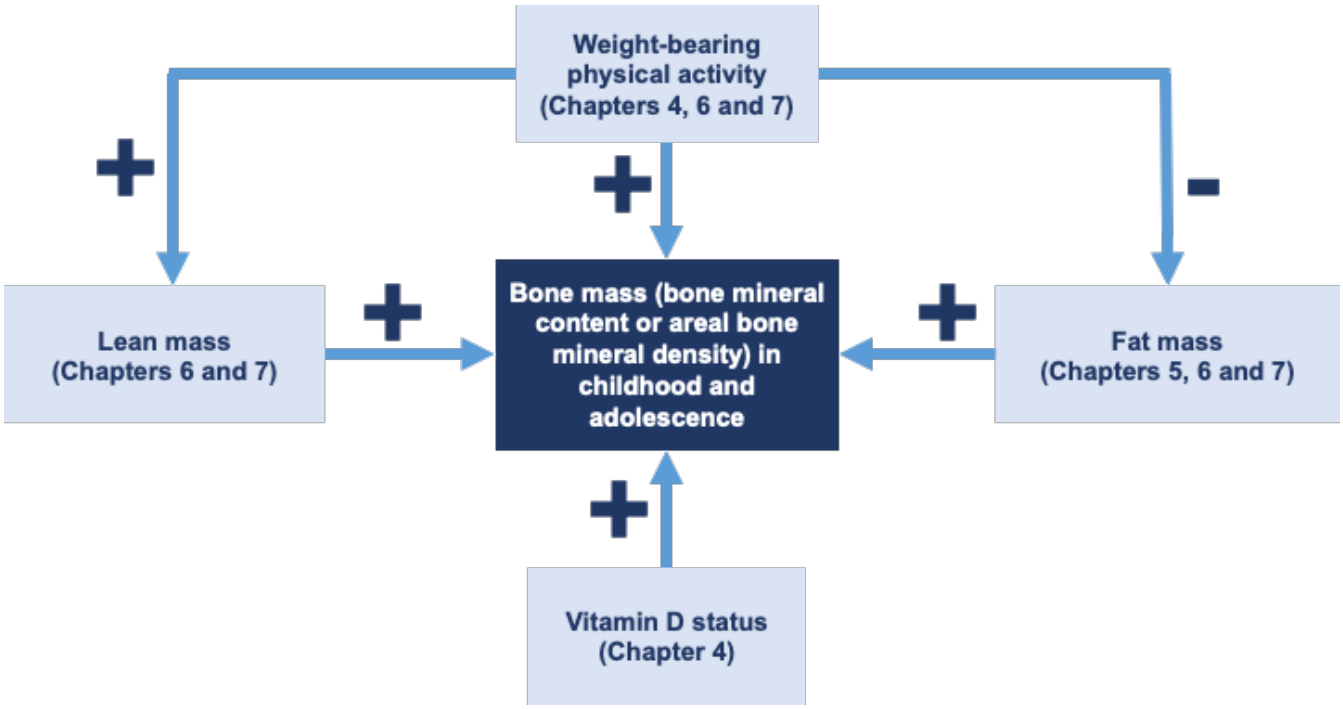

Figure 8.1 The interrelationships between physical activity, vitamin D status and body composition with bone mineral content and areal bone mineral density in childhood and adolescence. See text for discussion.

8.2.1 Physical Activity, Sedentary Behaviour, Sleep, and Bone

Collectively, this thesis investigated the relationships between PA and other movement behaviours, summarised with different assessment methods (i.e., combined heart rate and movement sensor data, acceleration only data, and questionnaire) with BMC and aBMD. The findings of this thesis generally indicated that PA is a positive determinant of BMC and aBMD in childhood and adolescence, though these observations differed dependent on the measures of PA used.

The assessment of PA using the combined heart rate and movement sensors utilised in the PANIC study facilitated the consideration of PA exposures based on combined heart and acceleration data and based on acceleration data only.

Whilst energy expenditure-based estimates of MVPA (minutes/day > 4 METs) from the combined heart rate and acceleration data were generally not associated with bone outcomes, MVPA justified based on acceleration (minutes/day > 0.75 m/s^2) and total PA volume from acceleration data were positively associated with bone outcomes. When energy expenditure-based PA intensity is the exposure of interest, the combined heart rate and acceleration data has advantages over using acceleration only, as the relationship between acceleration and energy expenditure is highly variable across activities such as walking on the level and incline, cycling, and during load-bearing activities (Brage *et al.*, 2004). However, energy expenditure-based estimates of PA are not necessarily relevant when considering bone as the outcome of interest.

Previous studies investigating the relationships between PA and bone have not assessed PA with combined heart rate and movement sensors, and therefore have been unable to assess relationships between both the combined sensor data and the acceleration only data. Both the ALSPAC (Tobias *et al.*, 2007; Elhakeem *et al.*, 2020) and the IBDS (Janz *et al.*, 2010; Janz *et al.*, 2014) characterised MVPA based on acceleration only, and observed positive associations with bone outcomes. As accelerometer data is positively correlated with GRFs (Janz *et al.*, 2003; Rowlands and Stiles, 2012; Meyer *et al.*, 2015), estimates of PA based on acceleration only data offers a proxy measure for mechanical loading. Our findings are in agreement with those of the ALSPAC (Tobias *et al.*, 2007; Elhakeem *et al.*, 2020) and IBDS (Janz *et al.*, 2010; Janz *et al.*, 2014), that weight-bearing PA is important for bone in childhood and adolescence, and extends these findings by indicating that increased energy expenditure alone is generally not important for bone outcomes. In terms of

future research, the findings of this thesis emphasise the importance of using PA exposures which are relevant to bone when investigating the relationships between PA and bone. In terms of practical recommendations, our findings highlight the importance of differentiating between weight-bearing and non-weight-bearing PA when making recommendations for bone health, with greater levels of weight-bearing habitual PA being associated with improved aBMD and BMC in children. This distinction is particularly important as the WHO PA guidelines (Chaput *et al.*, 2020) do not stipulate that MVPA should be weight-bearing, though the literature supporting the importance of MVPA for bone health is based on acceleration only data (Poitras *et al.*, 2016), and therefore is reflective of the relationship between weight-bearing MVPA and bone health. The findings of this thesis support the promotion of weight-bearing total PA and MVPA for bone health in children.

Although accelerometers are valuable in assessing the total volume and intensity of PA, and do not rely on participant recall, accelerometers are limited in assessing the type of PA, which may be particularly relevant when considering bone health as an outcome (Hildebrand and Ekelund, 2017). This thesis found that questionnaire-assessed sport and exercise participation, as a proxy for muscle and bone strengthening activity, was positively associated with BMC both cross-sectionally and longitudinally, whereas MVPA based on combined heart rate and movement data was not associated with BMC cross-sectionally, and was only associated with BMC longitudinally in females. Similarly, in the IBDS, male interscholastic sport participants and female interscholastic power sport participants had greater hip aBMD than their peers, though it should be highlighted that these findings are specific to athletes

performing at interscholastic level and may be biased by genetics (Ward *et al.*, 2019). In terms of recreational sport participation, in the Raine Study cohort, males who consistently participated in sports from age 5 to 17 years had greater whole-body BMC at age 20 than those who dropped out over time, though there were no differences in whole-body BMC in females based on their sport participation (McVeigh *et al.*, 2019). This thesis extends the previous studies by considering sport and exercise participation as a continuous variable and examining these relationships in a population sample, studying recreational sport and exercise rather than high performance sport participation only. The findings of this thesis support the inclusion of muscle and bone strengthening activity in the WHO PA guidelines (Chaput *et al.*, 2020). Further, given the specific importance of muscle and bone strengthening activity for bone health demonstrated in this thesis, and given that childhood and adolescence is a period of particular importance in maximising bone accrual (Gunter, Almstedt and Janz, 2012), the muscle and bone strengthening aspect of the PA recommendations deserves further attention in terms of research (Chaput *et al.*, 2020), surveillance (Strain *et al.*, 2020), and promotion. Although there is limited research relating to awareness of the PA guidelines in children, within adults the data suggests that awareness around the strength component of the guidelines is lacking. For example, only 32% of physiotherapists were aware of the strength PA recommendations, compared to 60% who were aware of the MVPA recommendations (Lowe *et al.*, 2017). More concerningly, other research investigating awareness of the PA guidelines did not assess awareness of the strength component (Dunlop and Murray, 2013; Hunter *et al.*, 2014). Further research into awareness of the guidelines in parents or guardians and children would be beneficial, with more emphasis on the importance of including

activities to strengthen muscles and bones in the messaging around the PA guidelines. The findings of this thesis indicate that specific, targeted activity is important for bone health in childhood and adolescence.

Sedentary time, based on the combined heart rate and acceleration data (minutes/day ≤ 1.5 METs) or based on acceleration only data (minutes/day ≤ 0.06 m/s^2) was not related to bone outcomes in our sample, and questionnaire-assessed screen time generally was not related with bone, except for a negative association in males at age 15 to 17 years. These findings are in agreement with systematic review evidence, which found no association between device-measured sedentary behaviour, adjusted for MVPA, with total body bone outcomes in children, adolescents and young adults (Koedijk *et al.*, 2017). However, there was insufficient evidence for an association between screen time and bone outcomes (Koedijk *et al.*, 2017). Overall, the findings of this thesis suggest that generally sedentary behaviour is not detrimental to bone health. Similarly, sleep was generally not related to bone in our sample, except for a positive association in females at age 15 to 17 years. The evidence base considering the relationship between sleep and bone in children and adolescents is sparse and contradictory (Casazza, Hanks and Fernandez, 2011; Cheng *et al.*, 2021), though the findings of this thesis extend the current evidence based by using device-measured sleep duration combined with DXA-assessed bone outcomes in a population sample spanning childhood and adolescence. However, although the findings of this thesis suggest sleep is largely not related to bone, it should be highlighted that the average sleep duration in our sample was within or close to the age-specific recommendations (Tremblay *et al.*, 2016), and it is possible that extreme sleep deficit remains

detrimental to bone. Although limiting sedentary behaviour and getting sufficient sleep may be important for adiposity-related outcomes, cardiometabolic and psychosocial health (Carson *et al.*, 2016; Chaput *et al.*, 2016), the findings of this thesis suggest that targeting sedentary behaviour and sleep is not sufficient for promoting bone health in childhood and adolescence.

The findings of this thesis can be explained by the Mechanostat hypothesis, which postulates that the skeleton adapts in response to mechanical loading (Frost, 2003). As, according to the Mechanostat hypothesis (Frost, 2003), mechanical loading is necessary for skeletal adaptation, this explains why associations were observed between acceleration only-based PA exposures and bone, but not between combined heart rate and acceleration-based PA exposures and bone. In addition to strain magnitude, insights from animal studies indicate that loading should be dynamic and applied quickly, with irregular spatial distribution (Forwood, 2008; Hart *et al.*, 2017). In practice, this means that activities like jumping, plyometrics, and sports which involve dynamic movements with quick changes in direction will be more osteogenic than slow, steady-state walking (Forwood, 2008). These movement characteristics are likely captured within our measure of sport and exercise participation, and therefore explains the positive association between sport and exercise participation and bone which we observed. These movement characteristics are also possible to capture with accelerometers, but given the 60-second epoch and the Actiheart dynamic range of ±2.5 *g* used in the PANIC study, it is likely that the acceleration signal associated with these high-intensity dynamic movements may have been diluted. In terms of sedentary behaviour, the Mechanostat hypothesis states that disuse remodelling occurs below a

certain strain threshold, leading to the removal of bone (Frost, 2003). Although sedentary behaviour represents a removal of skeletal loading, it is possible that within our sample the removal of loading was not enough to have a detrimental effect on bone. Taken together, the findings of this thesis indicate weight-bearing habitual PA and specific muscle and bone strengthening activity should be encouraged in childhood and adolescence for better bone health.

8.2.2 Vitamin D and Bone

Chapter 4 indicated that serum 25(OH)D was a positive determinant of aBMD in prepubertal children, though serum 25(OH)D did not moderate the relationship between PA intensity and aBMD. Similar to our findings, serum 25(OH)D was positively associated with DXA-assessed bone parameters in children aged 1 to 6 years (Hazell *et al.*, 2015), and in children and adolescents aged 7 to 19 years (Pekkinen *et al.*, 2012). However, in girls aged 10 to 12 years serum 25(OH)D was not associated with DXA-assessed bone parameters, though this was the only study not to adjust for stature in the analysis, potentially explaining the conflicting findings (Cheng *et al.*, 2003). The null interaction between serum 25(OH)D and PA intensity with aBMD that we observed is in line with the findings of Rønne and colleagues (Rønne *et al.*, 2018a), who found serum 25(OH)D did not interact with VPA to influence TBLH BMC. However, in female and male adolescents, those meeting the PA guidelines and with serum 25(OH)D ≥ 75 nmol/L had greater BMC than those meeting the PA guidelines with serum 25(OH)D < 75 nmol/L, suggesting that sufficient 25(OH)D levels may improve BMC in active adolescents only (Valtuena *et al.*, 2012). The different analysis strategies may account for the differences in findings. Whilst the analysis in this thesis and that of Rønne and colleagues (Rønne *et al.*,

2018a) considered the PA-vitamin D status interaction as a continuous variable, Valtuena and colleagues (Valtuena *et al.*, 2012) grouped participants based on activity levels and vitamin D status, and assessed differences between groups. The findings of this thesis indicate that promoting behaviours to encourage optimal vitamin D status is important in supporting skeletal development in childhood, but these strategies do not need to be used in conjunction with PA to be beneficial.

8.2.3 Body Composition and Bone

This thesis investigated body composition as a determinant of bone mass and considered how body composition altered the relationships between PA and bone. Lean mass is well established as a positive determinant of BMC and aBMD in childhood (Sioen *et al.*, 2016), and this thesis adds to previous evidence by indicating that fat mass is also a positive determinant of BMC in pre- and early-pubertal children, after taking lean mass into account. The positive relationship between fat mass and BMC observed in this thesis is in line with previous research in pre- and early-pubertal children (Leonard *et al.*, 2004b; Clark, Ness and Tobias, 2006). The relationship between lean mass and bone is explained by the Mechanostat theory, as aside from trauma, the largest loads, and therefore the largest bone strains, on the skeleton are from muscles (Frost and Schönau, 2000). Given this, greater lean mass leads to a greater BMC in children and adolescents (Frost and Schönau, 2000; Rauch *et al.*, 2004). This thesis found that the relationship between fat mass and BMC was largely not explained by insulin, leptin, adiponectin, DHEAS, testosterone and estradiol. It is likely that the positive relationship between fat mass and BMC is explained by the additional loading on the bones due to the additional weight

(Tobias, 2010). Although these findings are limited to pre- and early-pubertal children, and in a sample of children with largely a normal weight status, this thesis indicates that both total body lean mass and fat mass are positive determinants of total body bone mass in childhood.

Chapter 6 highlighted that adjusting for lean mass and fat mass altered the relationships between PA volume and bone, suggesting that part of the relationship between PA and bone is explained by the influence of PA on lean mass and fat mass. Whilst the associations between PA volume and bone were null when adjusting for lean mass, they were highly significant when adjusting for fat mass, and remained significant and positive when adjusting for lean and fat mass. The mediating role of lean mass in the PA-bone relationship is compatible with Mechanostat theory (Frost and Schönau, 2000). Muscle contractions during activity transfer the forces from PA to the bones, and the skeleton adapts in response to the strains (Zymbal *et al.*, 2019; Public Health England, 2021). As PA is positively associated with lean mass (Baxter-Jones *et al.*, 2008a; Zymbal *et al.*, 2019), and lean mass is positively associated with bone (Sioen *et al.*, 2016), this offers an explanation for how PA may influence bone. Similar to our findings that the PA-bone relationship was partially explained by lean mass, in the IBDS cohort, leg lean soft tissue explained 43-49% and 27-32% of the relationship between MVPA trajectories (from age 5 to 17) and proximal femur aBMD in females and males, respectively (Zymbal *et al.*, 2019). Similar findings have also been shown in adults (Xiang *et al.*, 2017; McMillan *et al.*, 2018). In practice, as this thesis combined with previous evidence indicates lean mass mediates the relationship between PA and bone, activities to increase lean mass should be considered in intervention studies to

improve BMC, providing PA is then engaged in to load the bone (Zymbal *et al.*, 2019).

In addition to lean mass, this thesis also highlighted the importance of considering fat mass when examining the relationship between PA and bone. The positive association between PA and bone was obscured when only adjusting for lean mass but was significant with additional adjustment for fat mass. Similar findings were presented from the ALSPAC cohort (Tobias *et al.*, 2007). When adjusted for lean mass, there was no relationship between total MVPA and TBLH BMC, but with additional adjustment for fat mass, the relationship between MVPA and TBLH BMC became positive (Tobias *et al.*, 2007). The PA-fat mass-bone relationship is complex, as PA is negatively associated with fat mass (Riddoch *et al.*, 2009), but fat mass is positively related with bone. This suggests that although the overall relationship between PA volume and BMC is positive, PA may also have a small negative influence on bone, by reducing fat mass, and therefore reducing mechanical loading on the bones due to fat mass. As such, both fat mass and lean mass should be considered when investigating the relationships between PA and bone, and when designing PA interventions with the goal of increasing bone.

8.2.4 Strengths and Limitations

The strengths of this thesis include the combination of cross-sectional and longitudinal study designs to investigate the determinants of BMC and aBMD in population sample of children and adolescents. The investigations in pre- and early-puberty capture a crucial time for skeletal development (Forwood, 2013). The use of DXA assessments of BMC and aBMD as the gold-standard for these

measures (Shalof *et al.*, 2021), and as recommended by the International Society for Clinical Densitometry for the assessment of paediatric bone health (International Society for Clinical Densitometry, 2019b), strengthens the findings of this thesis. The combination of different methods for summarising PA, justified both on the basis of energy expenditure and mechanical loading, in addition to the application of novel accelerometry metrics (average-acceleration, intensity-gradient, MX metrics) and questionnaire assessments provided extensive insight into the relationships between PA and bone. Finally, the adjustment for a range of relevant confounders further strengthens the inferences from the findings of this thesis.

There are limitations to this thesis, and the findings of this thesis should be considered in light of these. Firstly, given the nature of prospective cohort studies combined with a lifestyle intervention, selection bias is a possibility, whereby more health-conscious families and children enrol and remain in the study (Larsson, 2021). This poses a threat to the internal validity of the study when both the exposure and the outcome are related to initial participation and loss to follow-up (Tripepi *et al.*, 2010). However, at baseline the participants of the PANIC study did not differ in sex, age, height-SDS or BMI-SDS to all the children who started the first grade in Kuopio in 2007 to 2009. Even so, selection bias remains an unavoidable problem in epidemiological research (Tripepi *et al.*, 2010). In terms of external validity, the PANIC cohort were relatively active, particularly at age 6 to 9 years and age 9 to 11 years. In relation to the MVPA guidelines, 72% of females and 82% males met the guidelines at age 6 to 9 years, 66% of females and 82% of males met the guidelines at age 9 to 11 years, and 20% of females and 35% of males met the

guidelines at age 15 to 17 years. Given the 60-second epoch used, this is likely an underestimation of true PA levels (Baquet *et al.*, 2007). Further, the PANIC cohort had low levels of participants living with obesity across all measurement points. Therefore, the findings of this thesis may not be reflective of samples with lower activity levels and higher levels of participants living with obesity. Further, in all observational studies causality cannot be assumed, and the influence of unmeasured confounding on the observed relationships remains a possibility.

Due to the study design, there were no measures around the age of PHV, so the determinants of BMC and aBMD during this period of growth when bone is rapidly accrued are unknown (Baxter-Jones *et al.*, 2011). However, the PANIC study did include measures at age 6 to 9 years, age 9 to 11 years and age 15 to 17 years. Therefore, the BMC gain across measurement points is reflective of the amount of bone gained in the years surrounding PHV, with the average age of PHV as 11.8 years in females and 13.5 years in males (Baxter-Jones *et al.*, 2011). Given this, the longitudinal analysis (Chapter 7) presented in this thesis can provide insight into the determinants of BMC accrual across the pubertal years, though further longitudinal studies with more frequent measurement points would be valuable in understanding these relationships further.

The measurement methods used in the PANIC study are subject to limitations. The use of questionnaires to assess participation in sport and exercise and screen time is subject to recall bias, which may lead to an inaccurate measure of these behaviours based on accidental false recall, missed recall, or differential reporting accuracy of different domains of activity (Hildebrand and

Ekelund, 2017). Further, the use of questionnaires is subject to social desirability bias, which in general leads to overreporting of PA and underreporting of sedentary behaviours (Shephard, 2003). As there is not a criterion measure of sport and exercise participation, it is difficult to assess the validity of the questionnaire (Shephard, 2003). However, questionnaire assessments of PA have been suggested to adequately rank PA levels, and participation in discrete activities, such as sport and exercise, are generally more accurately recalled because the individual has made a conscious decision to engage in that activity (Hildebrand and Ekelund, 2017). Even so, it is possible that inaccurate measurements of PA and sedentary behaviour may mask or distort the associations between PA, sedentary behaviour, and bone health.

Accelerometer assessment of PA also has limitations which should be considered. Due to the technology available at the time of data collection, accelerometer data in the PANIC study was collected in 60-second epochs. As children's MPA and VPA commonly occurs in short bouts (< 10 seconds) (Baquet *et al.*, 2007), time spent in higher intensities of PA were likely misclassified as lower intensities, particularly across baseline and 2-year follow-up measurements when the participants were younger. An underestimation of high-intensity PA is particularly problematic when considering bone as an outcome, as evidence indicates that bone is especially responsive to high-intensity PA (Brailey *et al.*, 2022). Therefore, the relationships between accelerometer-assessed PA and bone may have been underestimated in the studies presented in this thesis. Further, it was not possible to accurately quantify the amount of high-intensity PA due to the epoch length, so the specific duration of high-intensity PA associated with improved bone outcomes remains

unknown. In addition, the dynamic range of the Actiheart (±2.5 *g*) may further lead to an underestimation of high-intensity activity, as peak acceleration during everyday PA, such as running and jumping, may exceed 5 *g* at the hip and the wrist (Rowlands and Stiles, 2012). Therefore, activities which exceed the dynamic range of the Actiheart are not accurately reflected in accelerometer data. The Actiheart accelerometers used in the PANIC study are not able to assess posture, and therefore the measures of sedentary time used in this thesis include stationary standing time as well as sedentary time in a seated or reclined position. It is possible that stationary standing time has a different relationship with bone to time spent sitting, reclined or lying, and therefore the true relationship between sedentary behaviour, which is characterised by an energy expenditure ≤ 1.5 METs whilst sitting, reclined or lying (Thivel *et al.*, 2018), and bone may be masked or distorted.

Although DXA is the gold-standard method for measuring bone in children, BMC and aBMD captured with DXA only represent one aspect of bone strength (Shalof *et al.*, 2021). In addition to bone mass, bone strength, defined as the ability of the bone to resist fracture, is influenced by bone microarchitecture and bone geometry (Forwood, 2013; Shalof *et al.*, 2021). DXA cannot provide measures of true vBMD or differentiate between cortical and trabecular bone (Forwood, 2013; Shalof *et al.*, 2021). Therefore, this thesis is unable to provide insight into the determinants of bone strength more broadly. In terms of reliability, DXA scans were not repeated within the PANIC dataset, meaning that measurement reliability could not be assessed. The reliability of our primary outcome measure is therefore unknown.

8.2.5 Addressing the Limitations with Accelerometer Assessment of Physical Activity

As highlighted throughout the thesis, there are limitations with the accelerometer data, particularly regarding the 60-second epoch, which may have influenced the results presented in this thesis. The influence of epoch length on summary measures of PA intensity (MPA, VPA) in well understood. However, it is unknown whether epoch length influences the intensity-gradient metric used in Chapter 6. This is of particular interest, as Chapter 6 found that intensity was not associated with BMC in females, and negatively associated with BMC in males, which was surprising given the physiology of how bone adapts to loading. The findings of Chapter 6 highlighted the need to investigate whether using average-acceleration and intensity-gradient to consider the relationships between PA and bone have utility when applied to PA data averaged over longer epochs. This led to a collaboration with another dataset to understand the impact of epoch length on average-acceleration and intensity-gradient. As such, an additional research study was undertaken alongside Chapter 7, to better understand the implications of the work in this thesis and to contribute to the wider literature regarding the use of average-acceleration and intensity-gradient in datasets with PA data collected in 60-second epochs going forwards (Skinner *et al.*, 2023a). This section will summarise the additional research study and place it in context regarding this thesis.

As discussed in Section 2.7.4.2 and throughout Chapter 6, the intensity-gradient and average-acceleration metrics were proposed by Rowlands *et al.* (2018) to overcome some of the limitations associated with cut-points (variation in cut-points between studies, justification on the basis of energy expenditure,

loss of information when condensing rich data into broad categories, and the cohort-specific nature of cut-points). The metrics have been applied in children and adults to investigate the associations between PA and a range of health outcomes, though previously with PA data averaged over 5-second epochs (Rowlands *et al.*, 2018; Fairclough *et al.*, 2019; Rowlands *et al.*, 2020). Although it is widely acknowledged that accelerometer data should be collected over the shortest epoch possible, there is a wealth of longitudinal data captured in 60-second epochs (Ness *et al.*, 2007; Janz *et al.*, 2010; Heil, Brage and Rothney, 2012; Loprinzi *et al.*, 2012; Haapala *et al.*, 2017). Given the surprising findings in Chapter 6 regarding the associations between intensity-gradient and BMC, it is important to understand whether the associations between intensity-gradient with health outcomes are influenced by epoch length, to inform whether these metrics have utility when applied to PA data with varying epoch lengths. Further, it is crucial to understand whether the observed associations when using shorter epochs are reflected when using longer epochs, thereby informing the use of intensity-gradient in PA data averaged over longer epochs, and aiding in the interpretation of the findings of Chapter 6. This has yet to be explored with intensity-gradient averaged over varying epoch lengths within the same sample.

The IBDS captured PA data from age 17 to 23 years (wave 6 to 9) using raw acceleration accelerometers. By averaging the PA data over various epoch lengths, the dataset would therefore facilitate examination of whether the relationship between intensity-gradient and average-acceleration with bone outcomes is dependent on the epoch used, thus informing the application of these novel accelerometer metrics in datasets with 60-second epoch PA data.

The aim of this study was to assess the mutually-adjusted associations between average-acceleration and intensity-gradient from PA data averaged over 1-second, 5-second, 15-second, 30-second, and 60-second epochs at age 17 to 23 years with bone outcomes (TBLH BMC, spine aBMD, total hip aBMD, femoral neck cross-sectional area, femoral neck section modulus) at age 23 years.

The study found that as epoch length increased from 1-second to 60-seconds, the intensity-gradient increased (became less negative). This likely reflects the dilution of activity at both extremes of the intensity continuum when activity is averaged over longer epochs, as the smoothing effect of the longer epoch (see Figure 2.4 in Chapter 2) shifts the data towards the middle of the intensity continuum. Further the correlation between intensity-gradient and average-acceleration increased as epoch length increased. This indicates that the metrics had increasing shared variance, suggesting they reflect similar properties of PA when averaged over longer epochs. The findings indicate that, like the influence of epoch length on summary measures of PA intensity, as discussed in Section 2.7.4.2, epoch length influences the intensity-gradient metric in adolescents and young adults, and this should be considered by researchers when using the intensity-gradient metric. Although the study presented in Chapter 6 was in children rather than adolescents and young adults, the findings presented here confirms the considerations presented in Chapter 6 that the intensity-gradient metric may be blunted by the 60-second epoch length.

Given that these findings show that epoch influences intensity-gradient, it is not surprising that this study also showed that epoch length influences the associations between intensity-gradient and average-acceleration with bone outcomes. The proportion of significant associations between PA and bone outcomes (out of a possible 5 bone outcomes; TBLH BMC, spine aBMD, total hip aBMD, femoral neck cross-sectional area, femoral neck section modulus) are presented for females in Figure 8.2 and for males in Figure 8.3.

In females, fewer independent associations between intensity-gradient with bone outcomes were significant with epoch lengths greater than 1-second, and no independent associations were significant with epochs greater than 5-seconds (Figure 8.2). In males, some independent associations between intensity-gradient with bone outcomes remained significant with 1-second, 5-second, 15-second, and 60-second epoch lengths, though similarly to the females, the most significant associations were observed when a 1-second epoch was used (Figure 8.3). The findings of this study indicate that the associations between intensity-gradient and bone outcomes in female and male young adults are influenced by epoch length. Furthermore, significant independent associations may be missed when PA is averaged over epoch lengths greater than 1-second. In light of these findings, it is likely that role of intensity was underestimated in Chapter 6, and it is plausible that with a shorter epoch length the findings presented in Chapter 6 would be different. The findings of this study strengthen the assertion presented in Chapter 6 that the results of Chapter 6 may not accurately reflect the true relationship between PA intensity and bone.

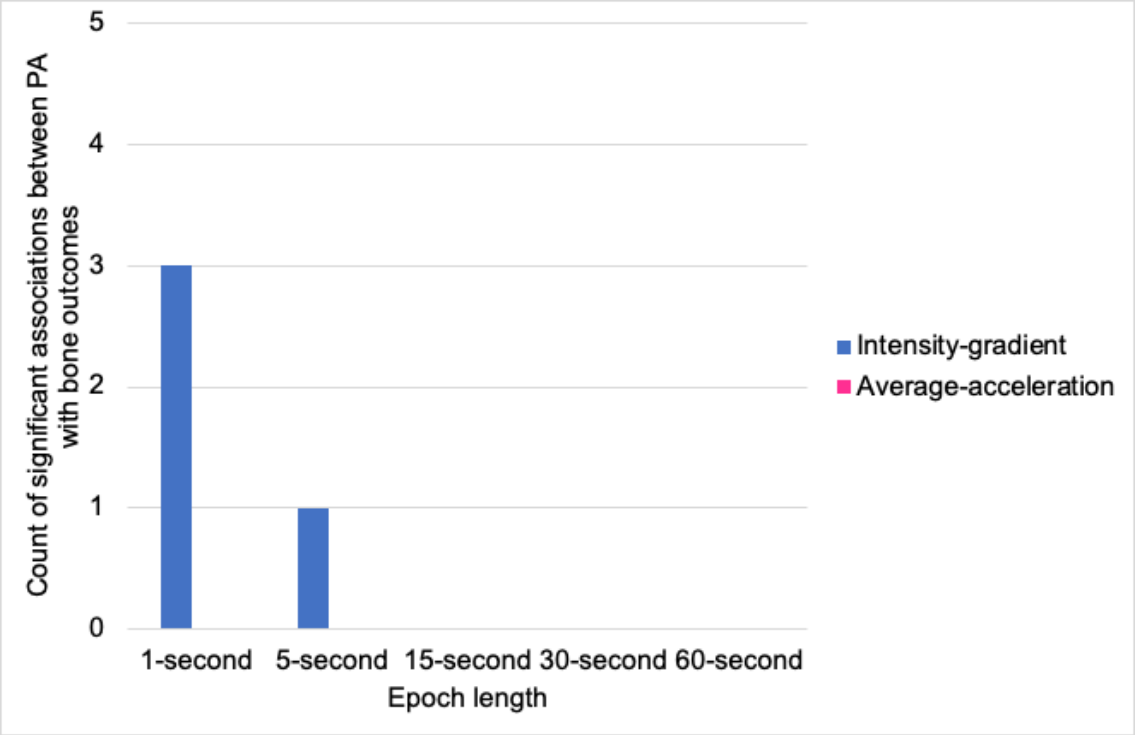

Figure 8.2 The number of significant associations between intensity-gradient and average-acceleration with bone outcomes in females. The graph shows that as epoch length increases, significant associations between intensity-gradient with bone outcomes are less likely to be observed, and there were no associations between average-acceleration with bone outcomes. All significant associations were positive. The associations were adjusted for age, stature, mass, years from PHV, proportion of the 24-hour cycle the accelerometer was worn, the mean age for PA measures, and the alternate activity metric (intensity-gradient or average-acceleration). The bone outcomes assessed were TBLH BMC, spine aBMD, total hip aBMD, femoral neck cross-sectional area, femoral neck section modulus.

aBMD, areal bone mineral density; BMC, bone mineral content; PHV, peak height velocity; TBLH, total body less head.

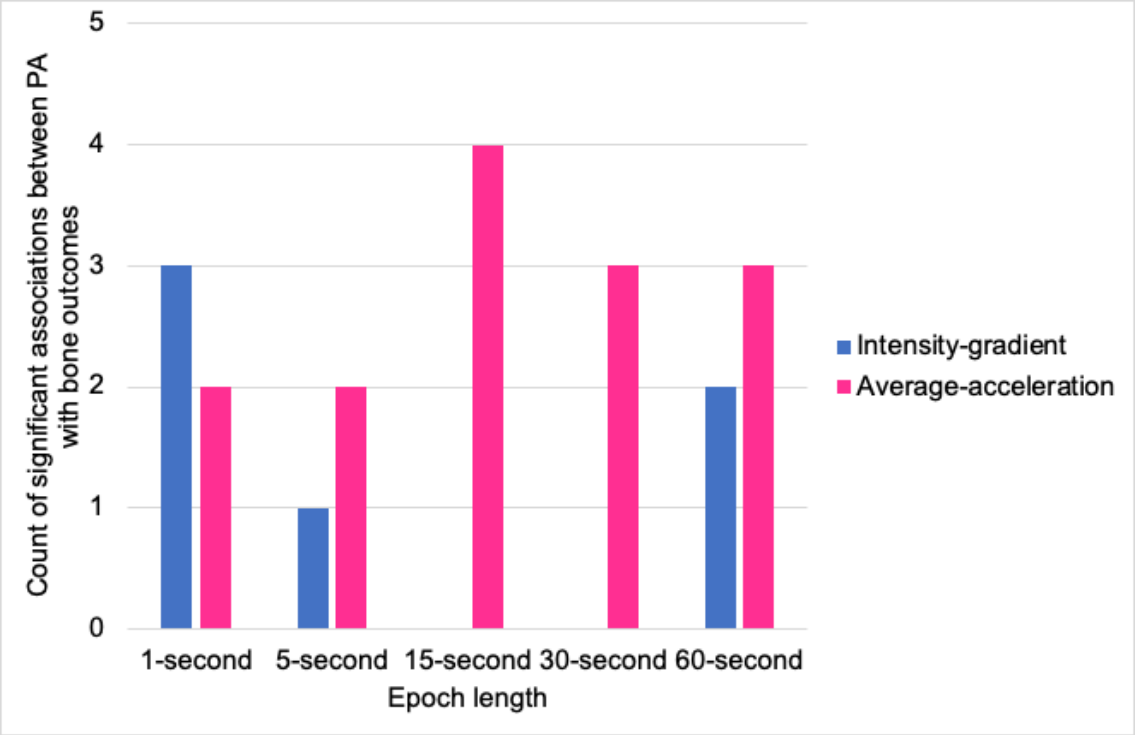

Figure 8.3 The number of significant associations between intensity-gradient and average-acceleration with bone outcomes in males. The graph shows that at epoch length > 5-seconds, average-acceleration is more likely to be associated with bone, and that the associations between intensity-gradient and bone differ depending on the epoch length. All significant associations were positive. The associations were adjusted for age, stature, mass, years from PHV, proportion of the 24-hour cycle the accelerometer was worn, the mean age for PA measures, and the alternate activity metric (intensity-gradient or average-acceleration). The bone outcomes assessed were TBLH BMC, spine aBMD, total hip aBMD, femoral neck cross-sectional area, femoral neck section modulus.

aBMD, areal bone mineral density; BMC, bone mineral content; PHV, peak height velocity; TBLH, total body less head.

In the IBDS cohort, when considering the associations between average-acceleration with bone outcomes in males, more independent associations between average-acceleration and bone were observed when the adjustment for intensity was from epochs longer than 1-second. This indicates that in addition to potentially underestimating the relationships between intensity and bone when using longer epochs, the independent relationships between volume and bone may be overestimated with longer epochs. This is particularly relevant given the findings of Chapter 6 that volume was positively related to TBLH BMC

in females and males. In light of the findings in the IBDS cohort, it is possible that the relationship between PA volume and TBLH BMC was overestimated in Chapter 6.

The study presented in Chapter 6 was the catalyst for the study in the IBDS cohort. The present results suggest that moving forward the intensity-gradient and average-acceleration should only be applied with PA data captured in 5-second epochs or less, and ideally 1-second epochs. However, this needs to be balanced against the value of large longitudinal datasets, which tend to have data in 60-second epochs. Even so, researchers should be mindful of the influence of epoch length when interpreting their data. The present study emphasises that the findings of Chapter 6 should be considered in light of the present study, as the relationships between intensity-gradient and bone may have been underestimated whilst the relationships between average-acceleration and bone may have been over-estimated.

8.2.6 Practical Implications

The findings of this thesis have several practical implications, which have been highlighted throughout this chapter. In terms of public health recommendations, the findings of this thesis support the promotion of weight-bearing MVPA and muscle and bone strengthening activities, and potentially total PA volume, though this may have been overestimated as discussed above, for improved BMC or aBMD in childhood and adolescence. The work presented in this thesis highlights the importance of further clarification and emphasis regarding weight-bearing MVPA and muscle and bone strengthening activity within the PA guidelines for children and adolescents. Assessing and raising awareness

regarding the importance of weight-bearing MVPA and muscle and bone strengthening activity within the PA guidelines for children and adolescents would be important for increasing engagement in these types of activities, therefore supporting bone development during the crucial period of childhood and adolescence. Further, the findings of this thesis support the promotion of behaviours to encourage optimal vitamin D status for improved aBMD in childhood, with previous research in the PANIC cohort indicating that dietary consumption of vitamin D, specifically fortified milk products, was an important positive determinant of vitamin D status (Soininen *et al.*, 2016) . In addition, this thesis has indicated the importance of fat mass as a positive determinant for skeletal development in childhood. In terms of research applications, this thesis has highlighted the importance of considering lean mass and fat mass when investigating the relationships between PA and bone and when designing PA interventions with the goal of increasing bone. When exploring the relationships between PA and bone, researchers should consider using PA exposures which are relevant to bone, based on mechanical loading rather than energy expenditure. The additional study presented in Section 8.2.5, which was designed based on the findings of Chapter 6, indicates that moving forwards the intensity-gradient and average-acceleration metrics should only be applied to PA data averaged over short epoch lengths (ideally 1-second but 5-seconds may be acceptable). Further, researchers should consider assessing the type of PA in addition to the intensity and volume, as the findings of this thesis suggest that muscle and bone strengthening activity is particularly important for bone in childhood and adolescence. Development and validation of a children's version of the BPAQ would be valuable, as it would allow researchers to better assess

bone-specific PA in children in order to explore the associations between bone-specific PA and bone health (Weeks and Beck, 2008)

8.2.7 Future Directions

Given the technological advancements since the PANIC study data collection, to further extend our understanding of the relationship between PA and bone, future research should use raw acceleration accelerometers to capture PA (Troiano *et al.*, 2014). Unlike when data collection for the PANIC study commenced, accelerometers are now available which can capture high frequency raw acceleration data and store this data over longer periods of time, making these a feasible option for capturing free-living PA data in population samples (Troiano *et al.*, 2014). Short bursts of high-intensity activity are known to be osteogenic, and these are not accurately captured when the PA data is averaged over longer epochs (Brailey *et al.*, 2022). The use of raw acceleration accelerometers in future research would allow the specific intensity and duration of PA associated with improved bone outcomes to be more accurately assessed and investigated, and allow further understanding of the relationships between intensity-gradient and average-acceleration with bone in children. In addition to better defining the intensity and duration of PA associated with better bone health, future research should also consider the pattern of PA. Mechanistically, the responsiveness of bone to mechanical loading declines as skeletal loading duration increases, governed by a law of diminishing returns (Forwood, 2013; Hart *et al.*, 2017). Periods of unloaded rest are needed for resensitisation to occur (Hart *et al.*, 2017). This means that the same loading volume delivered over several discrete bouts will be more osteogenic than a single bout (Forwood, 2013; Hart *et al.*, 2017). Future research should therefore

consider the pattern of PA alongside the duration and intensity. Further investigation into the relationships between muscle and bone strengthening activities and bone would be valuable (Public Health England, 2021). The specific dose of muscle and bone strengthening activity associated with improved bone remains unknown. Although the WHO PA guidelines (Chaput *et al.*, 2020), as well as PA guidelines from the US (Piercy *et al.*, 2018), Canada (Tremblay *et al.*, 2016), and Australia (Okely *et al.*, 2022) recommend muscle and bone strengthening activity three times a week for children and adolescents, the UK guidelines highlighted there was not enough evidence to support a frequency component (Department of Health and Social Care, 2019). In addition to the frequency, the duration of muscle and bone strengthening activity associated with improved bone outcomes should be considered in future studies. Further, given the importance of muscle and bone strengthening activity for bone health, future research should prioritise assessing adherence to this aspect of the PA guidelines in addition to the MVPA component. Given the aforementioned limitations with DXA assessments of bone health, future research using pQCT, which can provide measures of cortical and trabecular vBMD, bone geometry, and microarchitecture (Weaver *et al.*, 2016), would be valuable in better understanding the determinants of bone strength.

8.3 Conclusion

The studies presented in this thesis have provided novel insights into the relationships between PA, summarised with different assessment methods and metrics, body composition and associated endocrine factors, and vitamin D status, with BMC and aBMD in children and adolescents. The findings indicate that greater levels of MPA, MVPA and serum 25(OH)D are associated with

improved aBMD in prepubertal children, though PA intensity does not interact with vitamin D status to influence aBMD in this age group, and these findings should be considered in light of the limitations relating to epoch length. In pre- and early-pubertal children, fat mass is a positive predictor of BMC in females and males. The relationship between fat mass and BMC is suppressed by mediation through free leptin index in females and males, and moderated by adiponectin and free leptin index in females and males respectively, though these associations account for a relatively small contribution in terms of absolute values, and the relationship between fat mass and BMC is largely not explained by insulin, leptin, adiponectin, DHEAS, testosterone and estradiol. In pre- and early-pubertal children, PA volume is important for improving BMC in females and males, and increasing lean and reducing fat mass in males, whereas MVPA is important for favourable lean and fat outcomes in both sexes. However, given the limitations with epoch length, the role of volume may have been overestimated and the role of intensity may have been underestimated. Further, adjusting for lean and fat mass alters the relationships between PA with BMC, and should be considered by investigating the relationships between PA and bone. Engaging in the 24-hour movement behaviours in childhood, particularly sport and exercise to strengthen muscle and bone, is important in supporting bone and lean mass development in adolescence. The findings of this thesis can be used to inform future public health recommendations regarding the promotion of PA and behaviours to encourage optimal vitamin D status, and to direct future research to improve children's bone health during growth.

References

Aadland, E., Andersen, L. B., Anderssen, S. A., Resaland, G. K. and Kvalheim, O. M. (2020a) 'Accelerometer epoch setting is decisive for associations between physical activity and metabolic health in children', *J Sports Sci,* 38(3), pp. 256-263.

Aadland, E., Kvalheim, O. M., Hansen, B. H., Kriemler, S., Ried-Larsen, M., Wedderkopp, N., Sardinha, L. B., Møller, N. C., Hallal, P. C., Anderssen, S. A., Northstone, K. and Andersen, L. B. (2020b) 'The multivariate physical activity signature associated with metabolic health in children and youth: An International Children's Accelerometry Database (ICAD) analysis', *Prev Med,* 141, pp. 106266.

Aadland, E., Nilsen, A. K. O., Andersen, L. B., Rowlands, A. V. and Kvalheim, O. M. (2021) 'A comparison of analytical approaches to investigate associations for accelerometry-derived physical activity spectra with health and developmental outcomes in children', *J Sports Sci,* 39(4), pp. 430-438.

Adams, J. and Bishop, N. (2008) 'DXA in adults and children', *Primer on the metabolic bone diseases and disorders of mineral metabolism*: American Society for Bone and Mineral Research (ASBMR), Washington, DC, pp. 152-158.

Afghani, A. and Goran, M. I. (2009) 'The interrelationships between abdominal adiposity, leptin and bone mineral content in overweight Latino children', *Horm Res,* 72(2), pp. 82-7.

Agostinete, R. R., Fernandes, R. A., Narciso, P. H., Maillane-Vanegas, S., Werneck, A. O. and Vlachopoulos, D. (2020) 'Categorizing 10 sports according to bone and soft tissue profiles in adolescents', *Med Sci Sports Exerc,* 52(12), pp. 2673-2681.

Albanese, C. V., Diessel, E. and Genant, H. K. (2003) 'Clinical applications of body composition measurements using DXA', *J Clin Densitom,* 6(2), pp. 75-85.

Alin, A. (2010) 'Multicollinearity', *WIREs Comp Stats,* 2(3), pp. 370-374.

Allen, M. R. and Burr, D. B. (2014) 'Chapter 4 - Bone Modeling and Remodeling', in Burr, D.B. and Allen, M.R. (eds.) *Basic and Applied Bone Biology*. San Diego: Academic Press, pp. 75-90.

Allen, M. R. and Burr, D. B. (2019) 'Chapter 5 - Bone Growth, Modeling, and Remodeling', in Burr, D.B. and Allen, M.R. (eds.) *Basic and Applied Bone Biology.* 2nd ed: Academic Press, pp. 85-100.

Amarasekara, D. S., Kim, S. and Rho, J. (2021) 'Regulation of Osteoblast Differentiation by Cytokine Networks', *Int J Mol Sci,* 22(6), pp. 2851.

Andreoli, A., Monteleone, M., Van Loan, M., Promenzio, L., Tarantino, U. and De Lorenzo, A. (2001) 'Effects of different sports on bone density and muscle mass in highly trained athletes', *Med Sci Sports Exerc,* 33(4), pp. 507-11.

Ankarberg-Lindgren, C., Dahlgren, J., Carlsson, B., Rosberg, S., Carlsson, L., Albertsson-Wikland, K. and Norjavaara, E. (2001) 'Leptin levels show diurnal variation throughout puberty in healthy children, and follow a gender-specific pattern', *Eur J Endocrinol,* 145(1), pp. 43-51.

Arundel, P., Ahmed, S. F., Allgrove, J., Bishop, N. J., Burren, C. P., Jacobs, B., Mughal, M. Z., Offiah, A. C., Shaw, N. J. and British Paediatric and Adolescent Bone Group (2012) 'British Paediatric and Adolescent Bone Group's position statement on vitamin D deficiency', *Br Med J,* 345, pp. e8182.

Atkin, A. J., Corder, K., Ekelund, U., Wijndaele, K., Griffin, S. J. and van Sluijs, E. M. (2013) 'Determinants of change in children's sedentary time', *PLoS One,* 8(6), pp. e67627.

Auchus, R. J. and Rainey, W. E. (2004) 'Adrenarche – physiology, biochemistry and human disease', *Clin Endocrinol,* 60(3), pp. 288-296.

Avin, K. G., Bloomfield, S. A., Gross, T. S. and Warden, S. J. (2015) 'Biomechanical Aspects of the Muscle-Bone Interaction', *Curr Osteoporos Rep,* 13(1), pp. 1-8.

Ayabe, M., Kumahara, H., Morimura, K. and Tanaka, H. (2013) 'Epoch length and the physical activity bout analysis: An accelerometry research issue', *BMC Res Notes,* 6, pp. 20.

Bachrach, L. K., Gordon, C. M., AAP SECTION ON ENDOCRINOLOGY, Sills, I. N., Lynch, J. L., Casella, S. J., DiMeglio, L. A., Gonzalez, J. L., Wintergerst, K. and Kaplowitz, P. B. (2016) 'Bone Densitometry in Children and Adolescents', *Pediatr,* 138(4), pp. e20162398.

Bachran, R., Beyer, P., Klinkert, C., Heidtmann, B., Rosenbauer, J., Holl, R. W., German/Austrian DPV Initiative, The German Pediatric CSII Working Group and The BMBF Competence Network Diabetes (2012) 'Basal rates and circadian profiles in continuous subcutaneous insulin infusion (CSII) differ for preschool children, prepubertal children, adolescents and young adults', *Pediatr Diabetes,* 13(1), pp. 1-5.

Bailey, D. A. (1997) 'The Saskatchewan Pediatric Bone Mineral Accrual Study: bone mineral acquisition during the growing years', *Int J Sports Med,* 18 Suppl 3, pp. S191-4.

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', *J Bone Miner Res,* 14(10), pp. 1672-9.

Baird, J., Hill, C. M., Harvey, N. C., Crozier, S., Robinson, S. M., Godfrey, K. M., Cooper, C., Inskip, H. and The SWS Study Group (2016) 'Duration of sleep at 3 years of age is associated with fat and fat-free mass at 4 years of age: the Southampton Women's Survey', *J Sleep Res,* 25(4), pp. 412-418.

Banda, J. A., Haydel, K. F., Davila, T., Desai, M., Bryson, S., Haskell, W. L., Matheson, D. and Robinson, T. N. (2016) 'Effects of Varying Epoch Lengths, Wear Time Algorithms, and Activity Cut-Points on Estimates of Child Sedentary Behavior and Physical Activity from Accelerometer Data', *PLoS ONE,* 11(3), pp. e0150534.

Baquet, G., Stratton, G., Van Praagh, E. and Berthoin, S. (2007) 'Improving physical activity assessment in prepubertal children with high-frequency accelerometry monitoring: a methodological issue', *Prev Med,* 44(2), pp. 143-7.

Bass, S., Pearce, G., Bradney, M., Hendrich, E., Delmas, P. D., Harding, A. and Seeman, E. (1998) 'Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts', *J Bone Miner Res,* 13(3), pp. 500-7.

Bassey, E. J., Littlewood, J. J. and Taylor, S. J. (1997) 'Relations between compressive axial forces in an instrumented massive femoral implant, ground reaction forces, and integrated electromyographs from vastus lateralis during various 'osteogenic' exercises', *J Biomech,* 30(3), pp. 213-23.

Baxter-Jones, A. D. (2017) 'Growth and maturation', in Armstrong, N., van Mechelen, W., Armstrong, N. and van Mechelen, W. (eds.) *Oxford Textbook of Children's Sport and Exercise Medicine*: Oxford University Press, pp. 13-24.

Baxter-Jones, A. D., Eisenmann, J. C., Mirwald, R. L., Faulkner, R. A. and Bailey, D. A. (2008a) 'The influence of physical activity on lean mass accrual during adolescence: a longitudinal analysis', *J Appl Physiol (1985),* 105(2), pp. 734-41.

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', *J Bone Miner Res,* 26(8), pp. 1729-39.

Baxter-Jones, A. D., Kontulainen, S. A., Faulkner, R. A. and Bailey, D. A. (2008b) 'A longitudinal study of the relationship of physical activity to

bone mineral accrual from adolescence to young adulthood', *Bone,* 43(6), pp. 1101-7.

Baxter-Jones, A. D. G. and Jackowski, S. A. (2021) 'Sex differences in bone mineral content and bone geometry accrual: a review of the Paediatric Bone Mineral Accural Study (1991–2017)', *Ann Hum Biol,* 48(6), pp. 503-516.

Baxter-Jones, A. D. G., Mirwald, R. L., McKay, H. A. and Bailey, D. A. (2003) 'A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls', *Ann Hum Biol,* 30(2), pp. 160-175.

Bean, C. G., Pingel, R., Hallqvist, J., Berg, N. and Hammarström, A. (2019) 'Poor peer relations in adolescence, social support in early adulthood, and depressive symptoms in later adulthood—evaluating mediation and interaction using four-way decomposition analysis', *Ann Epidemiol,* 29, pp. 52-59.

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', *J Bone Miner Res,* 29(2), pp. 467-78.

Bellido, T., Plotkin, L. I. and Bruzzaniti, A. (2014) 'Chapter 2 - Bone Cells', in Burr, D.B. and Allen, M.R. (eds.) *Basic and Applied Bone Biology*. San Diego: Academic Press, pp. 27-45.

Bennie, J. A., Smith, J. J., Qian, W., Leatherdale, S. T. and Faulkner, G. (2022) 'Longitudinal trends and predictors of muscle-strengthening activity guideline adherence among Canadian youths', *J Sci Med Sport,* 25(3), pp. 230-234.

Bi, X., Loo, Y. T. and Henry, C. J. (2020) 'Relationships between adiponectin and bone: Sex difference', *Nutr,* 70, pp. 110489.

Binkovitz, L. A. and Henwood, M. J. (2007) 'Pediatric DXA: technique and interpretation', *Pediatr Radiol,* 37(1), pp. 21-31.

Blake, G. M. and Fogelman, I. (1997) 'Technical principles of dual energy X-ray absorptiometry', *Semin Nucl Med,* 27(3), pp. 210-228.

Bland, V. L., Heatherington-Rauth, M., Howe, C., Going, S. B. and Bea, J. W. (2020) 'Association of objectively measured physical activity and bone health in children and adolescents: a systematic review and narrative synthesis', *Osteoporos Int,* 31(10), pp. 1865-1894.

Blum, W. F., Englaro, P., Hanitsch, S., Juul, A., Hertel, N. T., Müller, J., Skakkebæk, N. E., Heiman, M. L., Birkett, M., Attanasio, A. M., Kiess, W. and Rascher, W. (1997) 'Plasma leptin levels in healthy children and

adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone', *J Clin Endocrinol Metab,* 82(9), pp. 2904-2910.

Bonjour, J. P., Chevalley, T., Ferrari, S. and Rizzoli, R. (2009) 'The importance and relevance of peak bone mass in the prevalence of osteoporosis', *Salud Publica Mex,* 51 Suppl 1, pp. S5-17.

Bonjour, J. P., Theintz, G., Law, F., Slosman, D. and Rizzoli, R. (1994) 'Peak bone mass', *Osteoporos Int,* 4 Suppl 1, pp. 7-13.

Boreham, C. A. and McKay, H. A. (2011) 'Physical activity in childhood and bone health', *Br J Sports Med,* 45(11), pp. 877-9.

Borgström, F., Karlsson, L., Ortsäter, G., Norton, N., Halbout, P., Cooper, C., Lorentzon, M., McCloskey, E. V., Harvey, N. C., Javaid, M. K., Kanis, J. A. and International Osteoporosis, F. (2020) 'Fragility fractures in Europe: burden, management and opportunities', *Arch Osteoporos,* 15(1), pp. 59-59.

Böttner, A., Kratzsch, J. r., Müller, G., Kapellen, T. M., Blüher, S., Keller, E., Blüher, M. and Kiess, W. (2004) 'Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels', *J Clin Endocrinol Metab,* 89(8), pp. 4053-4061.

Bowerman, B. L. and O'Connell, R. T. (1990) *Linear Statistical Models: An Applied Approach.* 2 edn.: Thomson Wadsworth.

Brage, S., Brage, N., Franks, P. W., Ekelund, U. and Wareham, N. J. (2005) 'Reliability and validity of the combined heart rate and movement sensor Actiheart', *Eur J Clin Nutr,* 59(4), pp. 561-70.

Brage, S., Brage, N., Franks, P. W., Ekelund, U., Wong, M. Y., Andersen, L. B., Froberg, K. and Wareham, N. J. (2004) 'Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure', *J Appl Physiol (1985),* 96(1), pp. 343-51.

Brage, S., Westgate, K., Wijndaele, K., Godinho, J., Griffin, S. and Wareham, N. 'Evaluation of a method for minimizing diurnal information bias in objective sensor data'. *International Conference on Ambulatory Monitoring of Physical Activity and Movement*, Massachusetts, USA.

Brailey, G., Metcalf, B., Lear, R., Price, L., Cumming, S. and Stiles, V. (2022) 'A comparison of the associations between bone health and three different intensities of accelerometer-derived habitual physical activity in children and adolescents: a systematic review', *Osteoporos Int,* 33(6), pp. 1191-1222.

Breen, M. E., Laing, E. M., Hall, D. B., Hausman, D. B., Taylor, R. G., Isales, C. M., Ding, K. H., Pollock, N. K., Hamrick, M. W., Baile, C. A. and Lewis, R. D. (2011) '25-Hydroxyvitamin D, Insulin-Like Growth Factor-I, and Bone Mineral Accrual during Growth', *J Clin Endocrinol Metab,* 96(1), pp. E89-E98.

Buchmann, N., Spira, D., Norman, K., Demuth, I., Eckardt, R. and Steinhagen-Thiessen, E. (2016) 'Sleep, Muscle Mass and Muscle Function in Older People', *Dtsch Arztebl Int,* 113(15), pp. 253-60.

Buck, D. W., II and Dumanian, G. A. (2012) 'Bone Biology and Physiology: Part I. The Fundamentals', *Plastic and Reconstructive Surgery,* 129(6), pp. 1314-1320.

Buckinx, F., Landi, F., Cesari, M., Fielding, R. A., Visser, M., Engelke, K., Maggi, S., Dennison, E., Al-Daghri, N. M., Allepaerts, S., Bauer, J., Bautmans, I., Brandi, M. L., Bruyère, O., Cederholm, T., Cerreta, F., Cherubini, A., Cooper, C., Cruz-Jentoft, A., McCloskey, E., Dawson-Hughes, B., Kaufman, J.-M., Laslop, A., Petermans, J., Reginster, J.-Y., Rizzoli, R., Robinson, S., Rolland, Y., Rueda, R., Vellas, B. and Kanis, J. A. (2018) 'Pitfalls in the measurement of muscle mass: a need for a reference standard', *J Cachexia Sarcopenia Muscle,* 9(2), pp. 269-278.

Burger, E. H., Klein-Nulend, J. and Cowin, S. C. (1998) 'Mechanotransduction in Bone', *Advances in Organ Biology*: Elsevier, pp. 123-136.

Burr, D. B. and Akkus, O. (2014) 'Chapter 1 - Bone Morphology and Organization', in Burr, D.B. and Allen, M.R. (eds.) *Basic and Applied Bone Biology*. San Diego: Academic Press, pp. 3-25.

Butte, N. F., Puyau, M. R., Wilson, T. A., Liu, Y., Wong, W. W., Adolph, A. L. and Zakeri, I. F. (2016) 'Role of physical activity and sleep duration in growth and body composition of preschool-aged children', *Obes,* 24(6), pp. 1328-1335.

Carson, V., Hunter, S., Kuzik, N., Gray, C. E., Poitras, V. J., Chaput, J.-P., Saunders, T. J., Katzmarzyk, P. T., Okely, A. D. and Connor Gorber, S. (2016) 'Systematic review of sedentary behaviour and health indicators in school-aged children and youth: an update', *Appl Physiol Nutr Metab,* 41(6 Suppl 3), pp. S240-S265.

Carter, D. R., Bouxsein, M. L. and Marcus, R. (1992) 'New approaches for interpreting projected bone densitometry data', *J Bone Miner Res,* 7(2), pp. 137-145.

Carter, P. J., Taylor, B. J., Williams, S. M. and Taylor, R. W. (2011) 'Longitudinal analysis of sleep in relation to BMI and body fat in children: the FLAME study', *Br Med J,* 342, pp. d2712.

Casazza, K., Hanks, L. J. and Fernandez, J. R. (2011) 'Shorter Sleep may be a Risk Factor for Impaired Bone Mass Accrual in Childhood', *J Clin Densitom,* 14(4), pp. 453-457.

Cashman, K. D., Hill, T. R., Cotter, A. A., Boreham, C. A., Dubitzky, W., Murray, L., Strain, J., Flynn, A., Robson, P. J., Wallace, J. M. and Kiely, M. (2008) 'Low vitamin D status adversely affects bone health parameters in adolescents', *Am J Clin Nutr,* 87(4), pp. 1039-44.

Caspersen, C. J., Powell, K. E. and Christenson, G. M. (1985) 'Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research', *Public Health Rep,* 100(2), pp. 126-31.

Cauley, J. A. (2015) 'Estrogen and bone health in men and women', *Steroids,* 99(Pt A), pp. 11-15.

Chaput, J.-P., Gray, C. E., Poitras, V. J., Carson, V., Gruber, R., Olds, T., Weiss, S. K., Gorber, S. C., Kho, M. E., Sampson, M., Belanger, K., Eryuzlu, S., Callender, L. and Tremblay, M. S. (2016) 'Systematic review of the relationships between sleep duration and health indicators in school-aged children and youth', *Appl Physiol Nutr Metab,* 41(6 (Suppl. 3)), pp. S266-S282.

Chaput, J.-P., Willumsen, J., Bull, F., Chou, R., Ekelund, U., Firth, J., Jago, R., Ortega, F. B. and Katzmarzyk, P. T. (2020) '2020 WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5–17 years: summary of the evidence', *Int J Behav Nutr Phys Act,* 17(1), pp. 141.

Chaves, L. G. C. M., Gonçalves, T. J. M., Bitencourt, A. G. V., Rstom, R. A., Pereira, T. R. and Velludo, S. F. (2022) 'Assessment of body composition by whole-body densitometry: what radiologists should know', *Radiol Bras,* 55(5), pp. 305-311.

Cheng, L., Pohlabeln, H., Ahrens, W., Russo, P., Veidebaum, T., Hadjigeorgiou, C., Molnár, D., Hunsberger, M., De Henauw, S., Moreno, L. A., Hebestreit, A. and on behalf of the IDEFICS and I.Family consortia (2021) 'Cross-sectional and longitudinal associations between sleep duration, sleep quality, and bone stiffness in European children and adolescents', *Osteoporos Int,* 32(5), pp. 853-863.

Cheng, S., Tylavsky, F., Kroger, H., Karkkainen, M., Lyytikainen, A., Koistinen, A., Mahonen, A., Alen, M., Halleen, J., Vaananen, K. and Lamberg-Allardt, C. (2003) 'Association of low 25-hydroxyvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls', *Am J Clin Nutr,* 78(3), pp. 485-92.

Chennaoui, M., Léger, D. and Gomez-Merino, D. (2020) 'Sleep and the GH/IGF-1 axis: Consequences and countermeasures of sleep loss/disorders', *Sleep Med Rev,* 49, pp. 101223.

Chevalley, T., Bonjour, J. P., Ferrari, S. and Rizzoli, R. (2009) 'Deleterious effect of late menarche on distal tibia microstructure in healthy 20-year-old and premenopausal middle-aged women', *J Bone Miner Res,* 24(1), pp. 144-52.

Chevalley, T. and Rizzoli, R. (2022) 'Acquisition of peak bone mass', *Best Pract Res Clin Endocrinol Metab,* 36(2), pp. 101616.

Clark, E. M., Ness, A. R., Bishop, N. J. and Tobias, J. H. (2006) 'Association between bone mass and fractures in children: a prospective cohort study', *J Bone Miner Res,* 21(9), pp. 1489-1495.

Clark, E. M., Ness, A. R. and Tobias, J. H. (2006) 'Adipose tissue stimulates bone growth in prepubertal children', *J Clin Endocrinol Metab,* 91(7), pp. 2534-41.

Clarke, B. (2008) 'Normal bone anatomy and physiology', *Clin J Am Soc Nephrol,* 3(Suppl 3), pp. S131-S139.

Clarke, B. L. and Khosla, S. (2009) 'Androgens and bone', *Steroids,* 74(3), pp. 296-305.

Cole, T. J., Bellizzi, M. C., Flegal, K. M. and Dietz, W. H. (2000) 'Establishing a standard definition for child overweight and obesity worldwide: international survey', *Br Med J,* 320(7244), pp. 1240-3.

Cole, T. J., Flegal, K. M., Nicholls, D. and Jackson, A. A. (2007) 'Body mass index cut offs to define thinness in children and adolescents: international survey', *Br Med J,* 335(7612), pp. 194.

Cole, T. J. and Lobstein, T. (2012) 'Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity', *Pediatr Obes,* 7(4), pp. 284-94.

Colley, R. C. and Tremblay, M. S. (2011) 'Moderate and vigorous physical activity intensity cut-points for the Actical accelerometer', *J Sports Sci,* 29(8), pp. 783-789.

Collings, P. J., Westgate, K., Väistö, J., Wijndaele, K., Atkin, A. J., Haapala, E. A., Lintu, N., Laitinen, T., Ekelund, U., Brage, S. and Lakka, T. A. (2017) 'Cross-Sectional Associations of Objectively-Measured Physical Activity and Sedentary Time with Body Composition and Cardiorespiratory Fitness in Mid-Childhood: The PANIC Study', *Sports Med,* 47(4), pp. 769-780.

Constable, A. M., Vlachopoulos, D., Barker, A. R., Moore, S. A., Soininen, S., Haapala, E. A., Väistö, J., Jääskeläinen, J., Voutilainen, R., Auriola, S., Häkkinen, M. R., Laitinen, T. and Lakka, T. A. (2022) 'The Mediating Role of Endocrine Factors in the Positive Relationship Between Fat Mass and Bone Mineral Content in Children Aged 9-11 Years: The Physical Activity and Nutrition in Children Study', *Front Endocrinol (Lausanne),* 13, pp. 850448.

Constable, A. M., Vlachopoulos, D., Barker, A. R., Moore, S. A., Soininen, S., Haapala, E. A., Väistö, J., Westgate, K., Brage, S., Mahonen, A. and Lakka, T. A. (2021) 'The independent and interactive associations of physical activity intensity and vitamin D status with bone mineral density in prepubertal children: the PANIC Study', *Osteoporos Int,* 32(8), pp. 1609-1620.

Corder, K., Brage, S., Mattocks, C., Ness, A., Riddoch, C., Wareham, N. J. and Ekelund, U. (2007) 'Comparison of two methods to assess PAEE during six activities in children', *Med Sci Sports Exerc,* 39(12), pp. 2180-8.

Corder, K., Brage, S., Wareham, N. J. and Ekelund, U. (2005) 'Comparison of PAEE from combined and separate heart rate and movement models in children', *Med Sci Sports Exerc,* 37(10), pp. 1761-7.

Córdoba-Rodríguez, D. P., Iglesia, I., Gómez-Bruton, A., Miguel-Berges, M. L., Flores-Barrantes, P., Casajús, J. A., Moreno, L. A. and Rodríguez, G. (2022) 'Associations between Spanish children's physical activity and physical fitness with lean body mass: The CALINA study', *J Sports Sci,* 40(4), pp. 401-412.

Corvalán, C., Uauy, R. and Mericq, V. (2013) 'Obesity is positively associated with dehydroepiandrosterone sulfate concentrations at 7 y in Chilean children of normal birth weight', *Am J Clin Nutr,* 97(2), pp. 318-325.

Cossio Bolaños, M. A., Andruske, C. L., de Arruda, M., Sulla-Torres, J., Urra-Albornoz, C., Rivera-Portugal, M., Luarte-Rocha, C., Pacheco-Carrillo, J. and Gómez-Campos, R. (2019) 'Muscle Mass in Children and Adolescents: Proposed Equations and Reference Values for Assessment', *Front Endocrinol (Lausanne),* 10, pp. 583.

Cullen, D. M., Smith, R. T. and Akhter, M. P. (2001) 'Bone-loading response varies with strain magnitude and cycle number', *J Appl Physiol (1985),* 91(5), pp. 1971-6.

Cummings, S. R. and Melton, L. J. (2002) 'Epidemiology and outcomes of osteoporotic fractures', *The Lancet,* 359(9319), pp. 1761-1767.

Dalskov, S., Ritz, C., Larnkjær, A., Damsgaard, C. T., Petersen, R. A., Sørensen, L. B., Ong, K. K., Astrup, A., Michaelsen, K. F. and Mølgaard, C. (2016) 'Associations between adiposity, hormones, and gains in height, whole-body height-adjusted bone size, and size-

adjusted bone mineral content in 8- to 11-year-old children', *Osteoporos Int,* 27(4), pp. 1619-1629.

Damilakis, J., Solomou, G., Manios, G. E. and Karantanas, A. (2013) 'Pediatric radiation dose and risk from bone density measurements using a GE Lunar Prodigy scanner', *Osteoporos Int,* 24(7), pp. 2025-31.

Davies, J. H., Evans, B. A. J. and Gregory, J. W. (2005) 'Bone mass acquisition in healthy children', *Arch Dis Child,* 90(4), pp. 373-378.

Deere, K., Sayers, A., Davey Smith, G., Rittweger, J. and Tobias, J. H. (2012) 'High impact activity is related to lean but not fat mass: findings from a population-based study in adolescents', *Int J Epidemiol,* 41(4), pp. 1124-1131.

Department of Health and Social Care (2019) *UK Chief Medical Officers' Physical Activity Guidelines*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/832868/uk-chief-medical-officers-physical-activity-guidelines.pdf (Accessed: 30/09/2019).

Dhuper, S., Warren, M. P., Brooks-Gunn, J. and Fox, R. (1990) 'Effects of Hormonal Status on Bone Density in Adolescent Girls', *J Clin Endocrinol Metab,* 71(5), pp. 1083-1088.

Diethelm, K., Bolzenius, K., Cheng, G., Remer, T. and Buyken, A. E. (2011) 'Longitudinal associations between reported sleep duration in early childhood and the development of body mass index, fat mass index and fat free mass index until age 7', *Int J Pediatr Obes,* 6(2-2), pp. e114-23.

Dimai, H. P. (2017) 'Use of dual-energy X-ray absorptiometry (DXA) for diagnosis and fracture risk assessment; WHO-criteria, T- and Z-score, and reference databases', *Bone,* 104, pp. 39-43.

Dimitri, P. (2018) 'Fat and bone in children - where are we now?', *Ann Pediatr Endocrinol Metab,* 23(2), pp. 62-69.

Dimitri, P. (2019) 'The impact of childhood obesity on skeletal health and development', *J Obes Metab Syndr,* 28(1), pp. 4-17.

Dimitri, P., Wales, J. K. and Bishop, N. (2011) 'Adipokines, bone-derived factors and bone turnover in obese children; evidence for altered fat-bone signalling resulting in reduced bone mass', *Bone,* 48(2), pp. 189-196.

Discacciati, A., Bellavia, A., Lee, J. J., Mazumdar, M. and Valeri, L. (2018) 'Med4way: a Stata command to investigate mediating and interactive mechanisms using the four-way effect decomposition', *Int J Epidemiol,* 48(1), pp. 15-20.

Donaldson, L. J., Reckless, I. P., Scholes, S., Mindell, J. S. and Shelton, N. J. (2008) 'The epidemiology of fractures in England', *J Epidemiol Community Health,* 62(2), pp. 174-80.

Dook, J. E., James, C., Henderson, N. K. and Price, R. I. (1997) 'Exercise and bone mineral density in mature female athletes', *Med Sci Sports Exerc,* 29(3), pp. 291-6.

Duncan, R. L. and Turner, C. H. (1995) 'Mechanotransduction and the functional response of bone to mechanical strain', *Calcif Tissue Int,* 57(5), pp. 344-358.

Dunlop, M. and Murray, A. D. (2013) 'Major limitations in knowledge of physical activity guidelines among UK medical students revealed: implications for the undergraduate medical curriculum', *Br J Sports Med,* 47(11), pp. 718-20.

Edwardson, C. L. and Gorely, T. (2010) 'Epoch length and its effect on physical activity intensity', *Med Sci Sports Exerc,* 42(5), pp. 928-34.

Eisman, J. A., Kelly, P. J., Morrison, N. A., Pocock, N. A., Yeoman, R., Birmingham, J. and Sambrook, P. N. (1993) 'Peak bone mass and osteoporosis prevention', *Osteoporos Int,* 3 Suppl 1, pp. 56-60.

Ekelund, U., Luan, J., Sherar, L. B., Esliger, D. W., Griew, P., Cooper, A. and International Children's Accelerometry Database (ICAD) Collaborators (2012) 'Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents', *JAMA,* 307(7), pp. 704-12.

El Maghraoui, A. and Roux, C. (2008) 'DXA scanning in clinical practice', *QJM,* 101(8), pp. 605-617.

Elhakeem, A., Frysz, M., Tilling, K., Tobias, J. H. and Lawlor, D. A. (2019) 'Association Between Age at Puberty and Bone Accrual From 10 to 25 Years of Age', *JAMA Netw Open,* 2(8), pp. e198918-e198918.

Elhakeem, A., Heron, J., Tobias, J. H. and Lawlor, D. A. (2020) 'Physical Activity Throughout Adolescence and Peak Hip Strength in Young Adults', *JAMA Netw Open,* 3(8), pp. e2013463.

Ellis, K. J. and Nicolson, M. (1997) 'Leptin levels and body fatness in children: effects of gender, ethnicity, and sexual development', *Pediatr Res,* 42(4), pp. 484-488.

Elmlinger, M. W., Kühnel, W. and Ranke, M. B. (2002) 'Reference Ranges for Serum Concentrations of Lutropin (LH), Follitropin (FSH), Estradiol (E2), Prolactin, Progesterone, Sex Hormone-Binding Globulin (SHBG), Dehydroepiandrosterone Sulfate (DHEAS), Cortisol and Ferritin in

Neonates, Children and Young Adults', *Clin Chem Lab Med,* 40(11), pp. 1151-1160.

Emaus, N., Berntsen, G. K. R., Joakimsen, R. and Fonnebø, V. (2006) 'Longitudinal Changes in Forearm Bone Mineral Density in Women and Men Aged 45–84 Years: The Tromsø Study, a Population-based Study', *Am J Epidemiol,* 163(5), pp. 441-449.

Faigenbaum, A. D., MacDonald, J. P., Stracciolini, A. and Rebullido, T. R. (2020) 'Making a Strong Case for Prioritizing Muscular Fitness in Youth Physical Activity Guidelines', *Curr Sports Med Rep,* 19(12), pp. 530-536.

Fairclough, S. J., Taylor, S., Rowlands, A. V., Boddy, L. M. and Noonan, R. J. (2019) 'Average acceleration and intensity gradient of primary school children and associations with indicators of health and well-being', *J Sports Sci,* 37(18), pp. 2159-2167.

Faulkner, R. A. and Bailey, D. A. (2007) 'Osteoporosis: a pediatric concern?', *Med Sport Sci,* 51, pp. 1-12.

Field, A. (2014) *Discovering Statistics Using IBM SPSS Statistics.* 4 edn. London: SAGE Publications.

Finkelstein, J. S., Neer, R. M., Biller, B. M., Crawford, J. D. and Klibanski, A. (1992) 'Osteopenia in men with a history of delayed puberty', *N Engl J Med,* 326(9), pp. 600-4.

Florencio-Silva, R., Sasso, G. R., Sasso-Cerri, E., Simoes, M. J. and Cerri, P. S. (2015) 'Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells', *Biomed Res Int,* 2015, pp. 421746.

Forwood, M. R. (2008) 'Physical activity and bone development during childhood: insights from animal models', *J Appl Physiol,* 105(1), pp. 334-341.

Forwood, M. R. (2013) 'Growing a Healthy Skeleton: The Importance of Mechanical Loading', *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, pp. 149-155.

Foster, C. and Armstrong, M. E. G. (2018) 'What types of physical activities are effective in developing muscle and bone strength and balance?', *J Frailty Sarcopenia Falls,* 3(2), pp. 58-65.

Fox, J. (1991) *Regression Diagnostics.* Thousand Oaks, California: SAGE Publications, Inc.

Fraser, B. J., Alishah, Z., Magnussen, C. G., Venn, A. J., Dwyer, T. and Cleland, V. (2021) 'Factors associated with change and stability in

adherence to muscle-strengthening guidelines among young Australian adults: A longitudinal study', *J Sci Med Sport,* 24(12), pp. 1261-1266.

Frost, H. M. (1987) 'Bone “mass” and the “mechanostat”: A proposal', *Anat Rec,* 219(1), pp. 1-9.

Frost, H. M. (1994) 'Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians', *Angle Orthod,* 64(3), pp. 175-188.

Frost, H. M. (1998) 'Changing concepts in skeletal physiology: Wolff's Law, the Mechanostat, and the “Utah Paradigm”', *Am J Hum Biol,* 10(5), pp. 599-605.

Frost, H. M. (2001) 'From Wolff's law to the Utah paradigm: insights about bone physiology and its clinical applications', *Anat Rec,* 262(4), pp. 398-419.

Frost, H. M. (2003) 'Bone's mechanostat: A 2003 update', *Anat Rec A Discov Mol Cell Evol Biol,* 275(2), pp. 1081-1101.

Frost, H. M. and Schönau, E. (2000) 'The "Muscle-Bone Unit" in Children and Adolescents: A 2000 Overview', *J Pediatr Endocrinol Metab,* 13(6), pp. 571.

Fuchs, R. K., Warden, S. J. and Turner, C. H. (2009) '2 - Bone anatomy, physiology and adaptation to mechanical loading', in Planell, J.A., Best, S.M., Lacroix, D. and Merolli, A. (eds.) *Bone Repair Biomaterials*: Woodhead Publishing, pp. 25-68.

Fulton, J. E., Dai, S., Steffen, L. M., Grunbaum, J. A., Shah, S. M. and Labarthe, D. R. (2009) 'Physical Activity, Energy Intake, Sedentary Behavior, and Adiposity in Youth', *Am J Prev Med,* 37(1 Suppl), pp. S40-S49.

Garnett, S. P., Högler, W., Blades, B., Baur, L. A., Peat, J., Lee, J. and Cowell, C. T. (2004) 'Relation between hormones and body composition, including bone, in prepubertal children', *Am J Clin Nutr,* 80(4), pp. 966-972.

Gilsanz, V., Chalfant, J., Kalkwarf, H., Zemel, B., Lappe, J., Oberfield, S., Shepherd, J., Wren, T. and Winer, K. (2011) 'Age at onset of puberty predicts bone mass in young adulthood', *J Pediatr,* 158(1), pp. 100-5, 105 e1-2.

Goji, K. (1993) 'Twenty-four-hour concentration profiles of gonadotropin and estradiol (E2) in prepubertal and early pubertal girls: the diurnal rise of E2 is opposite the nocturnal rise of gonadotropin', *J Clin Endocrinol Metab,* 77(6), pp. 1629-1635.

Goltzman, D. (2019) 'The Aging Skeleton', in Rhim, J.S., Dritschilo, A. and Kremer, R. (eds.) *Human Cell Transformation: Advances in Cell Models for the Study of Cancer and Aging*. Cham: Springer International Publishing, pp. 153-160.

Gomez-Bruton, A., Matute-Llorente, A., Gonzalez-Aguero, A., Casajus, J. A. and Vicente-Rodriguez, G. (2017) 'Plyometric exercise and bone health in children and adolescents: a systematic review', *World J Pediatr,* 13(2), pp. 112-121.

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. and 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(1), pp. 43-58.

Gordon, C. M., Zemel, B. S., Wren, T. A., Leonard, M. B., Bachrach, L. K., Rauch, F., Gilsanz, V., Rosen, C. J. and Winer, K. K. (2017) 'The Determinants of Peak Bone Mass', *J Pediatr,* 180, pp. 261-269.

Grabowski, P. (2015) 'Physiology of Bone', *Endocr Dev,* 28, pp. 33-55.

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. and Group, H. S. (2011) 'Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study', *Am J Prev Med,* 40(6), pp. 599-607.

Gracia-Marco, L., Ortega, F. B., Jimenez-Pavon, D., Rodriguez, G., Castillo, M. J., Vicente-Rodriguez, G. and Moreno, L. A. (2012) 'Adiposity and bone health in Spanish adolescents. The HELENA study', *Osteoporos Int,* 23(3), pp. 937-47.

Gross, T. S., Edwards, J. L., McLeod, K. J. and Rubin, C. T. (1997) 'Strain gradients correlate with sites of periosteal bone formation', *J Bone Miner Res,* 12(6), pp. 982-8.

Guinhouya, B. C., Samouda, H. and de Beaufort, C. (2013) 'Level of physical activity among children and adolescents in Europe: a review of physical activity assessed objectively by accelerometry', *Public Health,* 127(4), pp. 301-311.

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(4), pp. 710-8.

Gunter, K. B., Almstedt, H. C. and Janz, K. F. (2012) 'Physical activity in childhood may be the key to optimizing lifespan skeletal health', *Exerc Sport Sci Rev,* 40(1), pp. 13-21.

Guo, S. S., Chumlea, W. C., Roche, A. F. and Siervogel, R. M. (1997) 'Age- and maturity-related changes in body composition during adolescence into adulthood: the Fels Longitudinal Study', *Int J Obes Relat Metab Disord,* 21(12), pp. 1167-75.

Guss, C. E., McAllister, A. and Gordon, C. M. (2021) 'DXA in Children and Adolescents', *J Clin Densitom,* 24(1), pp. 28-35.

Haapala, E. A., Gao, Y., Vanhala, A., Rantalainen, T. and Finni, T. (2020) 'Validity of traditional physical activity intensity calibration methods and the feasibility of self-paced walking and running on individualised calibration of physical activity intensity in children', *Sci Rep,* 10(1), pp. 11031.

Haapala, E. A., Rantalainen, T., Hesketh, K. D., Rodda, C. P. and Duckham, R. L. (2022) 'Accelerometer-based osteogenic indices, moderate-to-vigorous and vigorous physical activity, and bone traits in adolescents', *J Musculoskelet Neuronal Interact,* 22(4), pp. 514-523.

Haapala, E. A., Vaisto, J., Veijalainen, A., Lintu, N., Wiklund, P., Westgate, K., Ekelund, U., Lindi, V., Brage, S. and Lakka, T. A. (2017) 'Associations of objectively measured physical activity and sedentary time with arterial stiffness in pre-pubertal children', *Pediatr Exerc Sci,* 29(3), pp. 326-335.

Häkkinen, M. R., Heinosalo, T., Saarinen, N., Linnanen, T., Voutilainen, R., Lakka, T., Jääskeläinen, J., Poutanen, M. and Auriola, S. (2018) 'Analysis by LC-MS/MS of endogenous steroids from human serum, plasma, endometrium and endometriotic tissue', *J Pharm Biomed Anal,* 152, pp. 165-172.

Hamlyn-Williams, C. C., Freeman, P. and Parfitt, G. (2014) 'Acute affective responses to prescribed and self-selected exercise sessions in adolescent girls: an observational study', *BMC Sports Sci Med Rehabil,* 6(1), pp. 35.

Hardy, L. L., Hills, A. P., Timperio, A., Cliff, D., Lubans, D., Morgan, P. J., Taylor, B. J. and Brown, H. (2013) 'A hitchhiker's guide to assessing sedentary behaviour among young people: Deciding what method to use', *J Sci Med Sport,* 16(1), pp. 28-35.

Hart, N. H., Nimphius, S., Rantalainen, T., Ireland, A., Siafarikas, A. and Newton, R. U. (2017) 'Mechanical basis of bone strength: influence of bone material, bone structure and muscle action', *J Musculoskelet Neuronal Interact,* 17(3), pp. 114-139.

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. and S.W.S. Study Group (2012) 'Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study', *Osteoporos Int,* 23(1), pp. 121-30.

Hawkins, S. A., Schroeder, E. T., Wiswell, R. A., Jaque, S. V., Marcell, T. J. and Costa, K. (1999) 'Eccentric muscle action increases site-specific osteogenic response', *Med Sci Sports Exerc,* 31(9), pp. 1287-92.

Hazell, T. J., Pham, T. T., Jean-Philippe, S., Finch, S. L., El Hayek, J., Vanstone, C. A., Agellon, S., Rodd, C. J. and Weiler, H. A. (2015) 'Vitamin D status is associated with bone mineral density and bone mineral content in preschool-aged children', *J Clin Densitom,* 18(1), pp. 60-7.

Heaney, R. P., Abrams, S., Dawson-Hughes, B., Looker, A., Marcus, R., Matkovic, V. and Weaver, C. (2000) 'Peak bone mass', *Osteoporos Int,* 11(12), pp. 985-1009.

Heil, D. P., Brage, S. and Rothney, M. P. (2012) 'Modeling physical activity outcomes from wearable monitors', *Med Sci Sports Exerc,* 44(1S).

Heino, T. J. and Hentunen, T. A. (2008) 'Differentiation of osteoblasts and osteocytes from mesenchymal stem cells', *Curr Stem Cell Res Ther,* 3(2), pp. 131-145.

Heinonen, A. (2001) 'Chapter 3 - Biomechanics', in Khan, K., Mckay, H., Kannus, P., Bailey, D., Wark, J. and Bennell, K. (eds.) *Physical Activity and Bone Health*. Champaign, IL: Human Kinetics.

Hernandez, C. J., Beaupre, G. S. and 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(10), pp. 843-7.

Hernlund, E., Svedbom, A., Ivergård, M., Compston, J., Cooper, C., Stenmark, J., McCloskey, E. V., Jönsson, B. and 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(1), pp. 136.

Hildebrand, M. and Ekelund, U. (2017) 'The assessment of physical activity', in Armstrong, N. and van Mechelen, W. (eds.) *Oxford Textbook of Children's Sport and Exercise Medicine*. Oxford, UK: Oxford University Press, pp. 303-313.

Hill, A. V. (1938) 'The heat of shortening and the dynamic constants of muscle', *Proceedings of the Royal Society of London. Series B-Biological Sciences,* 126(843), pp. 136-195.

Hind, K. and Burrows, M. (2007) 'Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials', *Bone,* 40(1), pp. 14-27.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M. and Endocrine Society (2011) 'Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline', *J Clin Endocrinol Metab,* 96(7), pp. 1911-30.

Hughes, J. M. and Petit, M. A. (2010) 'Biological underpinnings of Frost's mechanostat thresholds: the important role of osteocytes', *J Musculoskelet Neuronal Interact,* 10(2), pp. 128-35.

Hunter, R. F., Tully, M. A., Donnelly, P., Stevenson, M. and Kee, F. (2014) 'Knowledge of UK physical activity guidelines: Implications for better targeted health promotion', *Prev Med,* 65, pp. 33-39.

Hyde, E. T., Watson, K. B., Omura, J. D., Janz, K. F., Lee, S. M., Fulton, J. E. and Carlson, S. A. (2021) 'Surveillance of Meeting the Youth Physical Activity Guideline: Impact of Including Vigorous-Intensity and Bone-Strengthening Activities', *Res Q Exerc Sport,* 93(4), pp. 728-733.

Ilich, J. Z., Skugor, M., Hangartner, T., Baosh, A. and Matkovic, V. (1998) 'Relation of Nutrition, Body Composition and Physical Activity to Skeletal Development: A Cross-Sectional Study in Preadolescent Females', *J Am Coll Nutr,* 17(2), pp. 136-147.

Institute of Medicine (2011) *Committee to Review Dietary Reference Intakes for Vitamin, D and Calcium*, Washington (DC): National Academies Press (US) National Academy of Sciences. Available at: https://www.ncbi.nlm.nih.gov/books/NBK56070/pdf/Bookshelf_NBK56070.pdf (Accessed: 08/11/2018).

International Society for Clinical Densitometry (2019a) *2019 ISCD Official Positions - Pediatric*. Available at: https://iscd.org/wp-content/uploads/2021/09/2019-Official-Positions-Pediatric-1.pdf (Accessed: 25 Jul 2019).

International Society for Clinical Densitometry (2019b) *2019 ISCD Official Positions - Pediatric*. Available at: https://iscd.org/wp-content/uploads/2021/09/2019-Official-Positions-Pediatric-1.pdf (Accessed: 25/07/2019.

Ireland, A., Degens, H., Maffulli, N. and Rittweger, J. (2015) 'Tennis Service Stroke Benefits Humerus Bone: Is Torsion the Cause?', *Calcif Tissue Int,* 97(2), pp. 193-198.

Ireland, A., Rittweger, J. and Degens, H. (2014) 'The Influence of Muscular Action on Bone Strength Via Exercise', *Clin Rev Bone Miner Metab,* 12(2), pp. 93-102.

Iuliano-Burns, S., Mirwald, R. L. and Bailey, D. A. (2001) 'Timing and magnitude of peak height velocity and peak tissue velocities for early, average, and late maturing boys and girls', *Am J Hum Biol,* 13(1), pp. 1-8.

Jackowski, S. A., Erlandson, M. C., Mirwald, R. L., Faulkner, R. A., Bailey, D. A., Kontulainen, S. A., Cooper, D. M. L. and Baxter-Jones, A. D. G. (2011) 'Effect of maturational timing on bone mineral content accrual from childhood to adulthood: Evidence from 15 years of longitudinal data', *Bone,* 48(5), pp. 1178-1185.

Janicka, A., Wren, T. A. L., Sanchez, M. M., Dorey, F., Kim, P. S., Mittelman, S. D. and Gilsanz, V. (2007) 'Fat mass Is not beneficial to bone in adolescents and young adults', *J Clin Endocrinol Metab,* 92(1), pp. 143-147.

Janssen, I., Roberts, K. C. and Thompson, W. (2017) 'Adherence to the 24-Hour Movement Guidelines among 10- to 17-year-old Canadians', *Health Promot Chronic Dis Prev Can,* 37(11), pp. 369-375.

Janz, K. F., Burns, T. L., Torner, J. C., Levy, S. M., Paulos, R., Willing, M. C. and Warren, J. J. (2001) 'Physical activity and bone measures in young children: the Iowa bone development study', *Pediatr,* 107(6), pp. 1387-93.

Janz, K. F., Gilmore, J. M., Burns, T. L., Levy, S. M., Torner, J. C., Willing, M. C. and Marshall, T. A. (2006) 'Physical activity augments bone mineral accrual in young children: The Iowa Bone Development study', *J Pediatr,* 148(6), pp. 793-9.

Janz, K. F., Letuchy, E. M., Burns, T. L., Eichenberger Gilmore, J. M., Torner, J. C. and Levy, S. M. (2014) 'Objectively measured physical activity trajectories predict adolescent bone strength: Iowa Bone Development Study', *Br J Sports Med,* 48(13), pp. 1032-6.

Janz, K. F., Letuchy, E. M., Eichenberger Gilmore, J. M., Burns, T. L., Torner, J. C., Willing, M. C. and Levy, S. M. (2010) 'Early physical activity provides sustained bone health benefits later in childhood', *Med Sci Sports Exerc,* 42(6), pp. 1072-8.

Janz, K. F., Medema-Johnson, H. C., Letuchy, E. M., Burns, T. L., Gilmore, J. M. E., Torner, J. C., Willing, M. and Levy, S. M. (2008) 'Subjective and

objective measures of physical activity in relationship to bone mineral content during late childhood: the Iowa Bone Development Study', *Br J Sports Med,* 42(8), pp. 658-63.

Janz, K. F., Rao, S., Baumann, H. J. and Schultz, J. L. (2003) 'Measuring children's vertical ground reaction forces with accelerometry during walking, running, and jumping: The Iowa Bone Development Study', *Pediatr Exerc Sci,* 15(1), pp. 34-43.

Jaworski, M. and Pludowski, P. (2013) 'Precision errors, least significant change, and monitoring time interval in pediatric measurements of bone mineral density, body composition, and mechanostat parameters by GE lunar prodigy', *J Clin Densitom,* 16(4), pp. 562-9.

Jetté, M., Sidney, K. and Blümchen, G. (1990) 'Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity', *Clin Cardiol,* 13(8), pp. 555-565.

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., III, O'Neill, T., Pols, H., Reeve, J., Silman, A. and Tenenhouse, A. (2009) 'Predictive Value of BMD for Hip and Other Fractures', *J Bone Miner Res,* 20(7), pp. 1185-1194.

Judex, S., Boyd, S., Qin, Y. X., Turner, S., Ye, K., Müller, R. and Rubin, C. (2003) 'Adaptations of trabecular bone to low magnitude vibrations result in more uniform stress and strain under load', *Ann Biomed Eng,* 31(1), pp. 12-20.

Judex, S., Lei, X., Han, D. and Rubin, C. (2007) 'Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude', *J Biomech,* 40(6), pp. 1333-9.

Judex, S. and Zernicke, R. F. (2000) 'High-impact exercise and growing bone: relation between high strain rates and enhanced bone formation', *J Appl Physiol (1985),* 88(6), pp. 2183-91.

Kalkwarf, H. J., Gilsanz, V., Lappe, J. M., Oberfield, S., Shepherd, J. A., Hangartner, T. N., Huang, X., Frederick, M. M., Winer, K. K. and Zemel, B. S. (2010) 'Tracking of bone mass and density during childhood and adolescence', *J Clin Endocrinol Metab,* 95(4), pp. 1690-8.

Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., Lappe, J. M., Horlick, M., Oberfield, S., Mahboubi, S., Fan, B., Frederick, M. M., Winer, K. and Shepherd, J. A. (2007) 'The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race', *J Clin Endocrinol Metab,* 92(6), pp. 2087-99.

Kanis, J. A., Norton, N., Harvey, N. C., Jacobson, T., Johansson, H., Lorentzon, M., McCloskey, E. V., Willers, C. and Borgström, F. (2021) 'SCOPE 2021: a new scorecard for osteoporosis in Europe', *Arch Osteoporos,* 16(1), pp. 82.

Katzman, D. K., Bachrach, L. K., Carter, D. R. and Marcus, R. (1991) 'Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls', *J Clin Endocrinol Metab,* 73(6), pp. 1332-9.

Kelsey, M. M. and Zeitler, P. S. (2016) 'Insulin Resistance of Puberty', *Curr Diab Rep,* 16(7), pp. 64.

Khalatbari, H., Binkovitz, L. A. and Parisi, M. T. (2021) 'Dual-energy X-ray absorptiometry bone densitometry in pediatrics: a practical review and update', *Pediatr Radiol,* 51(1), pp. 25-39.

Khan, K. M., Bennell, K. L., Hopper, J. L., Flicker, L., Nowson, C. A., Sherwin, A. J., Crichton, K. J., Harcourt, P. R. and Wark, J. D. (1998) 'Self-reported ballet classes undertaken at age 10-12 years and hip bone mineral density in later life', *Osteoporos Int,* 8(2), pp. 165-73.

Khan, K. M., Green, R. M., Saul, A., Bennell, K. L., Crichton, K. J., Hopper, J. L. and Wark, J. D. (1996) 'Retired elite female ballet dancers and nonathletic controls have similar bone mineral density at weightbearing sites', *J Bone Miner Res,* 11(10), pp. 1566-74.

Khan, K. M., Thompson, A. M., Blair, S. N., Sallis, J. F., Powell, K. E., Bull, F. C. and Bauman, A. E. (2012) 'Sport and exercise as contributors to the health of nations', *Lancet,* 380(9836), pp. 59-64.

Kindblom, J. M., Lorentzon, M., Norjavaara, E., Hellqvist, A., Nilsson, S., Mellström, D. and Ohlsson, C. (2006) 'Pubertal timing predicts previous fractures and BMD in young adult men: the GOOD study', *J Bone Miner Res,* 21(5), pp. 790-5.

Kindler, J. M., Lewis, R. D. and Hamrick, M. W. (2015) 'Skeletal muscle and pediatric bone development', *Curr Opin Endocrinol Diabetes Obes,* 22(6), pp. 467-74.

Kindler, J. M., Lobene, A. J., Vogel, K. A., Martin, B. R., McCabe, L. D., Peacock, M., Warden, S. J., McCabe, G. P. and Weaver, C. M. (2019) 'Adiposity, insulin resistance, and bone mass in children and adolescents', *J Clin Endocrinol Metab,* 104(3), pp. 892-899.

Kirk, B., Feehan, J., Lombardi, G. and Duque, G. (2020) 'Muscle, Bone, and Fat Crosstalk: the Biological Role of Myokines, Osteokines, and Adipokines', *Curr Osteoporos Rep,* 18(4), pp. 388-400.

Klein, K. O., Larmore, K. A., de Lancey, E., Brown, J. M., Considine, R. V. and Hassink, S. G. (1998) 'Effect of obesity on estradiol level, and Its relationship to leptin, bone maturation, and bone mineral density in children', *J Clin Endocrinol Metab,* 83(10), pp. 3469-3475.

Klitsie, T., Corder, K., Visscher, T. L. S., Atkin, A. J., Jones, A. P. and van Sluijs, E. M. F. (2013) 'Children's sedentary behaviour: descriptive epidemiology and associations with objectively-measured sedentary time', *BMC Public Health,* 13, pp. 1092.

Koedijk, J., van Rijswijk, J., Oranje, W., van den Bergh, J., Bours, S., Savelberg, H. and Schaper, N. (2017) 'Sedentary behaviour and bone health in children, adolescents and young adults: a systematic review', *Osteoporos Int,* 28(9), pp. 2507-2519.

Kohrt, W. M. (1995) 'Body composition by DXA: tried and true?', *Med Sci Sports Exerc,* 27(10), pp. 1349-53.

Kratzsch, J., Lammert, A., Bottner, A., Seidel, B., Mueller, G., Thiery, J., Hebebrand, J. and Kiess, W. (2002) 'Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence', *J Clin Endocrinol Metab,* 87(10), pp. 4587-4594.

Lamberg-Allardt, C., Brustad, M., Meyer, H. E. and Steingrimsdottir, L. (2013) 'Vitamin D - a systematic literature review for the 5th edition of the Nordic Nutrition Recommendations', *Food Nutr Res,* 57, pp. 22671.

Lampinen, E. K., Eloranta, A. M., Haapala, E. A., Lindi, V., Vaisto, J., Lintu, N., Karjalainen, P., Kukkonen-Harjula, K., Laaksonen, D. and Lakka, T. A. (2017) 'Physical activity, sedentary behaviour, and socioeconomic status among Finnish girls and boys aged 6-8 years', *Eur J Sport Sci,* 17(4), pp. 462-472.

Lanyon, L. E. and Rubin, C. T. (1984) 'Static vs dynamic loads as an influence on bone remodelling', *J Biomech,* 17(12), pp. 897-905.

Lappe, J. M., Watson, P., Gilsanz, V., Hangartner, T., Kalkwarf, H. J., Oberfield, S., Shepherd, J., Winer, K. K. and Zemel, B. (2015) 'The longitudinal effects of physical activity and dietary calcium on bone mass accrual across stages of pubertal development', *J Bone Miner Res,* 30(1), pp. 156-64.

Larsson, H. (2021) 'The importance of selection bias in prospective birth cohort studies', *JCPP Adv,* 1(3), pp. e12043.

Lawlor, D. A., Sattar, N., Sayers, A. and Tobias, J. H. (2012) 'The association of fasting insulin, glucose, and lipids with bone mass in adolescents: findings from a cross-sectional study', *J Clin Endocrinol Metab,* 97(6), pp. 2068-2076.

Lee, J. J., Valeri, L., Kapur, K., Ibne Hasan, M. O. S., Quamruzzaman, Q., Wright, R. O., Bellinger, D. C., Christiani, D. C. and Mazumdar, M. (2018) 'Growth parameters at birth mediate the relationship between prenatal manganese exposure and cognitive test scores among a cohort of 2- to 3-year-old Bangladeshi children', *Int J Epidemiol,* 47(4), pp. 1169-1179.

Lehtonen, K., Oja, S. and Hakamäki, M. (2022) *Equality in sports and physical activity in Finland in 2021*. Available at: https://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/164129/OKM_2022_22.pdf?sequence=1&isAllowed=y.

Lehtonen-Veromaa, M. K., Mottonen, T. T., Nuotio, I. O., Irjala, K. M., Leino, A. E. and Viikari, J. S. (2002) 'Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study', *Am J Clin Nutr,* 76(6), pp. 1446-53.

Leonard, M. B., Shults, J., Elliott, D. M., Stallings, V. A. and Zemel, B. S. (2004a) 'Interpretation of whole body dual energy X-ray absorptiometry measures in children: comparison with peripheral quantitative computed tomography', *Bone,* 34(6), pp. 1044-1052.

Leonard, M. B., Shults, J., Wilson, B. A., Tershakovec, A. M. and Zemel, B. S. (2004b) 'Obesity during childhood and adolescence augments bone mass and bone dimensions', *Am J Clin Nutr,* 80(2), pp. 514-23.

Leppänen, M. H., Haapala, E. A., Väistö, J., Ekelund, U., Brage, S., Kilpeläinen, T. O. and Lakka, T. A. (2022) 'Longitudinal and cross-sectional associations of adherence to 24-hour movement guidelines with cardiometabolic risk', *Scand J Med Sci Sports,* 32(1), pp. 255-266.

Li, J., Papadopoulos, V. and Vihma, V. (2015) 'Steroid biosynthesis in adipose tissue', *Steroids,* 103, pp. 89-104.

Lintu, N., Tompuri, T., Viitasalo, A., Soininen, S., Laitinen, T., Savonen, K., Lindi, V. and Lakka, T. A. (2014) 'Cardiovascular fitness and haemodynamic responses to maximal cycle ergometer exercise test in children 6-8 years of age', *J Sports Sci,* 32(7), pp. 652-9.

Lintu, N., Viitasalo, A., Tompuri, T., Veijalainen, A., Hakulinen, M., Laitinen, T., Savonen, K. and Lakka, T. A. (2015) 'Cardiorespiratory fitness, respiratory function and hemodynamic responses to maximal cycle ergometer exercise test in girls and boys aged 9-11 years: the PANIC Study', *Eur J Appl Physiol,* 115(2), pp. 235-43.

Loprinzi, P. D., Lee, H., Cardinal, B. J., Crespo, C. J., Andersen, R. E. and Smit, E. (2012) 'The relationship of Actigraph accelerometer cut-points for estimating physical activity with selected health outcomes', *Res Q Exerc Sport,* 83(3), pp. 422-430.

Lowe, A., Littlewood, C., McLean, S. and Kilner, K. (2017) 'Physiotherapy and physical activity: a cross-sectional survey exploring physical activity promotion, knowledge of physical activity guidelines and the physical activity habits of UK physiotherapists', *BMJ Open Sport Exerc Med,* 3(1), pp. e000290.

Lubans, D. R., Hesketh, K., Cliff, D. P., Barnett, L. M., Salmon, J., Dollman, J., Morgan, P. J., Hills, A. P. and Hardy, L. L. (2011) 'A systematic review of the validity and reliability of sedentary behaviour measures used with children and adolescents', *Obes Rev,* 12(10), pp. 781-799.

MacKelvie, K. J., Khan, K. M. and McKay, H. A. (2002) 'Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review', *Br J Sports Med,* 36(4), pp. 250-257.

Malina, R. M., Bouchard, C. and Bar-Or, O. (2003) *Growth, maturation, and physical activity.* 2nd edn. Champaign, Ill.: Human Kinetics.

Mantyselka, A., Lindi, V., Viitasalo, A., Eloranta, A. M., Agren, J., Vaisanen, S., Voutilainen, R., Laitinen, T., Lakka, T. A. and Jaaskelainen, J. (2018) 'Associations of dehydroepiandrosterone sulfate with cardiometabolic risk factors in prepubertal children', *J Clin Endocrinol Metab,* 103(7), pp. 2592-2600.

Matthews, C. E., Hagströmer, M., Pober, D. M. and Bowles, H. R. (2012) 'Best practices for using physical activity monitors in population-based research', *Med Sci Sports Exerc,* 44(1 Suppl 1), pp. S68-76.

Maurel, D. B., Jähn, K. and Lara-Castillo, N. (2017) 'Muscle-bone crosstalk: emerging opportunities for novel therapeutic approaches to treat musculoskeletal pathologies', *Biomedicines,* 5(4), pp. 62.

Maylor, B. D., Rowlands, A. V., Dawkins, N. P. and Edwardson, C. L. (2021) *Radar Plot Generator (Version 2.0) [Source code]*. Available at: https://github.com/Maylor8/RadarPlotGenerator (Accessed: 24/02/2022.

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', *Br J Sports Med,* 39(1), pp. 10-4.

McMillan, L. B., Aitken, D., Ebeling, P., Jones, G. and Scott, D. (2018) 'The relationship between objectively assessed physical activity and bone health in older adults differs by sex and is mediated by lean mass', *Osteoporos Int,* 29(6), pp. 1379-1388.

McVeigh, J. A., Howie, E. K., Zhu, K., Walsh, J. P. and Straker, L. (2019) 'Organized Sport Participation From Childhood to Adolescence Is

Associated With Bone Mass in Young Adults From the Raine Study', *J Bone Miner Res,* 34(1), pp. 67-74.

Mellon, S. J. and Tanner, K. E. (2012) 'Bone and its adaptation to mechanical loading: a review', *Int Mater Rev,* 57(5), pp. 235-255.

Melton III, L. J., Atkinson, E. J., O'Connor, M. K., O'Fallon, W. M. and Riggs, B. L. (2000) 'Determinants of Bone Loss from the Femoral Neck in Women of Different Ages', *J Bone Miner Res,* 15(1), pp. 24-31.

Metcalf, B., Henley, W. and Wilkin, T. (2012) 'Effectiveness of intervention on physical activity of children: systematic review and meta-analysis of controlled trials with objectively measured outcomes (EarlyBird 54)', *Br Med J,* 345, pp. e5888.

Meyer, U., Ernst, D., Schott, S., Riera, C., Hattendorf, J., Romkes, J., Granacher, U., Göpfert, B. and Kriemler, S. (2015) 'Validation of two accelerometers to determine mechanical loading of physical activities in children', *J Sports Sci,* 33(16), pp. 1702-1709.

Migueles, J. H., Cadenas-Sanchez, C., Tudor-Locke, C., Löf, M., Esteban-Cornejo, I., Molina-Garcia, P., Mora-Gonzalez, J., Rodriguez-Ayllon, M., Garcia-Marmol, E., Ekelund, U. and Ortega, F. B. (2019) 'Comparability of published cut-points for the assessment of physical activity: Implications for data harmonization', *Scand J Med Sci Sports,* 29(4), pp. 566-574.

Ministry of Education and Culture (2021) *Recommendation on physical activity for children and adolescents aged 7 to 17 years*, Finland. Available at: https://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/162988/OKM_2021_21.pdf?sequence=4 (Accessed: 29/09/2021).

Mittag, U., Kriechbaumer, A. and Rittweger, J. (2018) 'Torsion - an underestimated form shaping entity in bone adaptation?', *J Musculoskelet Neuronal Interact,* 18(4), pp. 407-418.

Molgaard, C., Thomsen, B. L. and Michaelsen, K. F. (2001) 'The influence of calcium intake and physical activity on bone mineral content and bone size in healthy children and adolescents', *Osteoporos Int,* 12(10), pp. 887-94.

Moliner-Urdiales, D., Ortega, F. B., Vicente-Rodriguez, G., Rey-Lopez, J. P., Gracia-Marco, L., Widhalm, K., Sjöström, M., Moreno, L. A., Castillo, M. J. and Ruiz, J. R. (2010) 'Association of physical activity with muscular strength and fat-free mass in adolescents: the HELENA study', *Eur J Appl Physiol,* 109(6), pp. 1119-1127.

Moon, R. J., Harvey, N. C., Davies, J. H. and Cooper, C. (2014) 'Vitamin D and skeletal health in infancy and childhood', *Osteoporos Int,* 25(12), pp. 2673-2684.

Moore, S. A., Cumming, S. P., Balletta, G., Ramage, K., Eisenmann, J. C., Baxter-Jones, A. D. G., Jackowski, S. A. and Sherar, L. B. (2020) 'Exploring the relationship between adolescent biological maturation, physical activity, and sedentary behaviour: a systematic review and narrative synthesis', *Ann Hum Biol,* 47(4), pp. 365-383.

Moore, S. A., McKay, H. A., Macdonald, H., Nettlefold, L., Baxter-Jones, A. D. G., Cameron, N. and Brasher, P. M. A. (2015) 'Enhancing a Somatic Maturity Prediction Model', *Med Sci Sports Exerc,* 47(8), pp. 1755-1764.

Morgan, E. F., Barnes, G. L. and Einhorn, T. A. (2008) 'CHAPTER 1 - The Bone Organ System: Form and Function', in Marcus, R., Feldman, D., Nelson, D.A. and Rosen, C.J. (eds.) *Osteoporosis.* 3 ed. San Diego: Academic Press, pp. 3-25.

Mosley, J. R. and Lanyon, L. E. (1998) 'Strain rate as a controlling influence on adaptive modeling in response to dynamic loading of the ulna in growing male rats', *Bone,* 23(4), pp. 313-8.

MRC Epidemiology Unit (2022a) *Combined heart rate and motion sensors*. Diet, Anthropometry and Physical Activity Measurement Toolkit. Available at: https://beta.measurement-toolkit.org/physical-activity/objective-methods/combined-sensors (Accessed: 8/11/22).

MRC Epidemiology Unit (2022b) *Heart rate monitors*. Diet, Anthropometry and Physical Activity Measurement Toolkit. Available at: https://beta.measurement-toolkit.org/physical-activity/objective-methods/heart-rate-monitors (Accessed: 7/11/22).

Nagy, T. R., Gower, B. A., Trowbridge, C. A., Dezenberg, C., Shewchuk, R. M. and Goran, M. I. (1997) 'Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children', *J Clin Endocrinol Metab,* 82(7), pp. 2148-2152.

Narciso, P. H., Werneck, A. O., Luiz-de-Marco, R., Ventura Faustino-da-Silva, Y. d. S., Maillane-Vanegas, S., Agostinete, R. R. and Fernandes, R. A. (2020) 'Influential role of lean soft tissue in the association between training volume and bone mineral density among male adolescent practitioners of impact-loading sports: ABCD Growth study', *BMC Pediatr,* 20(1), pp. 496.

National Nutrition Council (2010) *Report of Finnish experts of vitamin D*. Available at: http://www.ravitsemusneuvottelukunta.fi/attachments/vrn/d-vitamiiniraportti2010.pdf (Accessed: 07/09/2020).

National Nutrition Council (2014) *Finnish Nutrition Recommendations – Health from Food (Suomalaiset ravitsemussuositukset–Terveyttä ruoasta)*, Helsinki: Juvenes Print. Available at: https://www.ruokavirasto.fi/en/foodstuffs/healthy-diet/nutrition-and-food-recommendations/ (Accessed: 07/09/2020).

Nelson, D. A. and Koo, W. W. (1999) 'Interpretation of absorptiometric bone mass measurements in the growing skeleton: issues and limitations', *Calcif Tissue Int,* 65(1), pp. 1-3.

Ness, A. R., Leary, S. D., Mattocks, C., Blair, S. N., Reilly, J. J., Wells, J., Ingle, S., Tilling, K., Smith, G. D. and Riddoch, C. (2007) 'Objectively measured physical activity and fat mass in a large cohort of children', *PLoS Med,* 4(3), pp. e97.

Nilsson, B. E. and Westlin, N. E. (1971) 'Bone Density in Athletes', *Clin Orthop Relat Res,* 77, pp. 179-82.

Nogueira, R. C., Weeks, B. K. and Beck, B. R. (2014) 'Exercise to Improve Pediatric Bone and Fat: A Systematic Review and Meta-analysis', *Med Sci Sports Exerc,* 46(3), pp. 610-21.

Office of the Surgeon General (2004) *Bone Health and Osteoporosis: A Report of the Surgeon General*, Rockville (MD): Office of the Surgeon General (US). Available at: https://www.ncbi.nlm.nih.gov/books/NBK45513/ (Accessed: 12/05/2021).

Okely, A. D., Ghersi, D., Loughran, S. P., Cliff, D. P., Shilton, T., Jones, R. A., Stanley, R. M., Sherring, J., Toms, N., Eckermann, S., Olds, T. S., Zhang, Z., Parrish, A.-M., Kervin, L., Downie, S., Salmon, J., Bannerman, C., Needham, T., Marshall, E., Kaufman, J., Brown, L., Wille, J., Wood, G., Lubans, D. R., Biddle, S. J. H., Pill, S., Hargreaves, A., Jonas, N., Schranz, N., Campbell, P., Ingram, K., Dean, H., Verrender, A., Ellis, Y., Chong, K. H., Dumuid, D., Katzmarzyk, P. T., Draper, C. E., Lewthwaite, H. and Tremblay, M. S. (2022) 'A collaborative approach to adopting/adapting guidelines. The Australian 24-hour movement guidelines for children (5-12 years) and young people (13-17 years): An integration of physical activity, sedentary behaviour, and sleep', *Int J Behav Nutr Phys Act,* 19(1), pp. 2.

Outila, T. A., Karkkainen, M. U. and Lamberg-Allardt, C. J. (2001) 'Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density', *Am J Clin Nutr,* 74(2), pp. 206-10.

Pekkinen, M., Viljakainen, H., Saarnio, E., Lamberg-Allardt, C. and Mäkitie, O. (2012) 'Vitamin D is a major determinant of bone mineral density at school age', *PLoS One,* 7(7), pp. e40090.

Peplies, J., Jiménez-Pavón, D., Savva, S. C., Buck, C., Günther, K., Fraterman, A., Russo, P., Iacoviello, L., Veidebaum, T., Tornaritis, M., De Henauw, S., Mårild, S., Molnár, D., Moreno, L. A., Ahrens, W. and on behalf of the IDEFICS consortium (2014) 'Percentiles of fasting serum insulin, glucose, HbA1c and HOMA-IR in pre-pubertal normal weight European children from the IDEFICS cohort', *Int J Obes (Lond),* 38 Suppl 2, pp. S39-S47.

Pereira, J. R., Cliff, D. P., Sousa-Sa, E., Zhang, Z. and Santos, R. (2019) 'Prevalence of objectively measured sedentary behavior in early years: Systematic review and meta-analysis', *Scand J Med Sci Sports,* 29(3), pp. 308-328.

Physical Activity Guidelines Advisory Committee (2018) *2018 Physical Activity Guidelines Advisory Committee Scientific Report*. Available at: https://health.gov/sites/default/files/2019-09/PAG_Advisory_Committee_Report.pdf (Accessed: 8/12/22).

Piercy, K. L., Troiano, R. P., Ballard, R. M., Carlson, S. A., Fulton, J. E., Galuska, D. A., George, S. M. and Olson, R. D. (2018) 'The physical activity guidelines for Americans', *JAMA,* 320(19), pp. 2020-2028.

Pitukcheewanont, P., Austin, J., Chen, P. and Punyasavatsut, N. (2013) 'Bone health in children and adolescents: risk factors for low bone density', *Pediatr Endocrinol Rev,* 10(3), pp. 318-35.

Pivonka, P., Park, A. and Forwood, M. R. (2018) 'Functional Adaptation of Bone: The Mechanostat and Beyond', in Pivonka, P. (ed.) *Multiscale Mechanobiology of Bone Remodeling and Adaptation CISM International Centre for Mechanical Sciences*. Cham: Springer International Publishing, pp. 1-60.

Poitras, V. J., Gray, C. E., Borghese, M. M., Carson, V., Chaput, J. P., Janssen, I., Katzmarzyk, P. T., Pate, R. R., Connor Gorber, S., Kho, M. E., Sampson, M. and Tremblay, M. S. (2016) 'Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth', *Appl Physiol Nutr Metab,* 41(6 Suppl 3), pp. S197-239.

Pomerants, T., Tillmann, V., Jürimäe, J. and Jürimäe, T. (2007) 'The influence of serum ghrelin, IGF axis and testosterone on bone mineral density in boys at different stages of sexual maturity', *J Bone Miner Metab,* 25(3), pp. 193-197.

Public Health England (2021) *Muscle and bone strengthening activities for children and young people (5 to 18 years): a rapid evidence review*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/949958/CYP_MSBS_Review__1_.pdf (Accessed: 12/01/2021).

Puyau, M. R., Adolph, A. L., Vohra, F. A. and Butte, N. F. (2002) 'Validation and Calibration of Physical Activity Monitors in Children', *Obes Res,* 10(3), pp. 150-157.

R Core Team (2021) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/ (Accessed: 22/07/2021).

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(5), pp. 771-775.

Reid, I. R. (2010) 'Fat and bone', *Arch Biochem Biophys,* 503(1), pp. 20-7.

Richards, J. B., Valdes, A. M., Burling, K., Perks, U. C. and Spector, T. D. (2007) 'Serum Adiponectin and Bone Mineral Density in Women', *J Clin Endocrinol Metab,* 92(4), pp. 1517-1523.

Riddoch, C. J. and Boreham, C. A. G. (1995) 'The Health-Related Physical Activity of Children', *Sports Med,* 19(2), pp. 86-102.

Riddoch, C. J., Leary, S. D., Ness, A. R., Blair, S. N., Deere, K., Mattocks, C., Griffiths, A., Davey Smith, G. and Tilling, K. (2009) 'Prospective associations between objective measures of physical activity and fat mass in 12-14 year old children: the Avon Longitudinal Study of Parents and Children (ALSPAC)', *Br Med J,* 339, pp. b4544.

Ridgers, N. D., Salmon, J., Ridley, K., O'Connell, E., Arundell, L. and Timperio, A. (2012) 'Agreement between activPAL and ActiGraph for assessing children's sedentary time', *Int J Behav Nutr Phys Act,* 9, pp. 15.

Riso, E.-M., Kull, M., Mooses, K. and Jürimäe, J. (2018) 'Physical activity, sedentary time and sleep duration: associations with body composition in 10–12-year-old Estonian schoolchildren', *BMC Public Health,* 18(1), pp. 496.

Rittweger, J. (2007) 'Phyysiological Targets of Artificial Gravity: Adaptive Processes in Bone', in Clément, G. and Bukley, A. (eds.) *Artificial Gravity*. New York, NY: Springer New York, pp. 191-231.

Roberts, K. C., Yao, X., Carson, V., Chaput, J.-P., Janssen, I. and Tremblay, M. S. (2017) 'Meeting the Canadian 24-hour movement guidelines for children and youth', *Health Rep,* 28(10), pp. 3-7.

Roemmich, J. N., Clark, P. A., Mantzoros, C. S., Gurgol, C. M., Weltman, A. and Rogol, A. D. (2003) 'Relationship of Leptin to Bone Mineralization in Children and Adolescents', *J Clin Endocrinol Metab,* 88(2), pp. 599-604.

Rollo, S., Antsygina, O. and Tremblay, M. S. (2020) 'The whole day matters: Understanding 24-hour movement guideline adherence and relationships with health indicators across the lifespan', *J Sport Health Sci,* 9(6), pp. 493-510.

Roman-Vinas, B., Chaput, J. P., Katzmarzyk, P. T., Fogelholm, M., Lambert, E. V., Maher, C., Maia, J., Olds, T., Onywera, V., Sarmiento, O. L., Standage, M., Tudor-Locke, C., Tremblay, M. S. and Group, I. R. (2016) 'Proportion of children meeting recommendations for 24-hour movement guidelines and associations with adiposity in a 12-country study', *Int J Behav Nutr Phys Act,* 13(1), pp. 123.

Rønne, M. S., Heidemann, M., Lylloff, L., Schou, A. J., Tarp, J., Bugge, A., Laursen, J. O., Jørgensen, N. R., Husby, S., Wedderkopp, N. and Mølgaard, C. (2019) 'Bone mass development is sensitive to insulin resistance in adolescent boys', *Bone,* 122, pp. 1-7.

Rønne, M. S., Heidemann, M., Lylloff, L., Schou, A. J., Tarp, J., Laursen, J. O., Jorgensen, N. R., Husby, S., Wedderkopp, N. and Molgaard, C. (2018a) 'Bone Mass Development in Childhood and Its Association with Physical Activity and Vitamin D Levels. The CHAMPS-Study DK', *Calcif Tissue Int,* 104(1), pp. 1-13.

Rønne, M. S., Heidemann, M., Schou, A., Laursen, J. O., Bojesen, A. B., Lylloff, L., Husby, S., Wedderkopp, N. and Mølgaard, C. (2018b) 'Tracking of bone mass from childhood to puberty: a 7-year follow-up. The CHAMPS study DK', *Osteoporos Int,* 29(8), pp. 1843-1852.

Rowlands, A. V. (2007) 'Accelerometer assessment of physical activity in children: an update', *Pediatr Exerc Sci,* 19(3), pp. 252-66.

Rowlands, A. V., Dawkins, N. P., Maylor, B., Edwardson, C. L., Fairclough, S. J., Davies, M. J., Harrington, D. M., Khunti, K. and Yates, T. (2019a) 'Enhancing the value of accelerometer-assessed physical activity: meaningful visual comparisons of data-driven translational accelerometer metrics', *Sports Med Open,* 5(1), pp. 47.

Rowlands, A. V., Edwardson, C. L., Davies, M. J., Khunti, K., Harrington, D. M. and Yates, T. (2018) 'Beyond cut points: Accelerometer metrics that capture the physical activity profile', *Med Sci Sports Exerc,* 50(6), pp. 1323-1332.

Rowlands, A. V., Edwardson, C. L., Dawkins, N. P., Maylor, B. D., Metcalf, K. M. and Janz, K. F. (2020) 'Physical activity for bone health: How much and/or how hard?', *Med Sci Sports Exerc,* 52(11), pp. 2331-2341.

Rowlands, A. V., Pilgrim, E. L. and Eston, R. G. (2008) 'Patterns of habitual activity across weekdays and weekend days in 9–11-year-old children', *Prev Med,* 46(4), pp. 317-324.

Rowlands, A. V., Schuna, J. M., Stiles, V. H. and Tudor-Locke, C. (2014) 'Cadence, Peak Vertical Acceleration, and Peak Loading Rate During Ambulatory Activities: Implications for Activity Prescription for Bone Health', *J Phys Act Health,* 11(7), pp. 1291-1294.

Rowlands, A. V., Sherar, L. B., Fairclough, S. J., Yates, T., Edwardson, C. L., Harrington, D. M., Davies, M. J., Munir, F., Khunti, K. and Stiles, V. H. (2019b) 'A data-driven, meaningful, easy to interpret, standardised accelerometer outcome variable for global surveillance', *J Sci Med Sport,* 22(10), pp. 1132-1138.

Rowlands, A. V. and Stiles, V. H. (2012) 'Accelerometer counts and raw acceleration output in relation to mechanical loading', *J Biomech,* 45(3), pp. 448-54.

Rubin, C., Turner, A. S., Mallinckrodt, C., Jerome, C., McLeod, K. and Bain, S. (2002) 'Mechanical strain, induced noninvasively in the high-frequency domain, is anabolic to cancellous bone, but not cortical bone', *Bone,* 30(3), pp. 445-52.

Rubin, C. T. and Lanyon, L. E. (1984) 'Regulation of bone formation by applied dynamic loads', *J Bone Jt Surg,* 66(3), pp. 397-402.

Saari, A., Sankilampi, U., Hannila, M.-L., Kiviniemi, V., Kesseli, K. and Dunkel, L. (2011) 'New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age', *Ann Med,* 43(3), pp. 235-248.

Sallinen, T., Viitasalo, A., Lintu, N., Väistö, J., Soininen, S., Jalkanen, H., Haapala, E. A., Mikkonen, S., Schwab, U., Lakka, T. A. and Eloranta, A.-M. (2022) 'The effects of an 8-year individualised lifestyle intervention on food consumption and nutrient intake from childhood to adolescence: the PANIC Study', *J Nutr Sci,* 11, pp. e40.

Santos-Rocha, R. A., Oliveira, C. S. and Veloso, A. P. (2006) 'Osteogenic index of step exercise depending on choreographic movements, session duration, and stepping rate', *Br J Sports Med,* 40(10), pp. 860-6.

Sayers, A., Timpson, N. J., Sattar, N., Deanfield, J., Hingorani, A. D., Davey-Smith, G. and Tobias, J. H. (2010) 'Adiponectin and its association with bone mass accrual in childhood', *J Bone Miner Res,* 25(10), pp. 2212-2220.

Schmidt, A. F. and Finan, C. (2018) 'Linear regression and the normality assumption', *J Clin Epidemiol,* 98, pp. 146-151.

Schoenau, E. and Frost, H. (2002) 'The" muscle-bone unit" in children and adolescents', *Calcif Tissue Int,* 70(5), pp. 405-407.

Schoenau, E., Land, C., Stabrey, A., Remer, T. and Kroke, A. (2004) 'The bone mass concept: problems in short stature', *Eur J Endocrinol,* 151 Suppl 1, pp. S87-91.

Shalof, H., Dimitri, P., Shuweihdi, F. and Offiah, A. C. (2021) 'Which skeletal imaging modality is best for assessing bone health in children and young adults compared to DXA? A systematic review and meta-analysis', *Bone,* 150, pp. 116013.

Shephard, R. J. (2003) 'Limits to the measurement of habitual physical activity by questionnaires', *Br J Sports Med,* 37(3), pp. 197-206.

Shepherd, J. A., Fan, B., Lu, Y., Wu, X. P., Wacker, W. K., Ergun, D. L. and Levine, M. A. (2012) 'A multinational study to develop universal standardization of whole-body bone density and composition using GE Healthcare Lunar and Hologic DXA systems', *J Bone Miner Res,* 27(10), pp. 2208-2216.

Shepherd, J. A., Ng, B. K., Sommer, M. J. and Heymsfield, S. B. (2017) 'Body composition by DXA', *Bone,* 104, pp. 101-105.

Sioen, I., Lust, E., De Henauw, S., Moreno, L. A. and Jiménez-Pavón, D. (2016) 'Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review', *Calcif Tissue Int,* 99(6), pp. 557-577.

Sizonenko, P. C. and Paunier, L. (1975) 'Hormonal changes in puberty III: Correlation of plasma dehydroepiandrosterone, testosterone, FSH, and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche', *J Clin Endocrinol Metab,* 41(5), pp. 894-904.

Skinner, A. M., Barker, A. R., Moore, S. A., Soininen, S., Haapala, E. A., Väistö, J., Westgate, K., Brage, S., Lakka, T. A. and Vlachopoulos, D. (2024) 'Cross-sectional and longitudinal associations between the 24-hour movement behaviours, including muscle and bone strengthening activity, with bone and lean mass from childhood to adolescence', *BMC Public Health,* 24(1), pp. 227.

Skinner, A. M., Rowlands, A. V., Vlachopoulos, D., Barker, A. R., Janz, K. F. and Moore, S. A. (2023a) 'The Influence of Accelerometer Epoch Length on Associations of Physical Activity Intensity and Volume with Bone Outcomes', *J Sports Sci Med,* 22(1), pp. 117-132.

Skinner, A. M., Vlachopoulos, D., Barker, A. R., Moore, S. A., Rowlands, A. V., Soininen, S., Haapala, E. A., Vaisto, J., Westgate, K., Brage, S. and Lakka, T. A. (2023b) 'Physical activity volume and intensity distribution in relation to bone, lean and fat mass in children', *Scand J Med Sci Sports,* 33(3), pp. 267-282.

Slemenda, C. W., Miller, J. Z., Hui, S. L., Reister, T. K. and Johnston, C. C., Jr. (1991) 'Role of physical activity in the development of skeletal mass in children', *J Bone Miner Res,* 6(11), pp. 1227-33.

Smith, J. J., Diallo, T. M. O., Bennie, J. A., Tomkinson, G. R. and Lubans, D. R. (2020) 'Factors associated with adherence to the muscle-strengthening activity guideline among adolescents', *Psychol Sport Exerc,* 51, pp. 101747.

Society for Adolescent Health and Medicine (2013) 'Recommended vitamin D intake and management of low vitamin D status in adolescents: a position statement of the society for adolescent health and medicine', *J Adolesc Health,* 52(6), pp. 801-3.

Soininen, S., Eloranta, A. M., Lindi, V., Venäläinen, T., Zaproudina, N., Mahonen, A. and Lakka, T. A. (2016) 'Determinants of serum 25-hydroxyvitamin D concentration in Finnish children: the Physical Activity and Nutrition in Children (PANIC) study', *Br J Nutr,* 115(6), pp. 1080-91.

Soininen, S., Sidoroff, V., Lindi, V., Mahonen, A., Kröger, L., Kröger, H., Jääskeläinen, J., Atalay, M., Laaksonen, D. E., Laitinen, T. and Lakka, T. A. (2018) 'Body fat mass, lean body mass and associated biomarkers as determinants of bone mineral density in children 6-8years of age - The Physical Activity and Nutrition in Children (PANIC) study', *Bone,* 108, pp. 106-114.

Sopher, A. B., Jean, A. M., Zwany, S. K., Winston, D. M., Pomeranz, C. B., Bell, J. J., McMahon, D. J., Hassoun, A., Fennoy, I. and Oberfield, S. E. (2011) 'Bone age advancement in prepubertal children with obesity and premature adrenarche: possible potentiating factors', *Obesity (Silver Spring),* 19(6), pp. 1259-64.

Sousa, F., Ishikawa, M., Vilas-Boas, J. P. and Komi, P. V. (2007) 'Intensity- and muscle-specific fascicle behavior during human drop jumps', *J Appl Physiol (1985),* 102(1), pp. 382-389.

Soyka, L. A., Fairfield, W. P. and Klibanski, A. (2000) 'Hormonal Determinants and Disorders of Peak Bone Mass in Children', *J Clin Endocrinol Metab,* 85(11), pp. 3951-3963.

Sport England (2022) *Active Lives Children and Young People Survey - Academic year 2021-22*. Available at: https://sportengland-production-files.s3.eu-west-2.amazonaws.com/s3fs-public/2022-12/Active%20Lives%20Children%20and%20Young%20People%20Survey%20Academic%20Year%202021-22%20Report.pdf?VersionId=R5_hmJHw5M4yKFsewm2vGDMRGHWW7q3E (Accessed: 20/02/23).

Sport England (2023) *Active Lives Children and Young People Survery Academic Year 2022-23*. Available at: https://sportengland-production-

files.s3.eu-west-2.amazonaws.com/s3fs-public/2023-12/Active%20Lives%20Children%20and%20Young%20People%20Survey%20-%20academic%20year%202022-23%20report.pdf?VersionId=3N7GGWZMKy88UPsGfnJVUZkaTklLwB_L (Accessed: 26/03/24).

Stegle, O., Fallert, S. V., MacKay, D. J. and Brage, S. (2008) 'Gaussian process robust regression for noisy heart rate data', *IEEE Trans Biomed Eng,* 55(9), pp. 2143-51.

Stewart, S., Darwood, A., Masouros, S., Higgins, C. and Ramasamy, A. (2020) 'Mechanotransduction in osteogenesis', *Bone Joint Res,* 9(1), pp. 1-14.

Strain, T., Fitzsimons, C., Kelly, P. and Mutrie, N. (2016) 'The forgotten guidelines: cross-sectional analysis of participation in muscle strengthening and balance & co-ordination activities by adults and older adults in Scotland', *BMC Public Health,* 16(1), pp. 1108.

Strain, T., Milton, K., Dall, P., Standage, M. and Mutrie, N. (2020) 'How are we measuring physical activity and sedentary behaviour in the four home nations of the UK? A narrative review of current surveillance measures and future directions', *Br J Sports Med,* 54(21), pp. 1269-1276.

Swanson, C. M., Blatchford, P. J., Stone, K. L., Cauley, J. A., Lane, N. E., Rogers-Soeder, T. S., Redline, S., Bauer, D. C., Wright, K. P., Wierman, M. E., Kohrt, W. M., Orwoll, E. S. and for The Osteoporotic Fractures in Men, S. (2021) 'Sleep duration and bone health measures in older men', *Osteoporos Int,* 32(3), pp. 515-527.

Swanson, C. M., Kohrt, W. M., Buxton, O. M., Everson, C. A., Wright, K. P., Orwoll, E. S. and Shea, S. A. (2018) 'The importance of the circadian system & sleep for bone health', *Metabolism,* 84, pp. 28-43.

Tamme, R., Jürimäe, J., Mäestu, E., Remmel, L., Purge, P., Mengel, E. and Tillmann, V. (2019) 'Association of Serum Testosterone at 12 Years with a Subsequent Increase in Bone Mineral Apparent Density at 18 Years: A Longitudinal Study of Boys in Puberty', *Horm Res Paediatr,* 91(6), pp. 400-405.

Tanner, J. M. (1986) 'Normal growth and techniques of growth assessment', *Clin Endocrinol Metab,* 15(3), pp. 411-451.

Thivel, D., Tremblay, A., Genin, P. M., Panahi, S., Rivière, D. and Duclos, M. (2018) 'Physical Activity, Inactivity, and Sedentary Behaviors: Definitions and Implications in Occupational Health', *Front Public Health,* 6, pp. 288.

Thrailkill, K. M., Lumpkin, C. K., Jr., Bunn, R. C., Kemp, S. F. and Fowlkes, J. L. (2005) 'Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues', *Am J Physiol Endocrinol Metab,* 289(5), pp. E735-E745.

Timpson, N. J., Sayers, A., Davey-Smith, G. and Tobias, J. H. (2009) 'How Does Body Fat Influence Bone Mass in Childhood? A Mendelian Randomization Approach', *J Bone Miner Res,* 24(3), pp. 522-533.

Tobias, J. H. (2010) 'Fat mass and bone development', *Expert Rev Endocrinol Metab,* 5(3), pp. 323-325.

Tobias, J. H., Steer, C. D., Mattocks, C. G., Riddoch, C. and 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', *J Bone Miner Res,* 22(1), pp. 101-109.

Tompuri, T. T., Lintu, N., Soininen, S., Laitinen, T. and Lakka, T. A. (2016) 'Comparison between parameters from maximal cycle ergometer test first without respiratory gas analysis and thereafter with respiratory gas analysis among healthy prepubertal children', *Appl Physiol Nutr Metab,* 41(6), pp. 624-30.

Torres-Costoso, A., Pozuelo-Carrascosa, D. P., Álvarez-Bueno, C., Ferri-Morales, A., Miota Ibarra, J., Notario-Pacheco, B. and Martínez-Vizcaíno, V. (2017) 'Insulin and bone health in young adults: The mediator role of lean mass', *PLoS ONE,* 12(3), pp. e0173874.

Tremblay, M. S., Carson, V., Chaput, J.-P., Gorber, S. C., Dinh, T., Duggan, M., Faulkner, G., Gray, C. E., Gruber, R., Janson, K., Janssen, I., Katzmarzyk, P. T., Kho, M. E., Latimer-Cheung, A. E., LeBlanc, C., Okely, A. D., Olds, T., Pate, R. R., Phillips, A., Poitras, V. J., Rodenburg, S., Sampson, M., Saunders, T. J., Stone, J. A., Stratton, G., Weiss, S. K. and Zehr, L. (2016) 'Canadian 24-Hour Movement Guidelines for Children and Youth: An Integration of Physical Activity, Sedentary Behaviour, and Sleep', *Appl Physiol Nutr Metab,* 41(6 (Suppl. 3)), pp. S311-S327.

Tremblay, M. S., Carson, V. and Chaput, J. P. (2016) 'Introduction to the Canadian 24-Hour Movement Guidelines for Children and Youth: An Integration of Physical Activity, Sedentary Behaviour, and Sleep', *Appl Physiol Nutr Metab,* 41(6 Suppl 3), pp. iii-iv.

Tripepi, G., Jager, K. J., Dekker, F. W. and Zoccali, C. (2010) 'Selection Bias and Information Bias in Clinical Research', *Nephron Clin Pract,* 115(2), pp. c94-c99.

Troiano, R. P., Berrigan, D., Dodd, K. W., MÂSse, L. C., Tilert, T. and McDowell, M. (2008) 'Physical Activity in the United States Measured by Accelerometer', *Med Sci Sports Exerc,* 40(1), pp. 181-8.

Troiano, R. P., McClain, J. J., Brychta, R. J. and Chen, K. Y. (2014) 'Evolution of accelerometer methods for physical activity research', *Br J Sports Med,* 48(13), pp. 1019-1023.

Trost, S. G., Loprinzi, P. D., Moore, R. and Pfeiffer, K. A. (2011) 'Comparison of accelerometer cut points for predicting activity intensity in youth', *Med Sci Sports Exerc,* 43(7), pp. 1360-8.

Turner, C. H. (1998) 'Three rules for bone adaptation to mechanical stimuli', *Bone,* 23(5), pp. 399-407.

Turner, C. H. and Burr, D. B. (1993) 'Basic biomechanical measurements of bone: A tutorial', *Bone,* 14(4), pp. 595-608.

Turner, C. H. and Pavalko, F. M. (1998) 'Mechanotransduction and functional response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation', *J Orthop Sci,* 3(6), pp. 346-55.

Turner, C. H. and Robling, A. G. (2003) 'Designing exercise regimens to increase bone strength', *Exerc Sport Sci Rev,* 31(1), pp. 45-50.

UCLA: Statistical Consulting Group (2021) *Regression with Stata Chapter 2 - Regression Diagnostics*. Available at: https://stats.oarc.ucla.edu/stata/webbooks/reg/chapter2/stata-webbooksregressionwith-statachapter-2-regression-diagnostics/ (Accessed: 20/01/23).

Vaisto, J., Eloranta, A. M., Viitasalo, A., Tompuri, T., Lintu, N., Karjalainen, P., Lampinen, E. K., Agren, J., Laaksonen, D. E., Lakka, H. M., Lindi, V. and Lakka, T. A. (2014) 'Physical activity and sedentary behaviour in relation to cardiometabolic risk in children: cross-sectional findings from the Physical Activity and Nutrition in Children (PANIC) Study', *Int J Behav Nutr Phys Act,* 11, pp. 55.

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. and Helena Study Group (2012) 'Vitamin D status and physical activity interact to improve bone mass in adolescents. The HELENA Study', *Osteoporos Int,* 23(8), pp. 2227-37.

van der Ploeg, H. P. and Hillsdon, M. (2017) 'Is sedentary behaviour just physical inactivity by another name?', *Int J Behav Nutr Phys Act,* 14(1), pp. 142.

VanderWeele, T. J. (2014) 'A unification of mediation and interaction: a 4-way decomposition', *Epidemiology,* 25(5), pp. 749-761.

VanderWeele, T. J. (2016) 'Mediation Analysis: A Practitioner's Guide', *Annu Rev Public Health,* 37, pp. 17-32.

Venalainen, T. M., Viitasalo, A. M., Schwab, U. S., Eloranta, A. M., Haapala, E. A., Jalkanen, H. P., de Mello, V. D., Laaksonen, D. E., Lindi, V. I., Agren, J. J. and Lakka, T. A. (2016) 'Effect of a 2-y dietary and physical activity intervention on plasma fatty acid composition and estimated desaturase and elongase activities in children: the Physical Activity and Nutrition in Children Study', *Am J Clin Nutr,* 104(4), pp. 964-972.

Viitasalo, A., Eloranta, A. M., Lintu, N., Vaisto, J., Venalainen, T., Kiiskinen, S., Karjalainen, P., Peltola, J., Lampinen, E. K., Haapala, E. A., Paananen, J., Schwab, U., Lindi, V. and Lakka, T. A. (2016) 'The effects of a 2-year individualized and family-based lifestyle intervention on physical activity, sedentary behavior and diet in children', *Prev Med,* 87, pp. 81-88.

Vlachopoulos, D., Barker, A. R., Ubago-Guisado, E., Williams, C. A. and Gracia-Marco, L. (2018) 'The effect of a high-impact jumping intervention on bone mass, bone stiffness and fitness parameters in adolescent athletes', *Arch Osteoporos,* 13(1), pp. 128.

Vlachopoulos, D., Barker, A. R., Williams, C. A., SA, A. R., Knapp, K. M., Metcalf, B. S., Fatouros, I. G., Moreno, L. A. and Gracia-Marco, L. (2017) 'The Impact of Sport Participation on Bone Mass and Geometry in Male Adolescents', *Med Sci Sports Exerc,* 49(2), pp. 317-326.

Wallace, I. J., Demes, B., Mongle, C., Pearson, O. M., Polk, J. D. and Lieberman, D. E. (2014) 'Exercise-Induced Bone Formation Is Poorly Linked to Local Strain Magnitude in the Sheep Tibia', *PLOS ONE,* 9(6), pp. e99108.

Ward, R. C., Janz, K. F., Letuchy, E. M., Peterson, C. and Levy, S. M. (2019) 'Contribution of high school sport participation to young adult bone strength', *Med Sci Sports Exerc,* 51(5), pp. 1064-1072.

Warden, S. J., Bogenschutz, E. D., Smith, H. D. and Gutierrez, A. R. (2009) 'Throwing induces substantial torsional adaptation within the midshaft humerus of male baseball players', *Bone,* 45(5), pp. 931-941.

Weaver, C. M., Gordon, C. M., Janz, K. F., Kalkwarf, H. J., Lappe, J. M., Lewis, R., O'Karma, M., Wallace, T. C. and 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(4), pp. 1281-1386.

Weber, D. R., Moore, R. H., Leonard, M. B. and Zemel, B. S. (2013) 'Fat and lean BMI reference curves in children and adolescents and their utility in identifying excess adiposity compared with BMI and percentage body fat', *Am J Clin Nutr,* 98(1), pp. 49-56.

Weeks, B. K. and Beck, B. R. (2008) 'The BPAQ: a bone-specific physical activity assessment instrument', *Osteoporos Int,* 19(11), pp. 1567-1577.

Wells, J. C. (2003) 'Body composition in childhood: effects of normal growth and disease', *Proc Nutr Soc,* 62(2), pp. 521-8.

Welt, C. K., Chan, J. L., Bullen, J., Murphy, R., Smith, P., DePaoli, A. M., Karalis, A. and Mantzoros, C. S. (2004) 'Recombinant human leptin in women with hypothalamic amenorrhea', *N Engl J Med,* 351(10), pp. 987-97.

Willers, C., Norton, N., Harvey, N. C., Jacobson, T., Johansson, H., Lorentzon, M., McCloskey, E. V., Borgström, F., Kanis, J. A. and the Scope review panel of the I.O.F. (2022) 'Osteoporosis in Europe: a compendium of country-specific reports', *Arch Osteoporos,* 17(1), pp. 23.

Williams, G. A., Wang, Y., Callon, K. E., Watson, M., Lin, J.-m., Lam, J. B. B., Costa, J. L., Orpe, A., Broom, N., Naot, D., Reid, I. R. and Cornish, J. (2009) 'In Vitro and in Vivo Effects of Adiponectin on Bone', *Endocrinology,* 150(8), pp. 3603-3610.

Winzenberg, T., Powell, S., Shaw, K. A. and Jones, G. (2011) 'Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis', *Br Med J,* 342, pp. c7254.

Wolff, J., Maquet, P. G. J. and Furlong, R. (1986) *The law of bone remodelling.* Springer Berlin / Heidelberg.

World Health Organisation (1994) *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis*, Geneva, Switzerland. Available at: http://apps.who.int/iris/bitstream/handle/10665/39142/WHO_TRS_843_eng.pdf;jsessionid=802855634473E8B6DD24B9C4A252A420?sequence=1.

Wren, T. A., Liu, X., Pitukcheewanont, P. and Gilsanz, V. (2005) 'Bone acquisition in healthy children and adolescents: comparisons of dual-energy x-ray absorptiometry and computed tomography measures', *J Clin Endocrinol Metab,* 90(4), pp. 1925-8.

Xiang, J., Chen, Y., Wang, Y., Su, S., Wang, X., Xie, B., Zhang, Q. and Liu, M. (2017) 'Lean Mass and Fat Mass as Mediators of the Relationship Between Physical Activity and Bone Mineral Density in Postmenopausal Women', *J Women's Health (Larchmt),* 26(5), pp. 461-466.

Yang, P.-F., Sanno, M., Ganse, B., Koy, T., Brüggemann, G.-P., Müller, L. P. and Rittweger, J. (2014) 'Torsion and Antero-Posterior Bending in the In Vivo Human Tibia Loading Regimes during Walking and Running', *PLOS ONE,* 9(4), pp. e94525.

Yang, Y., Wu, F., Winzenberg, T. and Jones, G. (2018) 'Tracking of Areal Bone Mineral Density From Age Eight to Young Adulthood and Factors Associated With Deviation From Tracking: A 17-Year Prospective Cohort Study', *J Bone Miner Res,* 33(5), pp. 832-839.

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. and 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(10), pp. 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. and 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(3), pp. 1265-1273.

Zerwekh, J. E., Ruml, L. A., Gottschalk, F. and Pak, C. Y. (1998) 'The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects', *J Bone Miner Res,* 13(10), pp. 1594-601.

Zymbal, V., Baptista, F., Letuchy, E. M., Janz, K. F. and Levy, S. M. (2019) 'Mediating effect of muscle on the relationship of physical activity and bone', *Med Sci Sports Exerc,* 51(1), pp. 202-210.