

1 **STUDY PROTOCOL**

Open Access

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

5 Dimitris Vlachopoulos¹, Alan R Barker¹, Craig A Williams¹, Karen M Knapp^{3,4}, Brad S Metcalf^{1,4}
6 and Luis Gracia-Marco^{1,2*}

11 **Abstract**

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

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

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

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

* Correspondence: l.a.gracia-marco@exeter.ac.uk

¹Children's Health and Exercise Research Centre. Sport and Health Sciences,
University of Exeter, Exeter, UK

²Growth, Exercise, Nutrition and Development Research Group, University of
Zaragoza, Zaragoza, Spain

Full list of author information is available at the end of the article

36 Background

37 Osteoporosis is a common skeletal disease associated with
38 high morbidity and mortality [1]. Approximately 2.7 mil-
39 lion European men and women suffer an osteoporotic
40 fracture every year [2]. The economic burden of osteopor-
41 osis in Europe is higher than most types of cancer (except
42 lung cancer), or chronic cardiorespiratory diseases [2,3]
43 and represents a direct annual cost of ~€31.7 billion to
44 health care and social services [1]. In order to improve the
45 outcome for osteoporosis, primary prevention remains the
46 most important policy action in public health. Although
47 contested [4], it is generally accepted that acquiring a high
48 bone mass during childhood and adolescence is a key de-
49 terminant of adult skeletal health [5-7]. Approximately
50 60% of osteoporotic cases in adult life are related to low
51 bone mineral content (BMC) in adolescence with up to
52 50% of total body (TB) bone mass achieved during this
53 period of life [8,9]. Peak bone mass attainment typically
54 occurs between the second and third decade of life, with
55 80-90% acquired by late adolescence, although this is skel-
56 etal site dependent [6,10]. Although bone mass is ~60-
57 80% genetically determined [11], there are other factors
58 strongly related with bone mass development. Environ-
59 mental and lifestyle factors such as physical activity (PA)
60 [12] and nutrition, i.e. calcium intake [13] and vitamin D
61 [14], are known to have important osteogenic effects and
62 have been the key focus in several interventions.

63 Exercise as a tool to improve bone health

64 Exercise has been proposed as a key factor for develop-
65 ing healthy bones in childhood and adolescence [15,16],
66 mainly when high-impact and weight-bearing PA occurs
67 [15] above a certain intensity and duration [15,17,18].
68 Longitudinal studies have shown that habitual PA is
69 positively associated with bone health in children and
70 adolescents because of its impact on bone development
71 [19,20]. The long-term positive effects of PA during ado-
72 lescence remain into young adulthood with active males
73 aged 24.2 years having 8 and 10% higher BMC at TB
74 and femoral neck (FN) respectively compared to non-
75 active peers, even when adjusted for maturation and size
76 [21]. Research conducted on former professional football
77 players showed that exercise is not only an important
78 factor in the accretion of, but also in the maintenance,
79 of bone mineral density (BMD) [22]. It has been shown
80 that moderate and readily accessible weight-bearing ex-
81 ercise before puberty may increase femoral volumetric
82 BMC, by increasing cortical thickness, and therefore
83 bone strength [23]. In addition, bone development is
84 dependent on the impact of mechanical load and pro-
85 cesses that trigger bone modelling and remodelling [24],
86 and possibly on structural adaptations related with tra-
87 becular microarchitecture [25].

Sport participation and bone health

88 It has been shown that sport participation is crucial for
89 healthy bone development, however not all sports have a
90 positive influence on the skeletal mass. According to
91 their characteristics, sports can be described as osteo-
92 genic (weight-bearing exercise) and non-osteogenic (non
93 weight-bearing exercise). Apart from numerous health
94 benefits [26], football is considered as an osteogenic
95 sport both in childhood and adolescence as bone mass is
96 augmented [27-30]. In contrast, sports such as cycling
97 [31-40] or swimming [41-46] are associated with no
98 change or a reduction in bone mass when compared to
99 controls. This could be a barrier for obtaining a high
100 peak bone mass which may compromise future bone
101 health [40,41,46,47].
102

Plyometric exercise intervention to increase bone health

103 To achieve the benefits of exercise and gain acceptance,
104 PA models must be effective, simple to administer, fea-
105 sible, inexpensive, short in duration and possible to per-
106 form at any location (i.e. at home, at the sports centre).
107 Plyometric jump training (PJT) may be a judicious choice
108 and experimental studies using animal models have re-
109 peatedly shown that short, discrete bouts of exercise inter-
110 spersed with rest periods is more effective than a single
111 longer bout of exercise for improving bone mass and
112 strength [48].
113

114 Research in early puberty has shown that a novel and eas-
115 ily implemented 8-month PJT (Bounce at the Bell; ~3 min/
116 day) enhanced bone mass at the weight bearing prox-
117 imal femur [49]. Mackelvie et al. showed that a 7-month
118 jumping intervention (10 min, 3 times/week) was asso-
119 ciated with more bone at the FN and lumbar spine (LS)
120 in early pubertal girls [50], and these results were main-
121 tained after 2 years [51]. In addition, prepubertal Asian
122 and Caucasian boys of average or low body mass index
123 (BMI) augmented bone mineral accrual at several re-
124 gions after a 7-month jumping intervention (10 min, 3
125 times/week). However, there are a lack of studies analys-
126 ing the effect of PJT in adolescent population, which is
127 crucial as adolescence is the period associated with the
128 greatest increments in BMC and BMD [52]. In addition,
129 this has not been studied in adolescents engaged in dif-
130 ferent sports (osteogenic vs. non osteogenic), which is
131 important to examine if peak bone mass during adoles-
132 cence may be maximized and therefore reduce the risk
133 for developing osteoporosis in adulthood.

Bone turnover markers and vitamin D

134 Bone development depends on its metabolic activity,
135 which includes bone formation, resorption and, as a
136 consequence bone turnover [53]. The relationship of PA
137 and sport participation with bone metabolism markers
138 has been shown previously in adolescents [54,55]. An
139

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

145 The role of vitamin D in bone metabolism is important
146 due to contribution of vitamin D in calcium homeostasis
147 and bone mineralization processes during growth. Evidence
148 shows that adequate vitamin D levels are necessary to ac-
149 quire bone mass and interact with exercise to enhance bone
150 growth [57,58]. The magnitude of the benefits in boys and
151 girls differ at sites of the skeleton and may depend on the
152 baseline levels of vitamin D and on previous loading experi-
153 ence [59]. The positive interaction of PA and vitamin D on
154 BMD in adolescents has been described [60,61] however
155 the association between vitamin D with osteogenic and
156 non-osteogenic sports has not been justified.

157 Objectives

158 The objectives of the PRO-BONE study are: 1) to longi-
159 tudinally assess, over 3 years, bone health and its me-
160 tabolism in adolescents engaged in osteogenic (football)
161 and non-osteogenic (cycling and swimming) sports, and
162 2) after 12 months of sport participation to examine
163 whether a short and inexpensive 9 months PJT inter-
164 vention programme is positively associated with bone-
165 related variables and its metabolism in adolescent foot-
166 ballers, cyclists and swimmers.

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

173 Methods/Design

174 Study design

175 PRO-BONE is a longitudinal design and involves four
176 cohorts of males aged 12–14 years at the beginning of
177 the study. These four cohorts consist of footballers, cy-
178 clists, swimmers and controls that will be followed over
179 a period of 33 months. The timeline of the PRO-BONE
F1 180 study can be seen in Figure 1.

181 Sample size

182 The sample size has been calculated according to the
183 primary interest variable, TB BMD (of cyclists (aged
184 15.5 years) [39] in order to achieve 90% of statistical
185 power to detect differences in the contrast of the null
186 hypothesis $H_0: \mu_1 = \mu_2$ through bilateral Student *t*, dif-
187 ference between two dependent means (matched pairs).
188 Taking into account a significance level of 5% and as-
189 suming that the mean of the reference group 1.133 units
190 (SD = 0.127) and the mean of the experimental group is

1.002 units (SD = 0.093), it will be necessary to include 9 191
units in the reference group and 9 units in the experi- 192
mental group, totalling 18 units. It is known that the 193
number of participants to recruit depends also on poten- 194
tial withdrawals [or could use drop-outs]: $n' = n/(1-p)$, so 195
that if the withdrawals were 40% the number of partici- 196
pants to be recruited would be $n' = 9/(1-0.4) = 15$ in 197
each group (e.g. 15 INT cyclists + 15 CON cyclists = 30 198
cyclists). Therefore, cyclists (n = 30), footballers (n = 30), 199
swimmers (n = 30) and controls (n = 15) will be re- 200
cruited, yielding a total N = 105. 201

202 Recruitment of the participants

203 Participants and parents/guardians will be contacted via 203
advert flyers, posters and social media to participate in 204
this study and by contacting sport clubs and schools 205
from the South West of England. Where possible, a 206
meeting will be held to explain the project as well as to 207
answer any questions. At the end of this meeting, con- 208
sent/assent forms and information sheets will be given 209
out and participants and parents/guardians will have 210
15 days to return the consent/assent forms. After these 211
15 days, a reminder (phone call or email) will be pro- 212
vided to those not sending the consent/assent to check 213
if they wish to participate. Seven more days will be given 214
to those that agreed to participate and in the 2nd re- 215
minder, they will be asked to send the interest and 216
consent/assent forms signed. 217

218 Participants will be screened for eligibility, based on 218
the inclusion/exclusion criteria outlined below, by a 219
member of the research team depending on the informa- 220
tion provided in the interest form. If eligible, the base- 221
line assessment will be scheduled for the participant. All 222
participants and parents involved in this project will be 223
carefully informed about the risks and benefits of the 224
study and will be required to sign the approved assent 225
and consent forms before their visit to the laboratory at 226
the Children's Health and Exercise Research Centre 227
(CHERC, University of Exeter). 228

229 Inclusion and exclusion criteria

230 Inclusion criteria include: 1) Males 12–14 years old, en- 230
gaged (≥ 3 h/week) in osteogenic (football) and/or non- 231
osteogenic (swimming and cycling) sports in the last 232
3 years or more; 2) Male adolescents 12–14 years old 233
not engaged in any of these sports (≥ 3 h/week) in the 234
last 3 or more years (control group). 235

236 Exclusion criteria include: 1) participation in another 236
clinical trial; 2) any acute infection lasting until < 1 week 237
before inclusion; 3) medical history of diseases or medi- 238
cations affecting bone metabolism or the presence of an 239
injury (before inclusion) that may affect participation in 240
their respective sports and/or any variable considered in 241
the present study (i.e. doing the PJT); 4) non-Caucasian 242

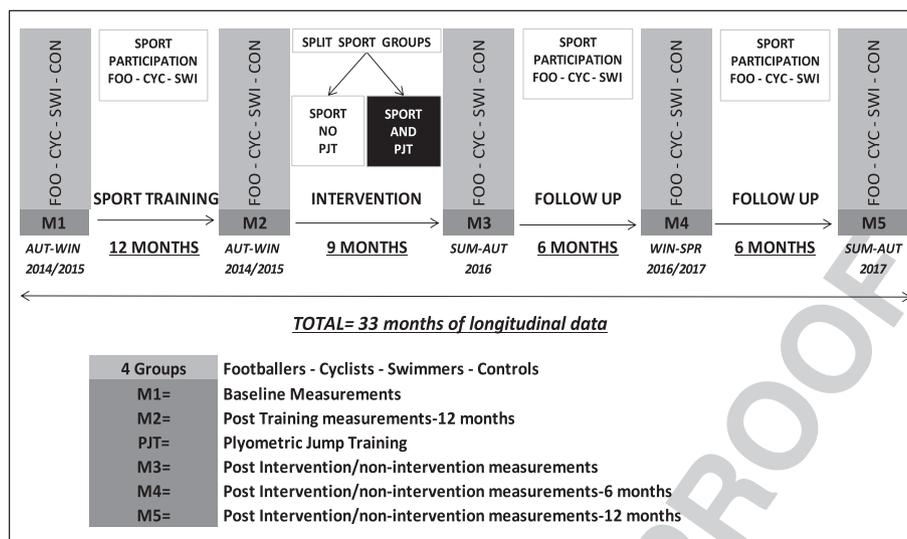


Figure 1 PRO-BONE timeline. FOO: Football players; CYC: Cyclists; SWI: Swimmers; PJT: Plyometric Jump Training.

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

247 **Ethics approval**

248 The methods and procedures of the PRO-BONE study
 249 have been checked and approved by: the Ethics Review
 250 Sector of Directorate-General of Research (European
 251 Commission, ref. number 618496), the Sport and Health
 252 Sciences Ethics Committee (University of Exeter, ref. num-
 253 ber 2014/766) and the National Research Ethics Service
 254 Committee (NRES Committee South West – Cornwall &
 255 Plymouth, ref. number 14/SW/0060). All data and informa-
 256 tion obtained will be confidential and access to database
 257 will be restricted to the researchers of the study. All mea-
 258 surements will be carried out by qualified and experienced
 259 researchers that will undergo a Disclosure and Barring
 260 Service check for approval to work with young people.

261 **Study protocol and measurements**

262 **Body composition**

263 **Anthropometry** Stature (cm), seated height (cm) and
 264 body mass (kg) will be measured by using a stadiometer
 265 (Harpenden, Holtain Ltd, Crymych, UK; precision
 266 0.1 cm; range 60–210 cm), a sitting height table
 267 (Harpenden, Holtain Ltd., Crymych, UK; precision 0.1 cm;
 268 range 32–109 cm) and an electronic scale (Seca 877, Seca
 269 Ltd, Birmingham, UK; precision 100 g; range 2–200 kg)
 270 respectively. Body mass index (BMI) will be calculated as
 271 body mass (kg) divided by the height (m) squared.

272 Waist circumference will measured at the midpoint
 273 between the lowest rib cage and the top of the iliac crest.
 274 Hip circumference will be measured around the widest

275 portion of the buttocks. All measurements will be 275
 276 undertaken by the same trained researcher using the 276
 277 type Seca 201 measuring tape (Seca Ltd, Birmingham, 277
 278 UK; precision 0.1 cm; range 0–205 cm). All anthropo- 278
 279 metrical measurements will be performed three times 279
 280 and the mean will be calculated. Pubertal maturation 280
 281 will be self-reported by the participants during each visit 281
 282 using adapted drawings of the five stages (Tanner) of pu- 282
 283 bertal hair development [63]. 283

284 **Dual-energy x-ray absorptiometry** Dual-energy x-ray 284
 285 absorptiometry scanner (GE Lunar Healthcare Corp., 285
 286 Madison, WI, USA) will be used to scan participants at 286
 287 four sites due to the evidence of site specific impact of 287
 288 sports participation [64–66]: 1) LS (mean of L1-L4), 2) 288
 289 right hip, 3) left hip, 4) TB. The DXA equipment will be 289
 290 calibrated at the start of each testing day by using a LS 290
 291 phantom as recommended by the manufacturer. The 291
 292 body will be segmented in accordance to standard pro- 292
 293 cedures to evaluate regional bone mass and fat distribu- 293
 294 tion. The scan modes will be automatically selected by 294
 295 the scanner software (standard or thick). All DXA scans 295
 296 and analyses will be performed using the GE enCORE 296
 297 software (2006, version 14.10.022). 297

298 Participants will be asked to remain still and they will be 298
 299 scanned in the supine position. The BMC (g) and BMD 299
 300 (g/cm²) with aged-matched Z-scores and age-matched % 300
 301 will be obtained. For LS regions area (cm²), width (cm) 301
 302 and height (cm) will be recorded and for TB regions, fat 302
 303 mass (g), lean mass (kg) and body fat (% and kg) will be 303
 304 obtained. Information about hip strength index, fat mass 304
 305 ratios (trunk/total, legs/total, arms and legs/trunk), an- 305
 306 droid and gynoid regions will also be obtained and have 306
 307 been previously validated in adolescents [67]. 307

308 This technique uses ionizing radiation that raises eth- 360
309 ical issues particularly for child participants. However, 361
310 this technique uses a minimal radiation dose (similar to 362
311 spending a day outside in the sunshine), and has been 363
312 widely used for research purposes with child participants 364
313 worldwide. The estimated lifetime risks of using GE 365
314 Lunar Prodigy DXA measurements in the paediatric 366
315 population was found to be negligible [68]. 367

316 **Air displacement plethysmography** Body volume will 368
317 be measured with BodPod (Body Composition System, 369
318 Life Measurement Instruments, Concord, California, 370
319 USA) as it can effectively predict visceral adipose tissue 371
320 in children [69] and determine the changes of body fat 372
321 percentage over time [70]. Two measurements will be 373
322 performed and if there is a difference of more than 374
323 150 mL in body volume, a third measurement will be 375
324 taken. The equipment will be calibrated at the com- 376
325 mencement of each testing day following the manufac- 377
326 turer's guidelines and using a cylinder of specific volume 378
327 (49.887 L). Participants will wear clothing according to 379
328 the manufacturer's recommendation (a swimsuit and a 380
329 swim cap) to rule out air trapped in clothes and hair. 381
330 Participants will be weighed on the BodPod calibrated 382
331 digital scale and then will enter into the BodPod cham- 383
332 ber. During the measurements participants will be asked 384
333 to remain in a seated position and to breathe normally. 385
334 A mean value for body volume will be obtained follow- 386
335 ing the manufacturer's recommendations [71] and this 387
336 value will be integrated into the calculation of lung vol- 388
337 ume. Percentage of TB fat mass will be calculated using 389
338 the equation reported by Siri [72,73]. 390

339 **Imaging bone ultrasonometer** Qualitative ultrasound 391
340 measurements will be performed with a Lunar Achilles 392
341 Insight and the OsteoReport PC software version 5.x + 393
342 (TM Insight GE Healthcare, Milwaukee, WI, USA). This 394
343 portable device measures bone stiffness using ultrasound 395
344 waves and is considered a valid and radiation-free 396
345 method to assess bone health in children [74,75]. The 397
346 same device will be used throughout the study and cali- 398
347 bration will be carried out prior to each visit. A standard 399
348 procedure will be followed according to manufacturer's 400
349 instructions. Participants will be placed on a stable chair 401
350 in a comfortable position directly in front of the Achilles 402
351 device. The position of the leg will rest lightly against 403
352 the calf support so the foot, calf and thigh are aligned 404
353 with the centre of the calf support and the positioner. 405

354 The qualitative ultrasound device provides three out- 406
355 come variables, the broadband ultrasound attenuation 407
356 (BUS), the speed of sound (SOS) and the stiffness index 408
357 (SI). The BUA indicates the absorption of sound waves 409
358 measured in decibels per megahertz. The SOS shows the 410
359 stiffness of a material by the ratio of the traversed 411

distance to the transit time, expressed in meters per sec- 360
ond. And the SI is calculated by a linear combination of 361
BUA and SOS: $SI = (0.67 \times BUA) + (0.28 \times SOS) - 420$. 362
The real-time image of the calcaneus and the region of 363
interest ensure that the measurement is precise [74]. 364
Both feet will be measured twice and the mean of the 365
two measurements will be calculated and used for 366
statistical analyses. 367

Bioelectrical impedance analysis The portable BIA de- 368
vice (Tanita BF-350, Tokyo, Japan; range 2–200 kg; preci- 369
sion 100 g; body fat % range 1–75%; body fat% increments 370
0.1%) will estimate the percentage of body fat by using the 371
values of resistance and reactance. Participants will be 372
measured in a fasting state and will remove any metal ob- 373
jects and socks prior the measurements. They will be posi- 374
tioned on the posterior surface barefoot according to 375
manufacturer's instructions. Despite the reported predic- 376
tion measurement error, BIA is considered a practical 377
method to assess body fat in addition to DXA and BodPod 378
in adolescents [76,77]. 379

Biochemical markers and blood collection 380

The measurement of bone turnover markers, in addition 381
to the measurement of bone mass, is an interesting op- 382
tion to obtain a more dynamic picture of bone tissue, 383
with the advantage that can be repeated at short inter- 384
vals [78]. Therefore, the combination of both measures 385
(bone mass and bone metabolism) is essential to obtain 386
a better understanding on changes in the skeletal mass. 387
In this regard, the International Osteoporosis Founda- 388
tion and the International Federation of Clinical Chem- 389
istry recommended the use of serum procollagen type 1 390
aminoterminal propeptide (P1NP) and isomer of the 391
Carboxi-terminal telopeptide of type 1 collagen (CTX-1) 392
as markers for formation and resorption, respectively 393
[79]. The role of vitamin D in bone metabolism is im- 394
portant due to contribution of vitamin D in calcium 395
homeostasis and bone mineralization processes during 396
growth. Assessment of vitamin D levels can be achieved 397
by measuring the serum 25-hydroxyvitamin D [25(OH) 398
D] in the blood [80]. For the scope of the present study 399
25(OH)D will be analysed as it has been shown to inter- 400
act with PA to improve bone mass in adolescents [14]. 401

Blood samples will be collected between 8:00 am and 402
9:00 am following a 12-hour fast period. A research team 403
experienced in sampling techniques will collect capillary 404
blood samples (~1.2 mL) from a pre-warmed hand into 405
heparin fluoride coated microvettes (CB 300 tubes, 406
Sarstedt Ltd, Leicester, UK) that will be placed immedi- 407
ately on ice. The microvettes will be centrifuged at 1000 × 408
G per min for 15 minutes at 4°C and plasma will be sepa- 409
rated in Eppendorf tubes of at least 60 µL, 110 µL and 410
60 µL and stored at –80°C for future analysis of P1NP, 411

412 CTX-1 and 25(OH)D respectively. The CTX-1 and 25
413 (OH)D biochemical markers will be analysed by using
414 IDS-iSYS CrossLaps (Immunodiagnostic Systems Ltd,
415 UK) and total P1NP by using ELISA kit (MyBioSource,
416 San Diego, California, USA).

417 Physical fitness assessment

418 A battery of tests will be used to assess attributes of
419 physical fitness that may play an important role in the
420 development of skeletal mass and strength during
421 growth and maturation. Cardiorespiratory fitness (aer-
422 obic performance) will be estimated using the 20 m
423 shuttle run test [81], which has been shown to be both
424 reliable and valid in youth [82]. The participants will be
425 tested at the end of the day following a standardized
426 warm up. They will be asked to run between two lines
427 set 20 m apart by following the pace of the audio signals
428 produced from a CD player. The starting speed will be
429 $8.5 \text{ km}\cdot\text{h}^{-1}$ and will be increased by $0.5 \text{ km}\cdot\text{h}^{-1}$ each mi-
430 nute. The participants will be encouraged to continue
431 the test until they reach maximal effort. The test will
432 end when the participant fails to reach the line two con-
433 secutive times. The last completed shuttle will indicate
434 the score of the test.

435 The standing long jump test and the Abalakov jump
436 test will be performed at least half an hour before the
437 20 m shuttle run test and following a standardized warm
438 up and with 2 minutes rest between the two tests. The
439 starting position of the standing long jump test will be
440 exactly behind a line and with feet at shoulder's width
441 apart. Participants will be allowed to swing their arms
442 during the eccentric contraction phase and they will be
443 advised to jump as far as possible in order to land with
444 both feet in a non-slippery hard surface. The distance
445 (cm) will be measured between the starting line and the
446 participant's heels. Participants will perform the
447 Abalakov jump test on a jump mat (Probotics Inc.,
448 Huntsville, USA) after having received instructions as to
449 how much can they bend their knees and the position of
450 their arms, they will be asked to jump as high as possi-
451 ble. Then, they will be placed in a standing position
452 with their feet shoulder width apart at the jump mat.
453 For both muscular tests the participants will perform 1
454 familiarization effort and 2 maximal effort jumps. The
455 mean height and distance (in cm) of the maximal efforts
456 will be used as criterion of measure. The reliability of
457 both tests in adolescents was previously described and is
458 acceptable to be used in this population [83]. The order
459 of all the measurements in each testing day can be seen
F2 460 in Figure 2.

461 Physical activity measurements

462 PA will be measured using two different methods: 1)
463 International Physical Activity Questionnaire and 2) a

wrist accelerometer (GENEActiv, GENE, UK). The vali- 464
465 dity and reliability of the accelerometer and of the Inter-
466 national Physical Activity Questionnaire has been
467 established previously in children and adolescents
468 [84,85]. GENEActiv accelerometers are waterproof so
469 are valid for the swimmers too. Both methods will be
470 used in order to obtain more precise data as, for ex-
471 ample, accelerometers do not properly measure PA in
472 cyclists as bouts of activity are not detected [86]. A diary
473 to complement accelerometer data will be administered
474 to the participants to obtain additional information such
475 as calcium and protein intake.

Dietary assessment Assessment of dietary intakes of 476
477 calcium, vitamin D and milk will be completed by using
478 two non-consecutive 24-h dietary recall questionnaires.
479 CompEat Pro software (Nutrition systems, VIS Visual
480 Information Systems Ltd., UK) will be used for the analysis.

481 Jumping intervention

482 Following 12 months of sport specific training, the ran-
483 domisation process will start in each sport group and par-
484 ticipants will be divided into two sub-groups to perform a
485 PJT programme as follows: 1) intervention programme
486 groups, (sport + PJT) and 2) sport groups (sport only). It
487 has been shown that 7 to 9 month PJT programmes can
488 effectively improve BMC and/or BMD at different skeletal
489 sites in children and adolescents and to maintain the ben-
490 efits for 3 years after the intervention [52,87]. Therefore, a
491 progressive PJT (~10 min/day) will be performed by inter-
492 vention groups 3 to 4 times/week (depending on progres-
493 sion) as shown in Table 1. Before the intervention, trained
494 staff will ensure that participants fully understand and cor-
495 rectly execute the different jumps and a research assistant
496 will meet with the participants to observe, demonstrate
497 and review the jumps. Participants will be instructed to
498 perform a number of countermovement jumps (CMJ) and
499 squat jumps (SJ) on a hard surface. Jumps will be per-
500 formed before and after school and before going to bed.
501 The CMJ will be performed by bending the knees immedi-
502 ately prior to the jump. The CMJ activates the stretch-
503 shortening cycle in the muscles, resulting in greater power
504 production in the legs compared to a SJ. For the SJ partici-
505 pants will squat down until the knees are bent at 90 de-
506 grees, then they will immediately jump vertically as high
507 as possible, landing back on the ground on both feet sim-
508 ultaneously. For this technique, the participant starts from
509 a stationary semi-squatting position, or pauses at the lower
510 level of the squat before jumping upwards. This removes
511 the factor of the stretch-shortening cycle. The reliability
512 and validity of the CMJ and SJ has been previously re-
513 ported [88,89].

514 These jumps are associated with important ground re-
515 action forces, i.e. for a countermovement it is about 5

Testing day	Blood Collection	Body Composition			Physical Fitness Tests		
	Anthropometry	DXA (LS-FN-TB)	BodPod	Ultrasonometer/ BIA	Jump Height	Long Jump	20 m shuttle run test
Time	8:00 am-9:00 am	9:00 am-12:00 pm				12:30 pm	

Figure 2 Order of the measurements at each testing day. DXA, dual energy x-ray absorptiometry; LS, lumbar spine; FN, femoral neck; TB, total body; BIA, bioelectrical impedance analysis.

516 times body weight (BW), compared to 3.5 times BW for
 517 jumping jacks. Similarly, the highest rates of change in
 518 force are 493 times BW/s for the CMJ, as shown in an
 519 independent sample of boys and girls [90]. A diary will
 520 be used to record the number of jumps performed each
 521 day. Both the intensity and number of jumps will be in-
 522 creased progressively in 3 levels of 12 weeks each. Inten-
 523 sity will be modified using ankle weights (from 1 kg at
 524 level 1 to 2.5 kg at level 3). With this an increase in BW
 525 between 2 to 5 kg will be achieved. In this regard, it has
 526 been shown that adolescents with higher BMI have
 527 higher levels of bone mass, because of the higher lean
 528 mass that they develop as a consequence of their higher
 529 fat mass [91].

530 **Discussion**

531 PRO-BONE will assess the longitudinal impact of osteo-
 532 genic (football) and non-osteogenic (cycling and swim-
 533 ming) sports on bone development in adolescents aged
 534 12–14 years old. In addition, it will investigate whether a
 535 simple, feasible and inexpensive PJT programme can im-
 536 prove bone development and if the effects will be main-
 537 tained a year after finishing the PJT programme. Several
 538 investigations have been conducted in order to improve
 539 bone health through exercise, strength, jumping or even

540 combinations among them [92]. However, to achieve im-
 541 pact and gain acceptance, the intervention must be ef-
 542 fective, simple to administer, feasible, inexpensive, short
 543 in duration and possible to perform at any place [49].
 544 PRO-BONE has been designed to meet all these require-
 545 ments and follow-up its effects after the withdrawal of
 546 the intervention.

547 Previous research has shown that exercise is positively
 548 associated with bone health [93]. However, there are
 549 some sports that due to the impact generated at the
 550 skeletal sites may have a minimal or negative effect on
 551 BMC and BMD [40,56]. As recent data have shown,
 552 jump training is associated with increases in BMC and
 553 BMD and may play an important role in the prevention
 554 of osteoporosis [94]. It is well known that early preven-
 555 tion is the most effective tool, therefore, it is crucial to
 556 analyse the effect of PJT at an early stage (i.e. adoles-
 557 cence). In this sense, it is important to examine if PJT
 558 can counteract the potential negative consequences of
 559 non-osteogenic sports on bone health and if there is
 560 enough stimuli to increase BMC and BMD in adoles-
 561 cents engaged in osteogenic sports.

562 PRO-BONE will employ different and well known
 563 technological devices and methods such as DXA, BodPod,
 564 imaging bone ultrasonometer and triaxial accelerometers

t1.1 **Table 1 Plyometric jump training progression**

t1.2	Level	Exercise	Ankle weights (kg)	Repetitions	³ Sets/day (⁴ rest)	⁵ Trainings/week	Jumps/week	
t1.3	1	¹ CMJ	-	10	3	3	180	
t1.4		² SJ		10	3	3		
t1.5	Total level 1 (12 weeks)						180 × 12 =	2160
t1.6	2	CMJ	1	10	4	3	240	
t1.7		SJ	1	10	4	3		
t1.8	Total level 2 (12 weeks)						240 × 12 =	2880
t1.9	3	CMJ	2.5	10	4	4	320	
t1.10		SJ	2.5	10	4	4		
t1.11	Total level 3 (12 weeks)						320 × 12 =	3840
t1.12	Total intervention (36 weeks)							8880

t1.13 ¹Countermovement jump, ²Squat jump, ³1 set = 10 CMJ + 10 SJ, ⁴Rest between sets = 30 seconds.

t1.14 Rest between exercises = 1 minute, ⁵When 3 sets/day, jumps will be performed in the morning before going to school (1 set), after school (1 set) and before going
 t1.15 to bed (1 set). When 4 series, jumps will be performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1 set).

among others. In addition, the PJT will include a progression in intensity with ankle weights to maximize the potential to augment bone. PRO-BONE is timely as there is a lack of studies analysing the effects of PJT on bone health during the crucial this period of life. It represents a golden opportunity to measure how a simple, feasible and inexpensive PJT is associated with bone health in adolescents engaged in different sports. It will also show if the effect of this intervention differs between sports, expecting a greater effect in cyclists and swimmers than footballers. In addition, PRO-BONE will allow us to compare within each group and investigate changes in body composition in groups doing the PJT plus training vs groups only training. Finally, PRO-BONE will examine whether PJT has any additional effect on footballers. Football is considered one of the most osteogenic sports, but this type of intervention has not yet been studied.

582 Abbreviations

583 BMC: Bone mineral content; BMD: Bone mineral density; BMI: Body mass
584 index; 25(OH)D: 25-hydroxyvitamin D; BIA: Bioelectrical impedance analysis;
585 BodPod: Air displacement plethysmography; BW: Body weight; SOC: Football
586 players; SWI: Swimmers; CYC: Cyclists; CON: Control; DXA: Dual energy x-ray
587 absorptiometry; PJT: Plyometric jump training; CMJ: Counter movement
588 jump; SJ: Squad jump; P1NP: Procollagen type 1 aminoterminal propeptide;
589 CTX-1: Carboxi-terminal telopeptide of type I collagen; LS: Lumbar spine;
590 FN: Femoral neck; TB: Total body.

591 Competing interests

592 The authors declare that they have no competing interests.

593 Authors' contributions

594 LGM (principal investigator), ARB and CAW contributed to the draft of the
595 study. DV wrote the initial draft of the manuscript under the supervision of
596 LGM, ARB and CAW. BSM, KMK will contribute to perform the analysis of the
597 data obtained. All authors have read and approved this work.

598 Acknowledgements

599 The authors gratefully acknowledge the adolescents, parents and sport
600 coaches and schools who helped and participated in this study. The authors
601 also gratefully acknowledge the CHERC research team who helped to recruit
602 and measure the adolescents.

603 Funding

604 The research leading to these results has received funding from the
605 European Union Seventh Framework Programme ([FP7/2007-2013] under
606 grant agreement n°. PCIG13-GA-2013-618496.

607 Author details

608 ¹Children's Health and Exercise Research Centre. Sport and Health Sciences,
609 University of Exeter, Exeter, UK. ²Growth, Exercise, Nutrition and
610 Development Research Group, University of Zaragoza, Zaragoza, Spain.
611 ³Department of Medical Imaging, College of Engineering, Mathematics and
612 Physical Sciences, University of Exeter, Exeter, UK. ⁴University of Exeter
613 Medical School, Exeter, UK.

614 Received: 10 February 2015 Accepted: 12 March 2015

615

616 References

- 617 1. Kanis JA, Johnell O. Requirements for DXA for the management of
618 osteoporosis in Europe. *Osteoporos Int.* 2005;16(3):229–38.
- 619 2. Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster JY, Borgstrom F, et al.
620 European guidance for the diagnosis and management of osteoporosis in
621 postmenopausal women. *Osteoporos Int.* 2008;19(4):399–428.
3. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability
associated with osteoporotic fractures. *Osteoporos Int.* 2006;17(12):1726–33.
4. Gafni RI, Baron J. Childhood bone mass acquisition and peak bone mass
may not be important determinants of bone mass in late adulthood.
Pediatrics. 2007;119 Suppl 2:S131–6.
5. Rizzoli R, Bianchi ML, Garabedean M, McKay HA, Moreno LA. Maximizing
bone mineral mass gain during growth for the prevention of fractures in
the adolescents and the elderly. *Bone.* 2010;46(2):294–305.
6. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone
mineral accrual from 8 to 30 years of age: an estimation of peak bone mass.
J Bone Miner Res. 2011;26(8):1729–39.
7. Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children.
Arch Dis Child. 2005;90(4):373–8.
8. Perez-Lopez FR, Chedraui P, Cuadros-Lopez JL. Bone mass gain during
puberty and adolescence: deconstructing gender characteristics. *Curr Med
Chem.* 2010;17(5):453–66.
9. Baroncelli GI, Bertelloni S, Sodini F, Saggese G. Osteoporosis in children and
adolescents: etiology and management. *Paediatr Drugs.* 2005;7(5):295–323.
10. Henry YM, Fatayerji D, Eastell R. Attainment of peak bone mass at the
lumbar spine, femoral neck and radius in men and women: relative
contributions of bone size and volumetric bone mineral density.
*Osteoporosis international: a journal established as result of cooperation
between the European Foundation for Osteoporosis and the National
Osteoporosis Foundation of the USA.* 2004;15(4):263–73.
11. Zofkova I. Role of genetics in prediction of osteoporosis risk. *Vnitr Lek.*
2011;57(1):78–84.
12. Branca F, Valtuena S. Calcium, physical activity and bone health—building
bones for a stronger future. *Public Health Nutr.* 2001;4(1A):117–23.
13. Vicente-Rodriguez G, Ezquerro J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP,
Casajus JA, et al. Independent and combined effect of nutrition and exercise
on bone mass development. *J Bone Miner Metab.* 2008;26(5):416–24.
14. Valtuena J, Gracia-Marco L, Vicente-Rodriguez G, Gonzalez-Gross M, Huybrechts
I, Rey-Lopez JP, et al. Vitamin D status and physical activity interact to improve
bone mass in adolescents. *Osteoporos Int: The HELENA Study.* 2012.
15. Vicente-Rodriguez G. How does exercise affect bone development during
growth? *Sports Med.* 2006;36(7):561–9.
16. Boreham CA, McKay HA. Physical activity in childhood and bone health. *Br J
Sports Med.* 2011;45(11):877–9.
17. Gracia-Marco L, Moreno LA, Ortega FB, Leon F, Sioen I, Kafatos A, et al.
Levels of physical activity that predict optimal bone mass in adolescents
the HELENA study. *Am J Prev Med.* 2011;40(6):599–607.
18. Heinonen A. *Biomechanics*. In., 1st edn. Edited by Khan K, McKay H; Kannus
P et al.: Champaign (IL). Physical activity and bone health: Human Kinetics;
2001: 23–34.
19. Gustavsson A, Thorsen K, Nordstrom P. A 3-year longitudinal study of the
effect of physical activity on the accrual of bone mineral density in healthy
adolescent males. *Calcified Tissue Int.* 2003;73(2):108–14.
20. DA Bailey MH, Mirwald RL, Crocker PR, Faulkner RA. A six year longitudinal
study of the relationship of PA to BMAccrual in growing children.pdf>
J Bone Miner Res. 1999;14(10):1672–9.
21. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal
study of the relationship of physical activity to bone mineral accrual from
adolescence to young adulthood. *Bone.* 2008;43(6):1101–7.
22. Uzunca K, Birtane M, Durmus-Altun G, Ustun F. High bone mineral density
in loaded skeletal regions of former professional football (soccer) players:
what is the effect of time after active career? *Br J Sports Med.*
2005;39(3):154–7.
23. Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, et al. Moderate
exercise during growth in prepubertal boys: changes in bone mass, size,
volumetric density, and bone strength: a controlled prospective study.
J Bone Miner Res. 1998;13(12):1814–21.
24. Wolff I, van Croonenborg JJ, Kemper HC, Kostense PJ, Twisk JW. The effect
of exercise training programs on bone mass: a meta-analysis of published
controlled trials in pre- and postmenopausal women. *Osteoporos Int.*
1999;9(1):1–12.
25. Seeman E. An exercise in geometry. *J Bone Miner Res.* 2002;17(3):373–80.
26. Krustrup P, Dvorak J, Junge A, Bangsbo J. Executive summary: the health
and fitness benefits of regular participation in small-sided football games.
Scand J Med Sci Sports. 2010;20 Suppl 1:132–5.
27. Ara I, Vicente-Rodriguez G, Perez-Gomez J, Jimenez-Ramirez J, Serrano-
Sanchez JA, Dorado C, et al. Influence of extracurricular sport activities on

- 693 body composition and physical fitness in boys: a 3-year longitudinal study. 764
 694 Int J Obes (Lond). 2006;30(7):1062–71. 765
- 695 28. Vicente-Rodríguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, 766
 696 Calbet JA. High femoral bone mineral density accretion in prepubertal 767
 697 soccer players. Med Sci Sports Exerc. 2004;36(10):1789–95. 768
- 698 29. Krstrup P, Hansen PR, Andersen LJ, Jakobsen MD, Sundstrup E, Randers MB, 769
 699 et al. Long-term musculoskeletal and cardiac health effects of recreational 770
 700 football and running for premenopausal women. Scand J Med Sci Sports. 771
 701 2010;20 Suppl 1:58–71. 772
- 702 30. Calbet JA, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez LP. High femoral 773
 703 bone mineral content and density in male football (soccer) players. Med Sci 774
 704 Sports Exerc. 2001;33(10):1682–7. 775
- 705 31. Rico H, Revilla M, Villa LF, Gomez-Castresana F, Alvarez del Buergo M. Body 776
 706 composition in postpubertal boy cyclists. J Sports Med Phys Fitness. 777
 707 1993;33(3):278–81. 778
- 708 32. Stewart AD, Hannan J. Total and regional bone density in male runners, 779
 709 cyclists, and controls. Med Sci Sports Exerc. 2000;32(8):1373–7. 780
- 710 33. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody JN, Howman-Giles R. 781
 711 Bone mineral density in adolescent female athletes: relationship to exercise 782
 712 type and muscle strength. Med Sci Sports Exerc. 2002;34(2):286–94. 783
- 713 34. Duncan CS, Blimkie CJ, Kemp A, Higgs W, Cowell CT, Woodhead H, et al. 784
 714 Mid-femur geometry and biomechanical properties in 15- to 18-yr-old 785
 715 female athletes. Med Sci Sports Exerc. 2002;34(4):673–81. 786
- 716 35. Warner SE, Shaw JM, Dalsky GP. Bone mineral density of competitive male 787
 717 mountain and road cyclists. Bone. 2002;30(1):281–6. 788
- 718 36. Nichols JF, Palmer JE, Levy SS. Low bone mineral density in highly trained 789
 719 male master cyclists. Osteoporos Int. 2003;14(8):644–9. 790
- 720 37. Barry DW, Kohrt WM. BMD decreases over the course of a year in 791
 721 competitive male cyclists. J Bone Miner Res. 2008;23(4):484–91. 792
- 722 38. Rector RS, Rogers R, Ruebel M, Hinton PS. Participation in road cycling vs 793
 723 running is associated with lower bone mineral density in men. Metabolism. 794
 724 2008;57(2):226–32. 795
- 725 39. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. 796
 726 Bone related health status in adolescent cyclists. PLoS One. 2011;6(9):e24841. 797
- 727 40. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez 798
 728 G. Cycling and bone health: a systematic review. BMC Med. 2012;10:168. 799
- 729 41. Andreoli A, Celi M, Volpe SL, Sorge R, Tarantino U. Long-term effect of 800
 730 exercise on bone mineral density and body composition in post-menopausal 801
 731 ex-elite athletes: a retrospective study. Eur J Clin Nutr. 2012;66(1):69–74. 802
- 732 42. Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health 803
 733 during late adolescence: effects of an 8-month training program on bone 804
 734 geometry in female athletes. Joint Bone Spine. 2013;80(1):57–63. 805
- 735 43. Greenway KG, Walkley JW, Rich PA. Does long-term swimming participation 806
 736 have a deleterious effect on the adult female skeleton? Eur J Appl Physiol. 807
 737 2012;112(9):3217–25. 808
- 738 44. Ferry B, Duclos M, Burt L, Therre P, Le Gall F, Jaffre C, et al. Bone geometry 809
 739 and strength adaptations to physical constraints inherent in different sports: 810
 740 comparison between elite female soccer players and swimmers. J Bone 811
 741 Miner Metab. 2011;29(3):342–51. 812
- 742 45. Dias Quiterio AL, Carnero EA, Baptista FM, Sardinha LB. Skeletal mass in 813
 743 adolescent male athletes and nonathletes: relationships with high-impact 814
 744 sports. J Strength Cond Res. 2011;25(12):3439–47. 815
- 745 46. Tenforde AS, Fredericson M. Influence of sports participation on bone 816
 746 health in the young athlete: a review of the literature. PM R. 2011;3(9):861–7. 817
- 747 47. Scofield KL, Hecht S. Bone health in endurance athletes: runners, cyclists, 818
 748 and swimmers. Curr Sports Med Rep. 2012;11(6):328–34. 819
- 749 48. Robling AG, Burr DB, Turner CH. Recovery periods restore mechanosensitivity 820
 750 to dynamically loaded bone. J Exp Biol. 2001;204(Pt 19):3389–99. 821
- 751 49. McKay HA, MacLean L, Petit M, MacKelvie-O'Brien K, Janssen P, Beck T, et al. 822
 752 "Bounce at the Bell": a novel program of short bouts of exercise improves 823
 753 proximal femur bone mass in early pubertal children. Br J Sports Med. 824
 754 2005;39(8):521–6. 825
- 755 50. Mackelvie KJ, McKay HA, Khan KM, Crocker PR. A school-based exercise 826
 756 intervention augments bone mineral accrual in early pubertal girls. J Pediatr. 827
 757 2001;139(4):501–8. 828
- 758 51. MacKelvie KJ, Khan KM, Petit MA, Janssen PA, McKay HA. A school-based 829
 759 exercise intervention elicits substantial bone health benefits: a 2-year 830
 760 randomized controlled trial in girls. Pediatrics. 2003;112(6 Pt 1):e447. 831
- 761 52. Witzke KA, Snow CM. Effects of plyometric jump training on bone mass in 832
 762 adolescent girls. Medicine and science in sports and exercise. 833
 763 2000;32(6):1051–7. 834
53. Jurimae J. Interpretation and application of bone turnover markers in 764
 children and adolescents. Curr Opin Pediatr. 2010;22(4):494–500. 765
54. Lima F, De Falco V, Baima J, Carazzato JG, Pereira RM. Effect of impact load 766
 and active load on bone metabolism and body composition of adolescent 767
 athletes. Med Sci Sports Exerc. 2001;33(8):1318–23. 768
55. Gracia-Marco L, Ortega FB, Jiménez-Pavón D, Rodríguez G, Valtueña J, 769
 Díaz-Martínez ÁE, et al. Contribution of bone turnover markers to bone 770
 mass in pubertal boys and girls. J Pediatr Endocrinol Metab. 2011;24:11–2. 771
56. Gomez-Bruton A, Gonzalez-Aguero A, Gomez-Cabello A, Casajus JA, Vicente- 772
 Rodriguez G. Is bone tissue really affected by swimming? a systematic 773
 review. PLoS One. 2013;8(8):e70119. 774
57. Stear SJ, Prentice A, Jones SC, Cole TJ. Effect of a calcium and exercise 775
 intervention on the bone mineral status of 16-18-y-old adolescent girls. 776
 Am J Clin Nutr. 2003;77(4):985–92. 777
58. Valtueña J, Gracia-Marco L, Vicente-Rodriguez G, Gonzalez-Gross M, 778
 Huybrechts I, Rey-Lopez JP, et al. Vitamin D status and physical activity interact to 779
 improve bone mass in adolescents The HELENA Study. Osteoporosis international 780
 : a journal established as result of cooperation between the European Foundation 781
 for Osteoporosis and the National Osteoporosis Foundation of the USA. 782
 2012;23(8):2227–37. 783
59. Specker B, Vukovich M. Evidence for an interaction between exercise and 784
 nutrition for improved bone health during growth. Med Sport Sci. 785
 2007;51:50–63. 786
60. Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. Best 787
 Pract Res Clin Endocrinol Metab. 2011;25(4):573–84. 788
61. Constantini NW, Dubnov-Raz G, Chodick G, Rozen GS, Giladi A, Ish-Shalom 789
 S. Physical activity and bone mineral density in adolescents with vitamin D 790
 deficiency. Med Sci Sport Exer. 2010;42(4):646–50. 791
62. Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral 792
 acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a 793
 longitudinal study. J ClinEndocrinol Metab. 1999;84(12):4702–12. 794
63. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, 795
 weight, height velocity, weight velocity, and stages of puberty. Arch Dis 796
 Child. 1976;51(3):170–9. 797
64. Ginty F, Rennie KL, Mills L, Stear S, Jones S, Prentice A. Positive, site-specific 798
 associations between bone mineral status, fitness, and time spent at 799
 high-impact activities in 16-to 18-year-old boys. Bone. 2005;36(1):101–10. 800
65. Magkos F, Kavouras SA, Yannakoulia M, Karipidou M, Sidossi S, Sidossis LS. 801
 The bone response to non-weight-bearing exercise is sport-, site-, and 802
 sex-specific. Clin J Sport Med. 2007;17(2):123–8. 803
66. Ishikawa S, Kim Y, Kang M, Morgan DW. Effects of weight-bearing exercise 804
 on bone health in girls: a meta-analysis. Sports Med. 2013;43(9):875–92. 805
67. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, El-Hajj Fuleihan G, Kecskemethy 806
 HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in 807
 children and adolescents: the revised 2013 ISCD Pediatric Official Positions. 808
 J Clin Densitom. 2014;17(2):225–42. 809
68. Damilakis J, Solomou G, Manios GE, Karantanas A. Pediatric radiation dose 810
 and risk from bone density measurements using a GE Lunar Prodigy 811
 scanner. Osteoporosis international: a journal established as result of 812
 cooperation between the European Foundation for Osteoporosis and the 813
 National Osteoporosis Foundation of the USA. 2013;24(7):2025–31. 814
69. Winsley RJ, Fulford J, MacLeod KM, Ramos-Ibanez N, Williams CA, Armstrong 815
 N. Prediction of visceral adipose tissue using air displacement plethysmogra- 816
 phy in children. Obes Res. 2005;13(12):2048–51. 817
70. Elberg J, McDuffie JR, Sebring NG, Salaita C, Keil M, Robotham D, et al. 818
 Comparison of methods to assess change in children's body composition. 819
 Am J Clin Nutr. 2004;80(1):64–9. 820
71. McCrory MA, Gomez TD, Bernauer EM, Mole PA. Evaluation of a new air 821
 displacement plethysmograph for measuring human body composition. 822
 Med Sci Sports Exerc. 1995;27(12):1686–91. 823
72. Siri WE. Body composition from fluid spaces and density: analysis of 824
 methods. In: Brozek J, Henschel A, editors. Techniques for measuring body 825
 composition. Washington, DC: National Academy of Sciences, National 826
 Research Council; 1961. p. 223–34. 827
73. Siri WE. Body composition from fluid spaces and density: analysis of 828
 methods. 1961. Nutrition. 1993;9(5):480–91. discussion 480, 492. 829
74. Jaworski M, Lebedzowski M, Lorenc RS, Trempe J. Ultrasound bone 830
 measurement in pediatric subjects. Calcif Tissue Int. 1995;56(5):368–71. 831
75. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral 832
 status in children: technical characteristics, performance, and clinical 833
 application. Pediatr Res. 2008;63(3):220–8. 834

- 835 76. Talma H, Chinapaw MJ, Bakker B, HiraSing RA, Terwee CB, Altenburg TM.
836 Bioelectrical impedance analysis to estimate body composition in children
837 and adolescents: a systematic review and evidence appraisal of validity,
838 responsiveness, reliability and measurement error. *Obesity reviews: an*
839 *official journal of the International Association for the Study of Obesity.*
840 2013;14(11):895–905.
- 841 77. Wu YT, Nielsen DH, Cassady SL, Cook JS, Janz KF, Hansen JR. Cross-validation of
842 bioelectrical impedance analysis of body composition in children and
843 adolescents. *Phys Ther.* 1993;73(5):320–8.
- 844 78. Jurimae J, Maestu J, Jurimae T. Bone turnover markers during pubertal
845 development: relationships with growth factors and adipocytokines. *Med*
846 *Sport Sci.* 2010;55:114–27.
- 847 79. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, et al.
848 International osteoporosis foundation and international federation of clinical
849 chemistry and laboratory medicine position on bone marker standards in
850 osteoporosis. *Clin Chem Lab Med.* 2011;49(8):1271–4.
- 851 80. Pérez-López F. Vitamin D and adolescent health. *Adolescent Health,*
852 *Medicine and Therapeutics.* 2010;1:1–8.
- 853 81. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle
854 run test for aerobic fitness. *J Sports Sci.* 1988;6(2):93–101.
- 855 82. Castro-Pinero J, Artero EG, Espana-Romero V, Ortega FB, Sjostrom M, Suni J,
856 et al. Criterion-related validity of field-based fitness tests in youth:
857 a systematic review. *Br J Sports Med.* 2010;44(13):934–43.
- 858 83. Ortega FB, Artero EG, Ruiz JR, Vicente-Rodriguez G, Bergman P, Hagstromer
859 M, et al. Reliability of health-related physical fitness tests in European
860 adolescents. The HELENA Study. *Int J Obes (Lond).* 2008;32 Suppl 5:S49–57.
- 861 84. Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEa accelerometer
862 for assessment of physical activity intensity in children. *J Sci Med Sport.*
863 2013;16(2):124–8.
- 864 85. Helmerhorst HJF, Brage S, Warren J, Besson H, Ekelund U: A systematic
865 review of reliability and objective criterion-related validity of physical activity
866 questionnaires. *Int J Behav Nutr Phy* 2012, 9
- 867 86. Slootmaker SM, Schuit AJ, Chinapaw MJ, Seidell JC, van Mechelen W.
868 Disagreement in physical activity assessed by accelerometer and self-report
869 in subgroups of age, gender, education and weight status. *Int J Behav Nutr*
870 *Phys Act.* 2009;6:17.
- 871 87. Gunter K, Baxter-Jones AD, Mirwald RL, Almstedt H, Fuller A, Durski S, et al.
872 Jump starting skeletal health: a 4-year longitudinal study assessing the
873 effects of jumping on skeletal development in pre and circum pubertal
874 children. *Bone.* 2008;42(4):710–8.
- 875 88. Acero RM, Fernandez-del Olmo M, Sanchez JA, Otero XL, Aguado X, Rodriguez
876 FA. Reliability of squat and countermovement jump tests in children 6 to
877 8 years of age. *Pediatr Exerc Sci.* 2011;23(1):151–60.
- 878 89. Markovic G, Dizdajic D, Jukic I, Cardinale M. Reliability and factorial validity of
879 squat and countermovement jump tests. *J Strength Cond Res.*
880 2004;18(3):551–5.
- 881 90. McKay H, Tsang G, Heinonen A, MacKelvie K, Sanderson D, Khan KM.
882 Ground reaction forces associated with an effective elementary school
883 based jumping intervention. *Br J Sports Med.* 2005;39(1):10–4.
- 884 91. Gracia-Marco L, Ortega FB, Jimenez Pavon D, Rodriguez G, Castillo MJ,
885 Vicente Rodriguez G, et al. Adiposity and bone health in Spanish
886 adolescents The HELENA study *Osteoporos Int.* 2012;23(3):937–47.
- 887 92. Guadalupe-Grau A, Perez-Gomez J, Olmedillas H, Chavarren J, Dorado C,
888 Santana A, et al. Strength training combined with plyometric jumps in
889 adults: sex differences in fat-bone axis adaptations. *J Appl Physiol.*
890 2009;106(4):1100–11.
- 891 93. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in
892 children and adolescents: a review of controlled trials. *Bone.* 2007;40(1):14–27.
- 893 94. MacKelvie KJ, McKay HA, Petit MA, Moran O, Khan KM. Bone mineral
894 response to a 7-month randomized controlled, school-based jumping
895 intervention in 121 prepubertal boys: associations with ethnicity and body
896 mass index. *J Bone Miner Res.* 2002;17(5):834–44.
- 897

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

