

**Please cite the Published Version**

Piasecki, Jessica, Ireland, A, Piasecki, M, Cameron, J, McPhee, J and Degens, Hans (2018) The strength of weight-bearing bones is similar in amenorrhoeic and eumenorrhoeic elite long-distance runners. *Scandinavian Journal of Medicine and Science in Sports*, 28 (5). pp. 1559-1568. ISSN 0905-7188

**DOI:** <https://doi.org/10.1111/sms.13062>

**Publisher:** Wiley

**Version:** Accepted Version

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17 **Abstract (246 words)**

18 **Background:** Regular intense endurance exercise can lead to amenorrhea with possible  
19 adverse consequences for bone health.

20 **Objective:** We compared whole-body and regional bone strength and skeletal muscle  
21 characteristics between amenorrheic (AA: n=14) and eumenorrheic (EA: n=15) elite adult  
22 female long distance runners and non-athletic controls (C: n=15).

23 **Study design and Participants:** Participants completed three-day food diaries, dual energy x-  
24 ray absorptiometry (DXA), magnetic resonance imaging (MRI), peripheral quantitative  
25 computed tomography (pQCT) and isometric maximal voluntary knee extension contraction  
26 (MVC).

27 **Results:** Both athlete groups had a higher caloric intake than controls, with no significant  
28 difference between athlete groups. DXA revealed lower bone mineral density (BMD) at the  
29 trunk, rib, pelvis and lumbar spine in the AA than EA and C. pQCT showed greater bone size  
30 in the radius and tibia in EA and AA than C. The radius and tibia of AA had a larger endocortical  
31 circumference than C. Tibia bone mass and moments of inertia (I<sub>x</sub> and I<sub>y</sub>) were greater in AA  
32 and EA than C, whereas in the radius only the proximal I<sub>y</sub> was larger in EA than C. Knee  
33 extensor MVC did not differ significantly between groups.

34 **Conclusions:** Amenorrheic adult female elite long-distance runners had lower BMD in the  
35 trunk, lumbar spine, ribs and pelvis than eumenorrheic athletes and controls. The radius and  
36 tibia bone size and strength indicators were similar in amenorrheic and eumenorrheic  
37 athletes, suggesting that long bones of the limbs differ in their response to amenorrhea from  
38 bones in the trunk.

39 **Key words:** eumenorrheic, amenorrheic, athletes, endocortical, periosteal, muscle.

40 **Introduction**

41 In elite endurance runners an appropriate balance between training, competition and  
42 recovery is important to maximise performance and prevent overtraining [1, 2]. When this  
43 balance is lost, injuries [2], such as stress fractures, caused by repeated stresses on the bone  
44 without appropriate recovery times can occur [1, 2].

45  
46 The mechanostat theory states that bone adapts to increased mechanical loading (impact  
47 exercise) by increasing bone mass, size and strength [3-5] while reduced mechanical  
48 deformation decreases [3] bone mass, size and strength. In line with the mechanostat theory,  
49 indicators of bone strength are 5-30% higher in post-pubertal athletes than non-athletes [5-  
50 9]. This suggests that physical activity is important for the development of high bone mass  
51 and strength, leading to 50-80% reduction in fracture risk [5].

52  
53 Oestrogen limits bone resorption by reducing osteoclast activity [10]. This may explain why a  
54 low concentration of oestrogen, occurring in the absence of menses [11], has a negative effect  
55 on bone mineral density (BMD) [12] and is associated with a greater risk of bone stress injuries  
56 [13-15]. The prevalence of 'athletic amenorrhea' or menstrual irregularities amongst active  
57 young women can be as high as 60% [14]. The associated low oestrogen levels can diminish,  
58 or negate, benefits of regular exercise on bone [6, 16, 17].

59  
60 Amenorrhoea is one of three features of the 'female athlete triad' that was originally defined  
61 in 1997 as a simultaneous occurrence of amenorrhea, inadequate food intake and high  
62 training volume [18] that all have a negative impact on bone health. Most studies that  
63 considered the effects of amenorrhea on bone used dual-energy x-ray absorptiometry (DXA)

64 (e.g. [16, 17]). Using DXA, higher BMD and strength indicators were found at the hip in  
65 eumenorrheic athletes than controls, while no such differences were seen between  
66 amenorrheic athletes and controls [16]. Something similar has been seen with high-  
67 resolution peripheral quantitative computerised tomography (HR-pQCT) [6, 7]. However, HR-  
68 pQCT does not give an indication of whole bone strength and cannot examine long bone shaft  
69 sites such as the tibia, which is particularly prone to stress fracture injury in athletes [19] but  
70 has received little attention in studies of amenorrheic athletes. Nevertheless, these studies  
71 suggest that there is a deficit in bone health in amenorrheic adolescent athletes and it is  
72 possible that symptoms are worse in adult elite level athletes due to a longer duration of  
73 amenorrhea than in adolescent athletes [20].

74

75 Reduced muscle mass, maximal force and quality (defined as maximal isometric force per unit  
76 muscle cross-sectional area) could be additional features of amenorrhea that impact on bone  
77 health due to a reduced mechanical stimulus to the bone [21]. It remains to be seen whether  
78 adult amenorrheic elite athletes have low muscle mass and/or quality of specific muscles  
79 associated with low strength in the bones these muscles act upon, and whether low bone  
80 strength is related to a low mass and/or quality of the muscles acting upon the corresponding  
81 bone. Such relationships can be examined using pQCT, along with imaging and dynamometry  
82 of muscle groups acting upon bone.

83

84 The aim of the present study was to examine the interrelationship of muscle and bone  
85 characteristics in female, adult elite-level endurance athletes affected by amenorrhea. The  
86 primary hypothesis was that amenorrheic athletes have lower indicators of bone strength  
87 than eumenorrheic athletes and controls in body segments with lower direct exposure to

88 weight-bearing impacts, whilst these indicators will be preserved in weight-bearing bones of  
89 the amenorrheic athlete.

90

91

## 92 **Materials and Methods**

### 93 *Participants*

94 Twenty-nine females, aged 17-42 years, were recruited after sending out a poster and  
95 participant information sheet to all athletes on an England Athletics email database. Of those  
96 that responded, only athletes that had represented their home country within the past two  
97 years in 1.5-10-km runs were eligible to participate and grouped according to their menstrual  
98 cycle history. All non-athletic controls were recruited from the local student population,  
99 performed less than 2 hours of physical activity per week and did not take part in athletic  
100 competitions. Participants were asked about the phase of menstrual cycle at the date of  
101 testing, use of oral contraceptive pills (OCP), any current medication, smoking habits, age of  
102 menarche and alcohol consumption. Based on self-reports, athletes were classified as  
103 amenorrheic (AA) if they had experienced an absence of menses for  $\geq 12$  months in a row  
104 within the past 12 months. None of the athletes had oligomenorrhea (4-9 cycles per year).  
105 Athletes with regular menstrual cycles ( $> 12$  in the past year) were classed as eumenorrheic  
106 (EA). Controls (C) had regular menstrual cycles, were recreationally active, but did not take  
107 part in competitive sports. As the study involves exposure to radiation during scanning any  
108 volunteers were excluded if they were pregnant or potentially pregnant. The Manchester  
109 Metropolitan University Ethics Committee approved the study and all participants gave  
110 written informed consent. Table 1 shows the participant characteristics.

111

112 *Experimental Protocol*

113 Sporting history was obtained by questionnaire. Participants completed a food diary on three  
114 consecutive days, specifying food and drink consumption. This was analysed using nutritional  
115 analysis software (Diet Plan 6 software, Forestfield Ltd, Horsham, UK and Nutritics software,  
116 Nutritics, Dublin, Ireland). Six food diaries were excluded (two from controls, one from the EA  
117 and three from the AA group) due to incomplete details for accurate analysis. The age-graded  
118 performance (AGP) for the main event was calculated using the World Master Association's  
119 Age-grading Calculator:

120 <http://www.howardgrubb.co.uk/athletics/wmalookup06.html>.

121

122 *DXA*

123 Scans (GE Medical, Lunar Prodigy Advance, version encore 10.50.086) were taken to  
124 determine whole body, lumbar spine (L1-4) and hip bone mineral density (BMD), and body  
125 fat and lean mass percentage. Geometric properties of the femoral neck were estimated using  
126 the advanced hip analysis (AHA) software (GE Medical, Lunar Prodigy Advance, version encore  
127 10.50.086). This calculated the cross-sectional area (CSA), the cross-sectional moment of  
128 inertia (CSMI: an index of structural rigidity), the width of the neck and shaft of the femur and  
129 the bone strength index, a ratio of estimated compressive yield strength of the femoral neck  
130 to an expected compressive strength of a fall onto the greater trochanter [17]. In our  
131 laboratory, the coefficient of variation for body, hip and lumbar spine scans (n=8) is 0.67%,  
132 2.02% and 0.9%, respectively.

133

134 *pQCT*

135 Scans were acquired at the non-dominant radius and dominant tibia with XCT-2000 and XCT-  
136 3000 pQCT scanners (Stratec Medizintechnik GmbH, Pforzheim, Germany) according to the  
137 manufacturer's protocols. Images obtained with the two scanners were cross-calibrated using  
138 functions derived from scans of different density regions within the same manufacturer-  
139 provided phantom on each scanner. The dominant arm was identified as the writing arm, and  
140 in any cases of ambidexterity, the dominant arm was defined as the favoured arm when  
141 playing racquet sports. The non-dominant leg was defined as the leg that was preferentially  
142 used for hopping. Scans were taken at 4 and 60% of the radius length, and 4 and 66% of the  
143 tibia length, where 0% indicates the most distal part of the bones. Radius length was  
144 measured between the olecranon process and the radial styloid process. Tibia length was the  
145 distance between the palpated medial knee joint cleft and medial malleolus.

146

147 Data were exported using the Automated Analysis Tools (Version 6.00). A peeling threshold  
148 of  $180 \text{ mg}\cdot\text{cm}^{-3}$  was applied to the epiphyseal slice. At the diaphyseal sites, a threshold of  $650$   
149  $\text{mg}\cdot\text{cm}^{-3}$  was used to separate cortical bone.

150

151 The following parameters examined in the 4% epiphyseal slice: total bone area ( $\text{Ar.tot}$ ,  $\text{mm}^2$ ),  
152 total bone mineral content ( $\text{vBMC.tot}$ ,  $\text{mg}\cdot\text{mm}^{-1}$ ) and trabecular bone mineral density  
153 ( $\text{vBMD.tb}$ ,  $\text{mg}\cdot\text{cm}^{-3}$ ). iaphyseal parameters examined were:  $\text{Ar.tot}$ ,  $\text{vBMC.tot}$ , cortical area  
154 ( $\text{Ar.ct}$ ,  $\text{mm}^2$ ), cortical density ( $\text{vBMD.ct}$ ,  $\text{mg}\cdot\text{cm}^{-3}$ ), cortical thickness ( $\text{Ct.Th}_{\text{der}}$  mm), periosteal  
155 ( $\text{PsC}$ , mm) and endocortical circumference ( $\text{EcC}$ , mm), antero-posterior ( $I_x$ ) and mediolateral  
156 ( $I_y$ ) moments of inertia representing bone bending stiffness. Cortical bone density values were  
157 corrected for the partial volume effect as described previously [22]. The coefficient of



158 variation of the pQCT measurements in our laboratory has been reported elsewhere [23] and  
159 was <0.5% for vBMC.tot, Ar.tot and Ar.ct.

160

### 161 *Magnetic Resonance Imaging (MRI)*

162 A 0.25-T G-scan MRI scanner (Esaote, Genova, Italy) was used to measure the volume of the  
163 quadriceps femoris and calf muscles. Serial cross sections (each 6.3 mm thick with a 50.4-mm  
164 inter-slice gap) were acquired from the lateral femoral condyle to the greater trochanter for  
165 the quadriceps and from the lateral femoral condyle to the lateral malleolus for the calf using  
166 a turbo 3-D T1 protocol [24]. Cross-sectional area was determined using Osirix software  
167 (Osirix medical imaging software, Atlanta, USA). The volumes of the muscle and femur bone  
168 were estimated as the integration of volume from each slice and inter-slice gap.

169

### 170 *Muscle strength measures*

171 Maximal voluntary isometric knee extensor torque of the quadriceps muscle was measured  
172 with a custom-built dynamometer [25]. Participants sat with hip and knee angles flexed at  
173 around 90° and straps fastened around the hip. Participants performed three maximum  
174 voluntary knee extension contractions, and the highest torque presented. Force was also  
175 expressed as force per quadriceps volume-

176

### 177 *Statistical Analysis*

178 Statistical analysis was performed on data normalised to object length or body height, to  
179 remove any variability caused by differences in these factors, with SPSSv19 (IBM, USA). Data  
180 was normally distributed as assessed using the Kolmogorov-Smirnov test. A one-way ANOVA  
181 was used to assess any significant differences between control, amenorrhoeic and

182 eumenorrhic athletes. To test whether the radius and the tibia showed the same differences  
183 from control in amenorrhic and eumenorrhic athletes we performed a repeated-measures  
184 ANOVA with bone as within-factor bone, and group as between-factor on the data of the bone  
185 parameters normalised to the corresponding average control values for each bone. If a main  
186 group effect was found, a post-hoc test with Bonferroni correction was performed to  
187 determine which groups differed from each other. There were no group\*bone interactions.  
188 Differences between groups were considered significant at  $p < 0.05$ . All data are presented as  
189 mean  $\pm$  standard error of the mean (SEM). All p-values shown in Tables 1-6 are those from  
190 post-hoc tests with Bonferroni correction.

191

192

## 193 **Results**

### 194 *Participants*

195 There were no significant differences between groups in age or height (Table 1). Body mass  
196 and BMI were lower in the athletes than the C ( $p < 0.05$ ). Body mass of EA was 10% higher than  
197 that of AA ( $p = 0.029$ ). Lean mass of EA, but not that of AA, was higher than C ( $p = 0.015$ ) and  
198 both athletic groups had lower absolute and percentage fat mass than C ( $p < 0.05$ ). The age-  
199 graded performance of EA and AA was within 15% of world record times, with no significant  
200 difference between the athlete groups. Onset of menarche was later in AA than C ( $p < 0.05$ ),  
201 with no significant differences between athlete groups or EA and C. Including the age of onset  
202 of menarche as a covariate did not change any statistical results and so was not included in  
203 final analysis (data not shown).

204

### 205 *Food Diaries*

206 Total daily energy ( $\text{kJ}\cdot\text{day}^{-1}$ ) intake was less in C than athlete groups (both  $p<0.05$ ; C;  
207  $6217\pm659$ , EA;  $10567\pm880$ , AA;  $9723\pm748$ ).

208

#### 209 *Muscle size and knee extensor strength*

210 Table 2 shows that there was no significant difference in forearm and tibia muscle cross-  
211 sectional area, and calf and quadriceps muscle volume between any groups. Both athlete  
212 groups had greater maximal voluntary knee extension torque than C ( $p<0.045$ ), (Table 2).  
213 Femur volume was higher in the athlete groups than C ( $p<0.05$ ), but did not differ significantly  
214 between EA and AA (Table 2).

215

216

#### 217 *DXA*

218 Total body, arms and hip BMD did not differ significantly between groups (Table 3). Trunk,  
219 rib, lumbar spine and pelvis BMD were lower in AA than EA and C (all  $p<0.05$ ). Leg BMD was  
220 significantly greater in EA than C ( $p<0.05$ ), with no significant difference between AA and C  
221 (Table 3).

222

223 Hip structure of the femurs was similar for both athlete groups (Table 4). Cortical width of the  
224 femur shaft was greater in both athletes than C ( $p<0.05$ ). There was no significant difference  
225 between any groups in the cortical width, cross-sectional area of the femur neck, bone  
226 strength index or cross-sectional moment of inertia.

227

#### 228 *pQCT*

229 Table 5 shows pQCT radius data. At the epiphyseal site the total bone area of the radius (Ar.tot)  
230 of both athlete groups was greater than C ( $p<0.05$ ). Total bone mineral content (vBMC.tot),  
231 trabecular bone mineral density (vBMD.tb) and bone strength index of the radius epiphysis  
232 showed no significant differences between groups.

233

234 At the diaphysis site of the radius, total area was larger in EA and AA than C( $p<0.004$ ), but  
235 there were no significant differences between groups in cortical bone mineral content and  
236 density (Table 5).

237

238 The periosteal circumference was larger in the athletes than the C ( $p\leq 0.01$ ; Figure 1A). The  
239 moment of inertia was significantly greater in EA than C in the y plane, but there was no  
240 significant difference between any groups in the x plane (Table 5).

241

242 Table 6 shows pQCT tibia data. Total bone mineral content for the epiphysis of the tibia was  
243 greater in EA than C ( $p<0.05$ ), with no significant difference between athlete groups or AA and  
244 C. Trabecular BMD and total area of the tibia epiphysis was greater in both athlete groups than  
245 C ( $p<0.05$ ), with no significant difference in bone strength index between groups.

246

247 Total area and total bone mineral content at the tibia diaphysis were larger in the AA and EA  
248 than C ( $p<0.05$ ). The trabecular BMD of the diaphysis was greater in C than AA ( $p=0.02$ ) and  
249 EA ( $p<0.0005$ ). The moment of inertia in the y- and x-plane at the tibia diaphysis was greater  
250 in the athletes than the C ( $p<0.05$ ; Table 6).

251

252 For the diaphysis of both the radius and the tibia the cortical thickness did not differ  
253 significantly between groups (Figure 1B), but the cortical area was larger in EA than C  
254 ( $p=0.005$ ; Figure 1C). The endocortical circumference (Figure 1D) was ~20% greater in AA than  
255 C ( $p=0.001$ ), with no significant difference between C and EA, or EA and AA. These changes  
256 are illustrated in figure 2.

257

## 258 **Discussion**

259 The main observations of the study are that amenorrheic adult female elite long-distance  
260 runners have a lower bone mineral density in the trunk, lumbar spine, ribs and pelvis than  
261 eumenorrheic athletes and controls. In contrast, tibia cortical bone strength indicators were  
262 greater in both athlete groups than controls but no such difference was seen in the radius.  
263 This suggests that long bones differ in their response to amenorrhea from bones in the trunk.  
264 Similar to eumenorrheic athletes, the amenorrheic athletes had a larger and stronger tibia  
265 and femur than controls indicating that the bone response to regular loading is not  
266 attenuated by amenorrhea. Yet, it is unlikely that loading can normalise bone remodelling in  
267 amenorrheic athletes entirely as both the unloaded radius and the loaded tibia exhibited an  
268 increase in endocortical circumference.

269

### 270 *Study participants*

271 The long-distance runners in the present study had represented their country at international  
272 athletic events. The average age-graded performance for both athlete groups was 85%; for a  
273 26-year-old female this equates to 35 mins for 10 km and 2 hours 40 mins for a marathon.  
274 This confirmed that the recruited athletes were indeed *elite* athletes. The athletes were

275 classified as amenorrheic if they self-reported an absence of menses for at least 12  
276 consecutive months in a row. In addition, none of the athletes were oligomenorrheic, the  
277 average duration of amenorrhea in the AA was 5.5 years and the EA athletes were on average  
278 12 years eumenorrheic, indicating that the EA and AA athletes represented distinct groups.  
279 The self-reported method to characterise amenorrhea is preferred to measurement of sex  
280 hormones, which are subject to fluctuations during the menstrual cycle and diurnal variations  
281 [26].

282

### 283 *Energy balance*

284 Persistent energy deficiency, occurring in up to 62% of elite female athletes, is considered an  
285 important cause of irregular or absent menstruation [18], both of which can lead to reduced  
286 bone health [20]. The common co-occurrence of amenorrhea and energy deficiency in  
287 athletes has made it difficult to disentangle the effects of amenorrhea and energy deficiency  
288 in previous studies [27]. In our study, the AA and EA reported similar total energy intake that  
289 exceeded that of the non-athletes by more than 30%, suggesting that energy deficit is unlikely  
290 to be the cause of bone differences between athletes and controls, or AA and EA, within our  
291 sample.

292

### 293 *Muscle mass and function*

294 According to the mechanostat theory [4], mechanical strain on bone, caused by muscle  
295 contraction, stimulates bone formation and increases bone strength [3, 4]. Effects of  
296 amenorrhea may thus be secondary to muscle weakness or a loss of muscle mass. We do not

297 think low muscle mass or weakness was a major consideration in our study because there  
298 were no significant differences in muscle mass and maximal strength between the  
299 eumenorrheic and amenorrheic athletes, although we did not determine the muscle forces  
300 during running and therefore cannot entirely rule out any differences between groups in the  
301 mechanical strain on bones during training.

302

### 303 *Non-weight-bearing bones*

304 The torso, lumbar spine, rib and hips of amenorrheic athletes had a lower BMD than those of  
305 the eumenorrheic athletes and controls. Bone area was also lower at these sites, and as a  
306 result amenorrheic athletes had large deficits in bone mineral content compared to the other  
307 two groups (data not shown). As these bones are not loaded during running, due to impact  
308 damping and limited direct contribution of the surrounding muscles to locomotion, it could  
309 be argued that the detrimental impact of amenorrhea on these bones is not compensated by  
310 the osteogenic effect of increased loading. Previous studies reported lower trabecular bone  
311 mineral density at the epiphysis of the radius in amenorrheic than eumenorrheic athletes and  
312 controls [6]. However in the current study it was observed that in contrast to the trunk  
313 skeleton, in the radius the bone mineral density was similar, and not less, in amenorrheic than  
314 eumenorrheic athletes and controls. Such a difference between bones in the response to  
315 amenorrhea has been observed previously; where bone mineral density was lower in the  
316 lumbar vertebrae, but not in the radius and the femur [28]. It has been suggested that the  
317 loss of bone mineral density in the lumbar vertebrae is due to loss of body mass rather than  
318 amenorrhea *per se* [29]. This indeed corresponds with the lower body mass of the  
319 amenorrheic athletes, but is at odds with the similar bone mineral density in the trunk

320 skeleton of eumenorrheic athletes and controls despite the lower body mass of the athletes.  
321 Also, in the radius, a lower body mass does not explain the absence of a lower bone mineral  
322 density in the the amenorrheic athletes. We speculate that the best explanation for the lower  
323 bone mineral density in the trunk skeleton, but maintained radius bone mineral density in  
324 amenorrheic athletes, is that long bones and the bones in the trunk respond differently to  
325 amenorrhea. Indeed, there are some indications in rat models that the responses to  
326 oestrogen on bone are site-specific [30], but this requires further investigation.

327

### 328 *Weight-bearing bones*

329 In the femur, bone CSA and the cortical width of the shaft were larger in both athlete groups  
330 than controls. This is consistent with previous observations [31] suggesting that the effects of  
331 loading are not attenuated in those with amenorrhea. Others have reported lower bone size  
332 and strength in amenorrheic compared to eumenorrheic athletes [32]. Part of the discrepancy  
333 may be related to the younger age of the athletes in previous studies. For instance, in one  
334 study the average age was 20 [33] and in another only 17 years [31], compared to the 26 years  
335 in our study, the age at which females have reached their maximum bone strength [34].

336

337 Although the tibia is a common stress fracture site in athletes, tibial diaphysis strength has  
338 been ignored in previous pQCT research involving amenorrheic and eumenorrheic athletes.  
339 In a monozygotic twin study it was found that regular physical activity resulted in an increase  
340 in BMD in the epiphysis of the tibia only [35]. This is similar to the larger BMD in the epiphysis,  
341 but not diaphysis, in the athletes than controls in our study and supports the notion that bone



342 adaptations to exercise may be site-specific [35]. Nevertheless, we found that bone size,  
343 strength and cortical bone area of the diaphysis was larger in athletes than controls, with no  
344 significant differences between amenorrheic and eumenorrheic athletes, except for the larger  
345 epiphyseal bone strength (indicated by total bone mass) over controls in eumenorrheic  
346 athletes only. This, similar to the observations in the femur, indicates that the effects of  
347 regular loading on bone [9, 36] are not attenuated by amenorrhea.

348

### 349 *Bone remodelling*

350 In both the radius and the tibia the endocortical circumference were larger in amenorrheic  
351 athletes than non-athletes, suggesting endocortical expansion (resorption) that could be  
352 attributable to their lack of oestrogen [37]. At the same time, both the radius and tibia had  
353 expanded. These findings are similar to that previously suggested by Mikkola et al [38], in that  
354 the effect of oestrogen is systemic with the tibia and radius being affected similarly. This effect  
355 also has some similarity to the decline in trabecular BMD [39] and increase in bone size [40]  
356 during pregnancy. This pregnancy-induced loss of BMD can be recovered during lactation  
357 when the child is weaned [39, 40] and if the underlying cause is similar, the expansion of the  
358 endocortical circumference in the amenorrheic athletes could most likely be recovered by  
359 normalisation of the menstrual cycle. In a study of monozygotic twins, hormone replacement  
360 therapy (HRT) was associated with larger cortical bone areas and smaller endocortical areas  
361 [38]. It is not known, however, if this would be effective in amenorrheic athletes as the  
362 duration of HRT in the twins study was on average 8 years. Although regular exercise was  
363 associated with a smaller endocortical area in monozygotic twins [35] it is unlikely that  
364 normalisation of the endocortical circumference in amenorrheic athletes can be realised by

365 increased loading, as both the unloaded radius and the loaded tibia exhibit this increase in  
366 endocortical circumference.

367

### 368 *Limitations*

369 It was not possible to include energy-deficient amenorrheic athletes in the current study,  
370 which may have offered further insights. However, this might equally be seen as a strength of  
371 our study because we were able to rule out the contribution of energy deficiency to our  
372 observations. Circulating levels of oestrogen were not measured which may have  
373 complemented the assessment of amenorrhea. However, oestrogen levels vary considerably  
374 during the menstrual cycle and diurnally, complicating distinction of eumonorrheic and  
375 amenorrheic athletes. Five of the athletes stated they were taking the oral contraceptive pill  
376 (OCP) for contraceptive reasons only. One AA who took OCP still suffered from amenorrhea  
377 and her bone parameters were all within the range of the group. The EA athletes all had  
378 regular cycles prior to using OCP and given these observations, we expect that OCP had no  
379 significant impact on our findings.

380

### 381 *Perspective*

382 The lower bone strength indicators in bones of the trunk but not the radius of amenorrheic  
383 athletes is not entirely explained by reduced loading, but rather suggests that the bone  
384 response to amenorrhea is site-specific. While the strength of weight bearing bones in the EA  
385 and AA are similar, the enlargement of the endocortical area, similar to that shown by Mikkola

386 et al [38], cannot be reversed by loading. We speculate that this can only be normalised by a  
387 return to a normal menstrual cycle.

388

389

## 390 **Acknowledgements**

391 We thank all the volunteers for participating in our study.

## 392 **References**

393

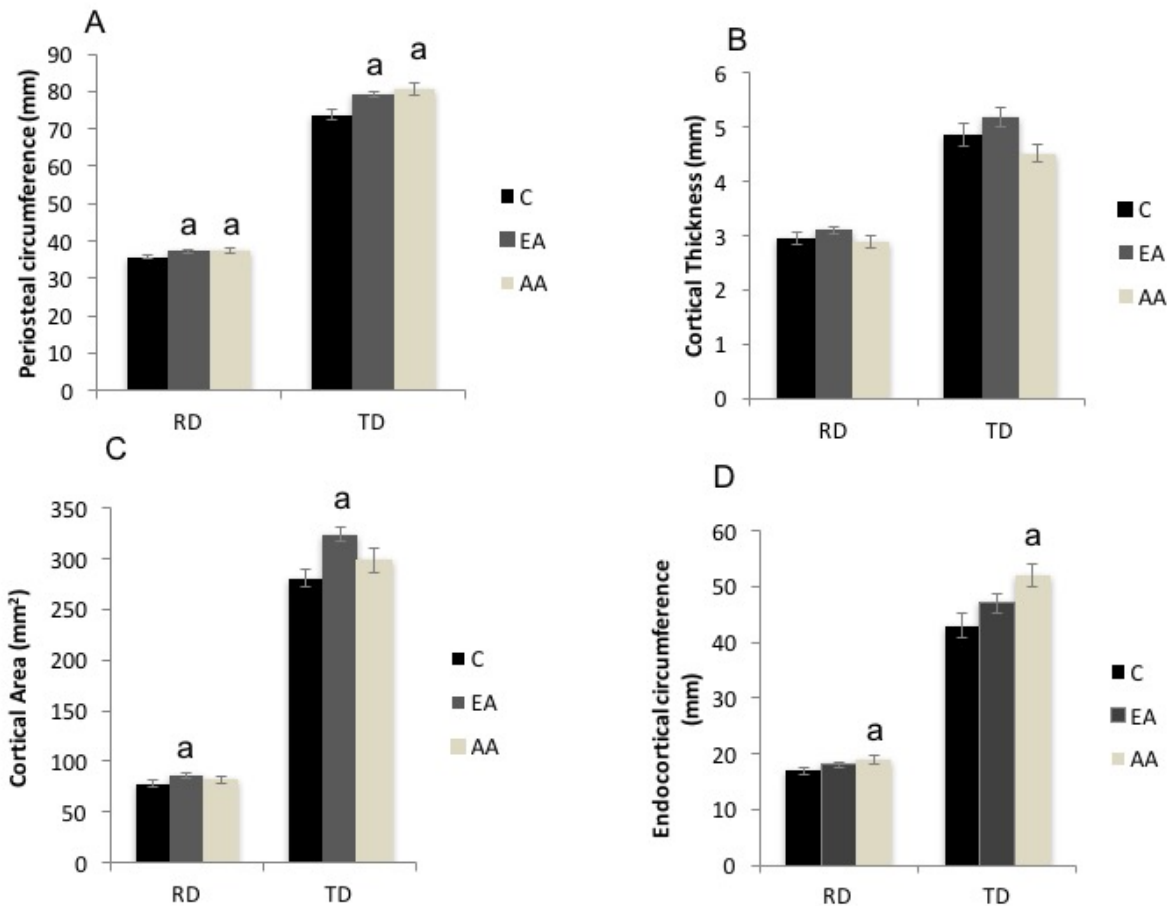
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484 **Figure 1: A)** Periosteal circumference (mm) for the radius diaphysis (RD) and tibia diaphysis

485 (TD) adjusted for object length; **B)** Cortical Thickness (mm) for the radius diaphysis (RD) and

486 tibia diaphysis (TD) adjusted for object length; **C)** Cortical Area (mm<sup>2</sup>) for the radius diaphysis

487 (RD) and the tibia diaphysis (TD) adjusted for object length; **D)** Endocortical Circumference

488 (mm) for the radius diaphysis (RD) and the tibia diaphysis (TD) adjusted for object length. C:

489 controls, EA: eumenorrheic athletes, AA: amenorrheic athletes. <sup>a</sup>: Significantly different from

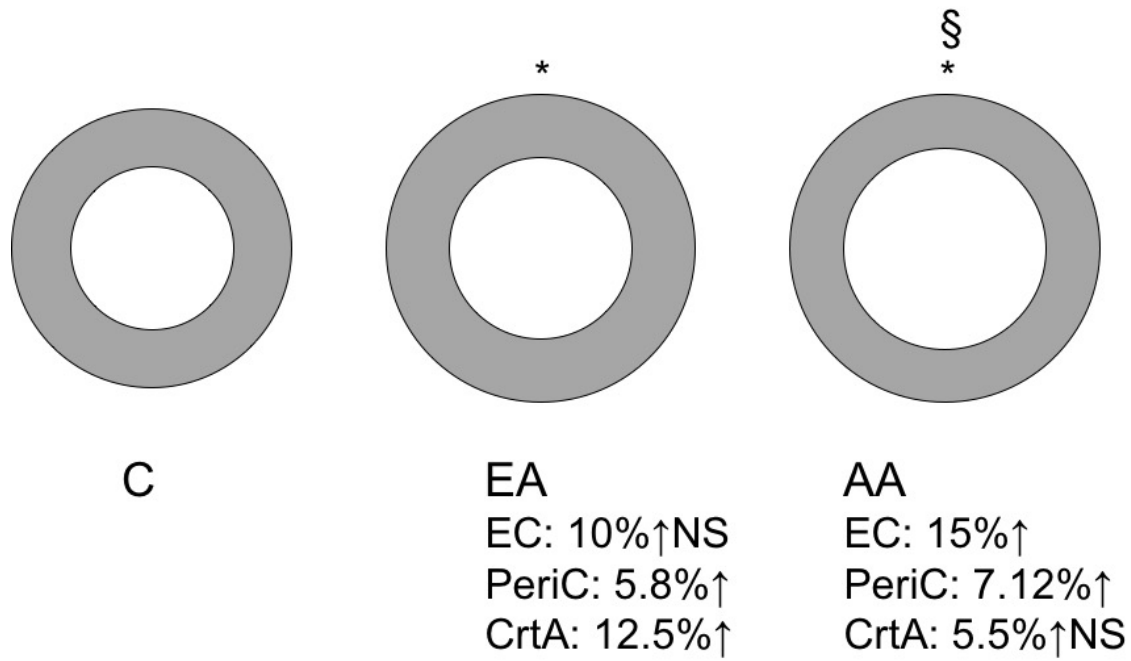
490 controls.

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496 **Figure 2:** A Schematic diagram to show the difference between groups in the endocortical  
 497 circumference (EC) and Periosteal Circumference (PeriC). AA have a significantly greater  
 498 circumferences' than both EA and controls with no difference between EA and controls.

499 \*=significantly different to controls; §=significantly different to EA.

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508 **Table 1.** Characteristics of controls (C), and eumenorrhic (EA) and amenorrhic athletes (AA).

	<b>C</b>	<b>EA</b>	<b>AA</b>	<b>P VALUE</b>	<b>P</b>	<b>P VALUE</b>
	<b>N=15</b>	<b>N=15</b>	<b>N=14</b>	<b>C VS. AA</b>	<b>VALUE</b>	<b>AA VS. EA</b>
	<b>C VS. EA</b>					
<b>Age (Years)</b>	26.8±0.9	27.6±2.1	26.4±0.8	0.863	0.714	0.594
<b>Height (m)</b>	1.66±0.17	1.66±0.02	1.64±0.02	0.590	0.862	0.479
<b>Mass (kg)</b>	59.6±1.5	54.5±1.3	49.6±1.6	<0.0005	0.037	0.029
<b>BMI (kg·m<sup>-2</sup>)</b>	21.7±0.6	19.8±0.4	18.3±0.4	<0.0005	0.009	0.045
<b>Lean mass (kg)</b>	39.0±1.6	44.5±1.1	42.0±1.2	0.112	0.015	0.215
<b>Fat mass (kg)</b>	18.5±1.5	8.1±0.7	5.3±0.6	<0.0005	<0.0005	0.054
<b>Body fat mass (%)</b>	30.6±2.1	14.9±1.2	10.7±1.0	<0.0005	<0.0005	0.065
<b>Lean mass (%)</b>	65.4±2.2	82.4±1.2	86.8±1.1	<0.0005	<0.0005	0.059
<b>AGP (%)</b>	N/A	86.9±1.0	86.6±1.2	N/A	N/A	0.890
<b>Age of menarche (years)</b>	13.0±0.34	14.1±0.35	14.9±0.54	0.01	0.051	0.275

509 Data are presented as mean ± SEM. AGP: Age-graded performance.

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519 **Table 2.** Muscle size and strength and femur size in controls (C), eumenorrheic athletes (EA)  
 520 and amenorrheic athletes (AA) as determined with MRI.

	C	EA	AA	P VALUE		
	n=15	n=15	n=14	C vs. AA	C vs. EA	AA vs. EA
<b>Forearm Muscle CSA (mm<sup>2</sup>)</b>	2617±93	2637±94	2516±101	0.555	0.876	0.458
<b>Lower Leg Muscle CSA (mm<sup>2</sup>)</b>	6457±221	7002±193	7099±242	0.225	0.944	0.198
<b>Calf Volume (cm<sup>3</sup>)</b>	1316±70	1317±74	1325±86	0.670	0.556	0.884
<b>Quadriceps Volume (cm<sup>3</sup>)</b>	1239±89	1469±92	1461±80	0.146	0.157	0.951
<b>Quadriceps Strength (Nm)</b>	171±6	164±7	163±10	0.314	0.304	0.992
<b>Normalised Force (Nm.cm<sup>-3</sup>)</b>	0.141±0.008	0.115±0.007	0.117±0.007	0.045	0.035	0.921

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 522 Data are presented as mean ± SEM. P-values reflect those related to the data adjusted for  
 523 Femur length in leg measures and radius length for forearm measures.

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525 **Table 3.** Bone mineral density as obtained with DXA data for controls (C) and eummenhoreic  
 526 (EA) and ammenorheic athletes (AA).

	C	EA	AA	P VALUE (AD FOR BODY HEIGHT)		
	n=15	n =15	n=14	C vs. AA	C vs. EA	AA vs. EA
<b>Total (g·cm<sup>-2</sup>)</b>	1.17±0.02	1.19±0.01	1.13±0.03	0.318	0.365	0.064
<b>Arms (g·cm<sup>-2</sup>)</b>	0.82±0.01	0.83±0.01	0.81±0.03	0.715	0.575	0.364
<b>Average Hip (g·cm<sup>-2</sup>)</b>	1.06±0.04	1.12±0.03	1.02±0.04	0.435	0.302	0.078
<b>Trunk (g·cm<sup>-2</sup>)</b>	0.91±0.03	0.91±0.02	0.82±0.02	0.002	0.909	0.003
<b>Ribs (g·cm<sup>-2</sup>)</b>	0.68±0.02	0.65±0.02	0.62±0.01	0.005	0.100	0.198
<b>Spine L1-4 (g·cm<sup>-2</sup>)</b>	1.19±0.03	1.16±0.03	1.04±0.04	0.004	0.585	0.015
<b>Pelvis (g·cm<sup>-2</sup>)</b>	1.11±0.01	1.14±0.02	0.99±0.03	0.004	0.568	0.001
<b>Legs (g·cm<sup>-2</sup>)</b>	1.25±0.03	1.33±0.02	1.26±0.03	0.555	0.032	0.122

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528 Data are presented as mean ± SEM.

529

530 **Table 4.** Hip and femur structural characteristics for controls (C) and eummenhoreic (EA) and  
 531 ammenorheic athletes (AA).

	C	EA	AA	p value (ad for FL)		
	n=15	n=15	n=14	C vs. AA	C vs. EA	AA vs. EA
<b>Cortical width shaft (mm)</b>	3.73±0.33	5.68±0.41	4.89±0.43	0.034	0.001	0.182
<b>Cortical width neck (mm)</b>	6.16±0.59	7.20±0.50	6.89±0.40	0.411	0.198	0.642
<b>CSA femoral neck (mm<sup>2</sup>)</b>	146±7.9	158±4.7	146±5.7	0.698	0.255	0.134
<b>Strength Index (BSI)</b>	1.69±0.10	1.81±0.07	1.89±0.11	0.161	0.398	0.570
<b>CSMI (mm<sup>4</sup>)</b>	9645±601	9840±676	8645±524	0.056	0.847	0.086
<b>Femur CSA (cm<sup>2</sup>)</b>	10.5±1.1	16.4±0.9	15.9±2.0	0.013	0.005	0.788
<b>Femur Volume (cm<sup>3</sup>)</b>	56.6±6.2	88.4±5.1	85.5±10.8	0.012	0.005	0.769

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 533 Data are presented as mean ± SEM. Cross-sectional moment of inertia (CSMI), cross-sectional  
 534 area (CSA) of the femur neck. P values displayed for data adjusted for femur length (FL).

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536 **Table 5.** Peripheral quantitative computer tomography (pQCT) data for the Radius epiphysis  
 537 (RE, 4%) and Radius diaphysis (RD, 60%) in controls (C), and eumenorrheic (EA) and  
 538 amenorrheic athletes (AA).

	C	EA	AA	P VALUE (AD FOR RADIUS LENGTH)		
	n=15	n=15	n=14	C vs. AA	C vs. EA	AA vs. EA
RE Ar.tot (mm <sup>2</sup> )	319±14	367±14	365±15	0.035	0.023	0.931
RE vBMC.tot (mg·mm <sup>-1</sup> )	101±4	109±4	102±6	0.861	0.220	0.304
RE vBMD.tb (mg·mm <sup>-3</sup> )	186±9	197±11	197±15	0.604	0.576	0.984
RD Ar.tot (mm <sup>2</sup> )	102±4	111±3	112±4	0.034	0.045	0.839
RD vBMC.tot (mg·mm <sup>-1</sup> )	93.0±4.0	103.2±4.0	98.9±4.3	0.997	0.336	0.529
RD vBMDct (mg·mm <sup>-3</sup> )	1132±14	1144±8	1142±11	0.819	0.721	0.907
RD I <sub>y</sub> (mm <sup>4</sup> )	138±7	158±7	156±7	0.067	0.032	0.801
RD I <sub>x</sub> (mm <sup>4</sup> )	135±8	149±8	151±8	0.165	0.190	0.896

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 542 RE: Radius epiphysis; RD: Radius diaphysis; vBMDct (mg·mm<sup>-3</sup>): Cortical bone mineral density;  
 543 vBMD.tb (mg·mm<sup>-3</sup>): Trabecular bone mineral density; Ar.tot (mm<sup>2</sup>); Ar.ct (mm<sup>2</sup>): Cortical  
 544 Area; EcC (mm): Endochondral circumference; I<sub>y</sub> and I<sub>x</sub> (mm<sup>4</sup>): moment of inertia indicating  
 545 bone's Stiffness in bending perpendicular to line of flexion/extension, in line with  
 546 flexion/extension and torsion respectively. Data are presented as mean ± SEM.

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550 **Table 6.** Peripheral quantitative computer tomography (pQCT) data for the Tibia epiphysis  
 551 (TE, 4%) and Tibia diaphysis (TD, 66%) in controls (C), and eumenorrheic (EA) and amenorrheic  
 552 athletes (AA).

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	C	EA	AA	P VALUE (AD FOR TIBIA LENTGH)		
	n=15	n=15	n=14	C vs. AA	C vs. EA	AA vs. EA
TE vBMC.tot (mg·mm <sup>-1</sup> )	296±11	337±11	324±12	0.147	0.012	0.858
TE vBMD.tb (mg·mm <sup>-3</sup> )	232±12	263±10	265±10	0.024	0.028	0.091
TE Ar.tot (mm <sup>2</sup> )	977±36	1067±32	1056±34	0.032	0.032	0.437
TD Ar.tot (mm <sup>2</sup> )	436±17	500±11	522±22	<0.0005	0.004	0.213
TD vBMC.tot (mg·mm <sup>-1</sup> )	312±9	390±8	364±10	0.006	<0.0005	0.153
TD vBMD.ct (mg·mm <sup>-3</sup> )	1127±7	1122±7	1112±8	0.02	<0.0005	0.280
TD I <sub>x</sub> (mm <sup>4</sup> )	1288±58	1580±60	1696±63	<0.0005	0.001	0.237
TD I <sub>y</sub> (mm <sup>4</sup> )	863±41	1077±43	1071±45	0.004	<0.0005	0.599

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555 TE: Tibia epiphysis; TD: Tibia diaphysis; vBMDct (mg·mm<sup>-3</sup>): Cortical bone mineral density;  
 556 vBMD.tb (mg·mm<sup>-3</sup>): Trabecular bone mineral density; Ar.tot (mm<sup>2</sup>); Ar.ct (mm<sup>2</sup>): Cortical  
 557 Area: EcC (mm): Endochondral circumference; I<sub>y</sub> and I<sub>x</sub> (mm<sup>4</sup>): moment of inertia indicating  
 558 bone's stiffness in bending perpendicular to line of flexion/extension, in line with  
 559 flexion/extension and torsion respectively. Data are presented as mean ± SEM.

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