The strength of weight-bearing bones is similar in amenorrheic and eumenorrheic elite long-distance runners

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Abstract (246 words)

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

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

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

Results: Both athlete groups had a higher caloric intake than controls, with no significant difference between athlete groups. DXA revealed lower bone mineral density (BMD) at the trunk, rib, pelvis and lumbar spine in the AA than EA and C. pQCT showed greater bone size in the radius and tibia in EA and AA than C. The radius and tibia of AA had a larger endocortical circumference than C. Tibia bone mass and moments of inertia (Ix and Iy) were greater in AA and EA than C, whereas in the radius only the proximal Iy was larger in EA than C. Knee extensor MVC did not differ significantly between groups.

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

Key words: eumennorheic, amenorrheic, athletes, endocortical, periosteal, muscle.

Introduction

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

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

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

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

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

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

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

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

Materials and Methods

Participants

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

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*

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

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

The following parameters examined in the 4% epiphyseal slice: total bone area (Ar.tot, mm^2), total bone mineral content (vBMC.tot, $mg \cdot mm^{-1}$) and trabecular bone mineral density (vBMD.tb, $mg \cdot cm^{-3}$). iaphyseal parameters examined were: Ar.tot, vBMC.tot, cortical area (Ar.ct, mm^2), cortical density (vBMD.ct, $mg \cdot cm^{-3}$), cortical thickness ($Ct.Th_{der}$ mm), periosteal (PsC, mm) and endocortical circumference (EcC, mm), antero-posterior (I_x) and mediolateral (I_y) moments of inertia representing bone bending stiffness. Cortical bone density values were 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 90^0 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 Kolmogrov-Smirnov test. A one-way ANOVA
181 was used to assess any significant differences between control, amenorrheic and

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

Results

Participants

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

Food Diaries

206 Total daily energy (kJ·day^{-1}) intake was less in C than athlete groups (both $p<0.05$; C;
207 6217±659, EA; 10567±880, AA; 9723±748).
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*

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

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

The periosteal circumference was larger in the athletes than the C ($p\leq0.01$; Figure 1A). The moment of inertia was significantly greater in EA than C in the y plane, but there was no significant difference between any groups in the x plane (Table 5).

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

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

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

Discussion

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

Study participants

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

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

Energy balance

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

Muscle mass and function

According to the mechanostat theory [4], mechanical strain on bone, caused by muscle contraction, stimulates bone formation and increases bone strength [3, 4]. Effects of 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

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

Weight-bearing bones

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

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

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

Bone remodelling

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

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

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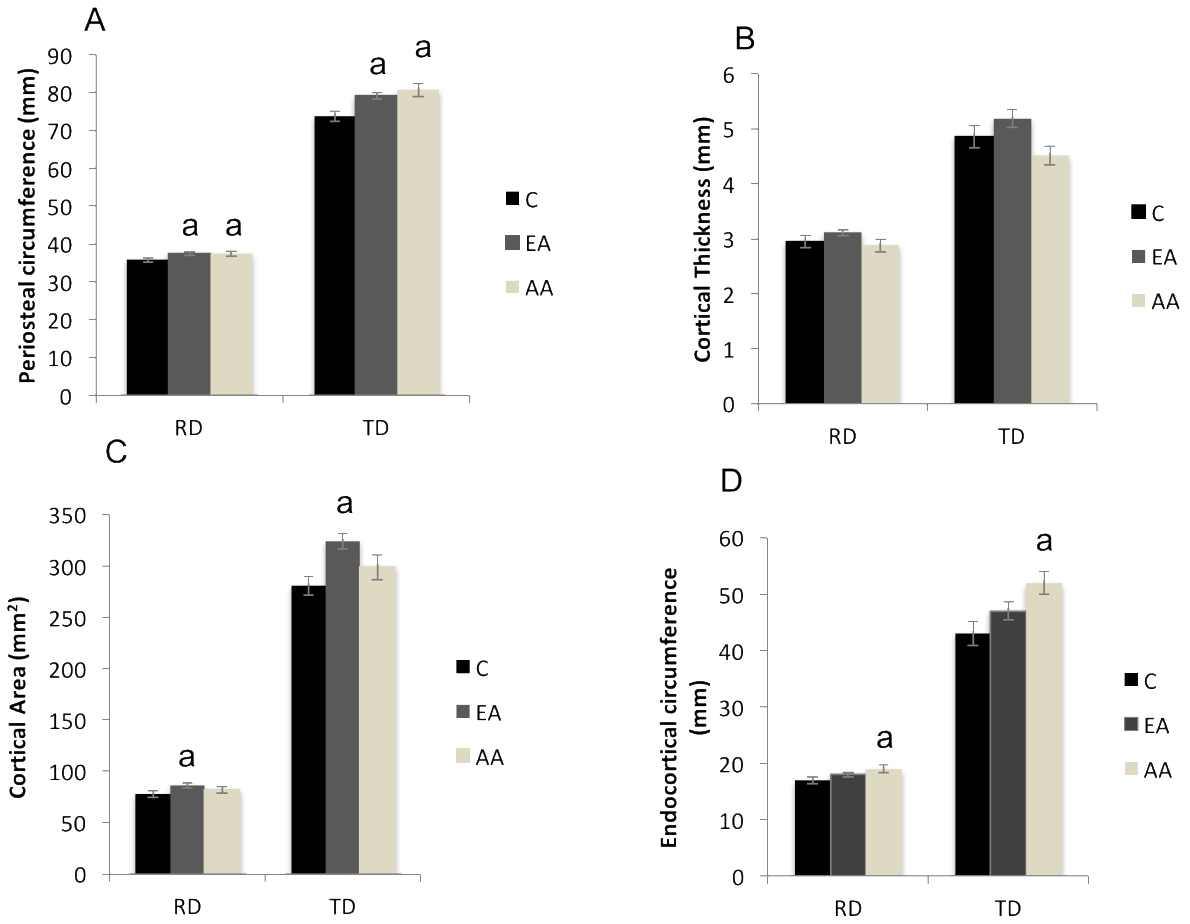

Figure 1: A) Periosteal circumference (mm) for the radius diaphysis (RD) and tibia diaphysis (TD) adjusted for object length; **B**) Cortical Thickness (mm) for the radius diaphysis (RD) and tibia diaphysis (TD) adjusted for object length; **C)** Cortical Area (mm^2) for the radius diaphysis (RD) and the tibia diaphysis (TD) adjusted for object length; **D)** Endocortical Circumference (mm) for the radius diaphysis (RD) and the tibia diaphysis (TD) adjusted for object length. C: controls, EA: eumenorrheic athletes, AA: amenorrheic athletes. [a] Significantly different from controls.

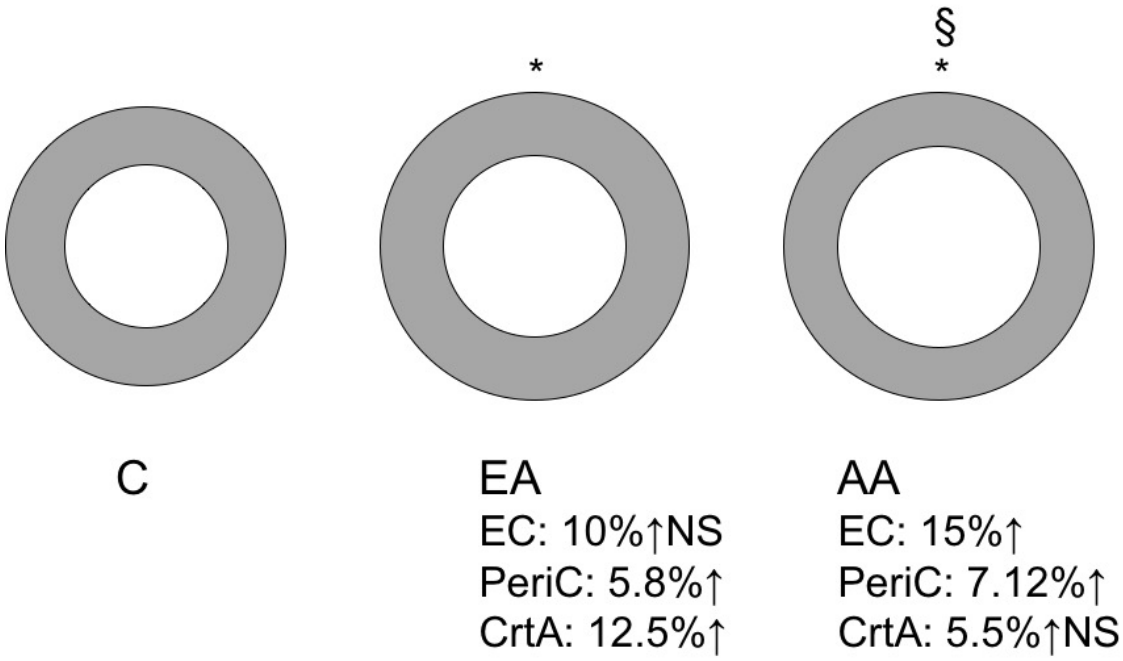

FIGURE LEGEND

Figure 2: A Schematic diagram to show the difference between groups in the endocortical circumference (EC) and Periosteal Circumference (PeriC) of the tibia. AA have a significantly greater circumferences' than both EA and controls with no difference between EA and controls. *=significantly different to controls; §=significantly different to EA. % increase is shown as an average of tibia and radius increase compared to controls.

Table 1. Characteristics of controls (C), and eumenorrheic (EA) and amenorrheic athletes (AA).

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

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

Table 2. Muscle size and strength and femur size in controls (C), eumenorrheic athletes (EA) 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
Forearm Muscle CSA (mm^2)	2617±93	2637±94	2516±101	0.555	0.876	0.458
Lower Leg Muscle CSA (mm^2)	6457±221	7002±193	7099±242	0.225	0.944	0.198
Calf Volume (cm^3)	1316±70	1317±74	1325±86	0.670	0.556	0.884
Quadriceps Volume (cm^3)	1239±89	1469±92	1461±80	0.146	0.157	0.951
Quadriceps Strength (Nm)	171±6	164±7	163±10	0.314	0.304	0.992
Normalised Force ($Nm.cm^{-3}$)	0.141±0.008	0.115±0.007	0.117±0.007	0.045	0.035	0.921

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

Table 3. Bone mineral density as obtained with DXA data for controls (C) and eummenhoreic (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
Total ($g \cdot cm^{-2}$)	1.17±0.02	1.19±0.01	1.13±0.03	0.318	0.365	0.064
Arms ($g \cdot cm^{-2}$)	0.82±0.01	0.83±0.01	0.81±0.03	0.715	0.575	0.364
Average Hip ($g \cdot cm^{-2}$)	1.06±0.04	1.12±0.03	1.02±0.04	0.435	0.302	0.078
Trunk ($g \cdot cm^{-2}$)	0.91±0.03	0.91±0.02	0.82±0.02	0.002	0.909	0.003
Ribs ($g \cdot cm^{-2}$)	0.68±0.02	0.65±0.02	0.62±0.01	0.005	0.100	0.198
Spine L1-4 ($g \cdot cm^{-2}$)	1.19±0.03	1.16±0.03	1.04±0.04	0.004	0.585	0.015
Pelvis ($g \cdot cm^{-2}$)	1.11±0.01	1.14±0.02	0.99±0.03	0.004	0.568	0.001
Legs ($g \cdot cm^{-2}$)	1.25±0.03	1.33±0.02	1.26±0.03	0.555	0.032	0.122

Data are presented as mean ± SEM.

Table 4. Hip and femur structural characteristics for controls (C) and eummenhoreic (EA) and 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
Cortical width shaft (mm)	3.73±0.33	5.68±0.41	4.89±0.43	0.034	0.001	0.182
Cortical width neck (mm)	6.16±0.59	7.20±0.50	6.89±0.40	0.411	0.198	0.642
CSA femoral neck (mm^2)	146±7.9	158±4.7	146±5.7	0.698	0.255	0.134
Strength Index (BSI)	1.69±0.10	1.81±0.07	1.89±0.11	0.161	0.398	0.570
CSMI (mm^4)	9645±601	9840±676	8645±524	0.056	0.847	0.086
Femur CSA (cm^2)	10.5±1.1	16.4±0.9	15.9±2.0	0.013	0.005	0.788
Femur Volume (cm^3)	56.6±6.2	88.4±5.1	85.5±10.8	0.012	0.005	0.769

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

Table 5. Peripheral quantitative computer tomography (pQCT) data for the Radius epiphysis (RE, 4%) and Radius diaphysis (RD, 60%) in controls (C), and eumenorrheic (EA) and 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^2)	319±14	367±14	365±15	0.035	0.023	0.931
RE vBMC.tot ($mg.mm^{-1}$)	101±4	109±4	102±6	0.861	0.220	0.304
RE vBMD.tb ($mg.mm^{-3}$)	186±9	197±11	197±15	0.604	0.576	0.984
RD Ar.tot (mm^2)	102±4	111±3	112±4	0.034	0.045	0.839
RD vBMC.tot ($mg.mm^{-1}$)	93.0±4.0	103.2±4.0	98.9±4.3	0.997	0.336	0.529
RD vBMDct ($mg.mm^{-3}$)	1132±14	1144±8	1142±11	0.819	0.721	0.907
RD I_y (mm^4)	138±7	158±7	156±7	0.067	0.032	0.801
RD I_x (mm^4)	135±8	149±8	151±8	0.165	0.190	0.896

RE: Radius epiphysis; RD: Radius diaphysis; vBMDct ($mg{\cdot}mm^{-3}$): Cortical bone mineral density; vBMD·tb ($mg{\cdot}mm^{-3}$): Trabecular bone mineral density; Ar·tot (mm^2); Ar·ct (mm^2): Cortical Area: EcC (mm): Endochondral circumference; I_y and I_x, (mm^4): moment of inertia indicating bone's Stiffness in bending perpendicular to line of flexion/extension, in line with flexion/extension and torsion respectively. Data are presented as mean ± SEM.

549 **Table 6.** Peripheral quantitative computer tomography (pQCT) data for the Tibia epiphysis (TE, 4%) and Tibia diaphysis (TD, 66%) in controls (C),

550 and eumenorrheic (EA) and amenorrheic 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^{-1})	296±11	337±11	324±12	0.147	0.012	0.858
TE vBMD.tb (mg·mm^{-3})	232±12	263±10	265±10	0.024	0.028	0.091
TE Ar.tot (mm^2)	977±36	1067±32	1056±34	0.032	0.032	0.437
TD Ar.tot (mm^2)	436±17	500±11	522±22	<0.0005	0.004	0.213
TD vBMC.tot (mg·mm^{-1})	312±9	390±8	364±10	0.006	<0.0005	0.153
TD vBMD.ct (mg·mm^{-3})	1127±7	1122±7	1112±8	0.02	<0.0005	0.280
TD I_x (mm^4)	1288±58	1580±60	1696±63	<0.0005	0.001	0.237
TD I_y (mm^4)	863±41	1077±43	1071±45	0.004	<0.0005	0.599

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553 TE: Tibia epiphysis; TD: Tibia diaphysis; vBMDct (mg·mm^{-3}): Cortical bone mineral density; vBMD·tb (mg·mm^{-3}): Trabecular bone mineral density;

554 Ar·tot (mm^2); Ar·ct (mm^2): Cortical Area: EcC (mm): Endochondral circumference; I_y and $I_{x,}$ (mm^4): moment of inertia indicating bone's stiffness

555 in bending perpendicular to line of flexion/extension, in line with flexion/extension and torsion respectively. Data are presented as mean ± SEM.

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