Section: Original Research

Article Title: Running event, age and competitive level as predictors of dual energy x-ray absorptiometry-derived body composition and bone health markers in female runners

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1 **ABSTRACT:**

2 The aim of this study was to assess the impact of running discipline, competitive level (COMP), 3 and age on body composition measures in female athletes. A total of n=51 female runners 4 (age: 30.9 ± 5.7 years, stature: 166.7 ± 5.7 cm, body mass (BM): 57.1 ± 8.2 kg) completed a 5 full body dual energy x-ray absorptiometry (DXA) scan in a cross-sectional design. One-way 6 ANOVA or Kruskal–Wallis was used to identify differences in DXA measures and independent 7 variables. Stepwise regression determined the contribution of independent variables on DXA 8 measures. Body fat percentage (BF%) and fat mass (FM) differed based on COMP (BF%: H₍₂₎ 9 = 17.451; FM: H₍₂₎ = 17.406, both p≤0.0001). COMP modestly predicted BF% and FM (BF%: 10 $R_{adj}^2 = 0.316$, $F_{(1,50)} = 22.660$; FM: $R_{adj}^2 = 0.300$, $F_{(1,50)} = 21.029$, both p≤0.0001). Bone mineral 11 density (BMD) and BMD Z-score (BMDz) did not differ between age, running discipline or 12 COMP (Age: BMD: F_(2,50) = 2.825, BMD_Z: F_(2,50) = 2.215; running discipline: BMD: F_(3,50) = 1.145, BMD_Z: $F_{(3,50)}$ = 1.474; COMP: BMD: $F_{(2,50)}$ = 0.074, BMD_Z: $F_{(2,50)}$ = 1.297, all p≤0.05). 13 Age and running discipline modestly predicted BMD and BMD_Z (BMD: R^{2}_{adj} = 0.179, $F_{(1,50)}$ = 14 15 5.264; BMD_Z: R^{2}_{adj} = 0.173, $F_{(1,50)}$ = 4.545, both p≤0.05). These findings indicate COMP may 16 be a predictor of BF% and FM. Age and running discipline appear predictors of bone health 17 markers. Such findings may enable medical and sport science practitioners to tailor 18 interventions relating to realisation of training adaptations, performance and health.

19

20 **Keywords:** DXA, Lean Mass, Bone Mineral Density, Endurance Athletes

21 **INTRODUCTION:**

22 Body composition is often assessed to provide an indication of an athlete's fitness and health 23 status (44). Traditionally, body composition is estimated using two- (e.g., skinfolds; SF, 24 bioelectrical impedance; BIA, air displacement plethysmography; ADP) or, three- (e.g., BIA, 25 dual-energy X-ray absorptiometry; DXA) compartment models to calculate fat-free mass 26 (FFM), lean mass (LM), fat mass (FM), and in the case of DXA, bone mineral density (BMD) 27 and bone mineral content (BMC) (43,44). Multicompartment model measurement techniques 28 provide an accurate measure of body composition, and tracking changes in such indices can 29 be useful for evaluating the effectiveness of dietary and/or conditioning interventions, with 30 such body composition alterations associated with enhancements in cardiorespiratory fitness 31 (2,5) and strength (26,39) which may be deemed favorable in certain sporting contexts.

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33 When compared to other methodologies of measuring body composition, DXA is widely 34 regarded as the gold standard non-invasive method of measuring FM, FFM and separating 35 FFM into LM and bone (40). Likewise, the method has also shown greater accuracy when 36 ascertaining body composition measures relative to ADP (21,22), BIA (33) and SF (45) 37 analyses, with DXA being highly correlated with both magnetic resonance imaging and 38 computed tomography when measuring muscle mass (11). For these reasons, and by 39 accounting for the variability in bone density that often exists in female populations, DXA may 40 be a superior methodology for use with athletic females (44). In relation to female athletes, 41 DXA scan technology has been previously used to monitor both body composition and bone 42 health in collegiate and high-level endurance runners (4,6,9,13,16) however further data 43 pertaining to body composition and bone health markers within these cohorts is still relatively 44 scarce compared to their male counterparts. Accurate measurement and assessment of bone 45 health in female athlete populations is of increasing importance due to the growing awareness 46 of low energy availability (LEA) and the consequences of this on bone mineral content (18,35). 47 Negative bone health consequences potentially arising from LEA are well identified in 48 physically active women (30,32) with female athletes experiencing LEA more likely to develop

49 low BMD (30,32). For this reason, it is therefore beneficial to have accurate information
50 concerning measures of body composition in athletes for both health and performance
51 purposes.

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53 With this in mind, the aims of this study were to a) profile whole body and regional body 54 composition and bone health markers via DXA in female middle distance and endurance 55 runners within the United Kingdom across differing age, running discipline and competitive 56 level (COMP), b) determine potential differences between whole body and regional body 57 composition and bone health markers within these cohorts and c) to determine whether the 58 running discipline undertaken, age or COMP are predictors of whole body and regional body 59 composition and bone health markers within these cohorts. Such outcomes may enable 60 medical, sport science and nutrition practitioners to tailor suitable interventions and advice 61 relating to the realization of training adaptations and performance.

62 **METHODS**:

63 Experimental approach to the problem:

Using a cross-sectional approach, body composition markers of female middle and endurance runners were measured via DXA scans. The study obtained institutional ethical approval and informed consent was sought from participants prior to study involvement. Whole body and regional body composition variables of body mass, FM, LM, BMD and BMC were collected via DXA scans as a one-off measure. Notably, DXA technology offers high precision and reliability when compared to other body composition methods such as BIA and anthropometry (11,29). All scans were performed by the same qualified technician, between July and August 2021.

71

72 **Participants:**

73 Participants were recruited from local athletics or running clubs via social media 74 advertisements and word of mouth, using a convenience sampling method. Participants were 75 athletes aged 18 - 40 years, who participated in regular running activities at a recreational or 76 competitive level, who were not currently pregnant, with no injuries nor experiencing any peri 77 menopausal or menopausal symptoms. Participants were asked to self-report their COMP. As 78 per the methods of Sharps et al. (38), professional athletes (COMP_{PRO}) were defined as any 79 athlete undertaking ≥10 h of training per week whose athletic performance has achieved the 80 highest level of competition (e.g. Olympics, international/national representation) and 81 receiving a full-time wage for sport undertaken. Competitive athletes (COMP_{COMP}) were 82 defined as any athlete undertaking ≥6 hours of training per week with a view to participate in 83 official competitions (e.g. university, club level athletes or higher) and whose full-time job was 84 not that of a full-time athlete (23,38). Recreational athletes (COMPREC) were defined as those 85 undertaking ≥4 hours of training per week who did not receive any money for partaking in sport 86 and participated for enjoyment (23,38). Participants were grouped into one of the following 87 age categories; 18 – 24 years, 25 – 30 years and 31 – 40 years. Primary running discipline undertaken was also self-reported (<3000m, 3000 m to 10 km, 10 miles to half-marathon, and 88

marathon/ultramarathon) and then categorized based on participant responses as per the
methods of Dervish *et al.* (8) for the purpose of analysis.

91

92 **Dual-energy X-ray absorptiometry (DXA):**

93 For each measurement, participants were asked to attend the laboratory in a rested state 94 (minimum of 12 hours since last high intensity exercise session) and having fasted overnight 95 (minimum of 12 hours), to eliminate changes in lean and total mass that corresponded to a 96 volume of food/drink consumed prior to scanning (27,28,29). Prior to scans being undertaken, 97 participants were screened for any existing injuries and/or pregnancy that may have precluded 98 them from the scan. Anthropometric variables of stature and body mass were measured via 99 portable stadiometer (Seca, Hamburg, Germany) to the nearest 1 mm and a calibrated 100 weighing scales (Seca, Hamburg, Germany) to the nearest 0.1 kg, respectively. These data 101 were inputted into the DXA computer for initial participant characteristics.

102 DXA scans (DPX-L Lunar Prodigy, GE Medical Systems, Lunar Madison, Wisconsin, USA) assessed whole body and regional FM, LM, body fat percentage (BF%), BMD and BMC 103 104 through tissue X-ray absorption from two X-ray energy peaks (enCORE 2008, version 105 12.30.008 software) (40). During the DXA procedure, participants were exposed to low levels 106 of ionizing radiation (0.4 µGy per 1 full-body scan); thus posing minimal risk to health with 107 exposures being comparable to that of everyday activity over a 24 h period at sea level (28,29). 108 During the scans, participants were required to lay supine on the DXA bed, with their hands 109 in a pronated position by their sides (as per manufacturer instructions) and to wear minimal 110 clothing to improve the accuracy of scan results as per the methods of (28,29). Regions of interest were automatically created with Encore[™] software, before being manually checked, 111 112 and then adjusted if necessary, by the same researcher each time to avoid measurement 113 errors.

114 Statistical Analysis:

115 All data were analyzed via SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for 116 Windows, Version 25.0. Armonk, NY: IBM Corp). Normality was assessed via Shapiro-Wilks 117 test. A one-way ANOVA was used to identify differences in whole body DXA variables (LM, 118 BMD, BMD Z-scores (BMD_z) and BMC means between primary running discipline, age 119 category and COMP) and regional DXA variables for the arms, legs, trunk, android and gynoid 120 (regional fat percentage; RF%, LM, BMC). Kruskal-Wallis was used to identify differences in 121 whole body DXA variables (body mass (BM), BF% and FM means between primary running 122 discipline, age category and COMP) and regional DXA variables for the arms, legs, trunk, 123 android and gynoid (RF%, Total Tissue, FM, BMC). Post-hoc testing was conducted where 124 appropriate. Following this, a stepwise regression analysis was carried out to determine the 125 contribution of age category, primary running discipline and COMP to body composition and 126 bone variables from the DXA scan. A variance inflation value (VIF) of less than five was 127 considered acceptable (34).

128 **RESULTS**:

Participant demographics can be seen in Table 1. A total of n=51 female runners were recruited. All 51 female runners (age: 30.9 ± 5.7 years, stature: 166.7 ± 5.7 cm, body mass (BM): 57.1 ± 8.2 kg) completed the study.

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133 Whole Body - Body Composition Markers:

134 Results of body composition markers via a whole body DXA scan can be seen in Table 1. LM 135 did not differ based on age (LM: $F_{(2,50)} = 0.789$, p≥0.05), running discipline (LM: $F_{(3,50)} = 1.974$, 136 p≥0.05) or COMP (LM: F_(2,50) = 0.074, p≥0.05). BM differed based upon age (Age: H₍₂₎ = 7.362, 137 p≤0.05) and COMP (Comp: H₍₂₎ = 14.433, p≤0.005). BF% and FM differed based upon COMP 138 (BF%; Comp: H₍₂₎ = 17.451, p≤0.0001, FM; Comp: H₍₂₎ = 17.406, p≤0.0001). Post-hoc pairwise 139 comparisons indicated that COMP_{COMP} had lower BF% and FM vs. COMP_{REC} (both p≤0.0001). 140 Stepwise multiple regression demonstrated COMP modestly predicted BF% and FM (BF%: R^{2}_{adj} = 0.316, $F_{(1,50)}$ = 22.660, p≤0.0001, VIF = 1.00; FM: R^{2}_{adj} = 0.300, $F_{(1,50)}$ = 21.029, 141 142 p≤0.0001, VIF = 1.00; Table 2). Both age and COMP modestly predicted BM (R^{2}_{adj} = 0.313, 143 F_(2,50) = 12.386, p≤0.0001, VIF = 1.00; Table 2).

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145 Whole Body - Bone Health Markers:

146 Results of bone health markers via a whole body DXA scan can be seen in Table 1. BMD and 147 BMD_Z did not differ based on age (BMD: $F_{(2,50)} = 2.825$, BMD_Z: $F_{(2,50)} = 2.215$, both p≥0.05), 148 running discipline (BMD: $F_{(3,50)} = 1.145$, BMD_Z: $F_{(3,50)} = 1.474$, both p≥0.05) or COMP (BMD: 149 $F_{(2.50)} = 0.074$, BMD_Z: $F_{(2.50)} = 1.297$, both p≥0.05). BMC differed based on age (Age: $F_{(2.50)} =$ 150 4.273, p≤0.05) and COMP (COMP: F_(2,50) = 5.347, p≤0.05). Tukey's post-hoc tests showed 151 that COMP_{COMP} athletes had lower BMC than COMP_{REC} athletes (p≤0.05). Stepwise multiple regression demonstrated age and running discipline modestly predicted BMD and BMD_z 152 153 (BMD: R_{adj}^2 = 0.179, $F_{(1,50)}$ = 5.264, p≤0.05, VIF = 1.00, BMD_Z: R_{adj}^2 = 0.173, $F_{(1,50)}$ = 4.545,

154 p≤0.05, VIF = 1.00; Table 2), with both age and COMP modestly predicted BMC (R^{2}_{adj} = 0.256, 155 F_(2.66) = 9.471, p≤0.001, VIF = 0.94; Table 2).

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157 **Regional Analysis – Body Composition Markers:**

158 Results of regional body composition analysis via a whole body DXA scan be seen in Table 159 3. RF% for arms, legs, android or gynoid did not differ based upon age (RF%_{Arms}: $F_{(3,47)}$ = 160 0.289, RF%_{Legs}: $F_{(3,47)} = 2.182$, RF%_{Android}: $F_{(3,47)} = 1.406$, RF%_{Gynoid}: $F_{(3,47)} = 2.293$, all p≥0.05) 161 and running distance (RF%_{Arms}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 2.099$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 2.099$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 2.099$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 2.099$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 2.099$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 1.929$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 1.929$, RF%_{Android}: $F_{(3,47)} = 1.929$, RF%_{Leas}: $F_{(3,47)} = 1.929$, RF%_{Android}: $F_{(3,47)} = 1.929$, R 162 2.272, RF%_{Gynoid}: $F_{(3,47)}$ = 2.140, all p≥0.05), however did differ based upon COMP (RF%_{Arms}: $\mathsf{F}_{(2,47)} = \text{ 7.198, } \mathsf{RF}\%_{\mathsf{Legs}} : \mathsf{F}_{(2,47)} = \text{ 7.347, } \mathsf{RF}\%_{\mathsf{Android}} : \mathsf{F}_{(3,47)} = \text{ 5.229, } p \ge 0.05 \; \mathsf{RF}\%_{\mathsf{Gynoid}} : \mathsf{F}_{(2,47)} = 0.05 \; \mathsf{RF}\%_{\mathsf{Gynoid$ 163 164 7.135, all p≤0.05). RF%_{Trunk} did differ based upon COMP (RF%_{Trunk}: H₍₂₎ = 9.696, p≤0.05). Total 165 tissue for arms, legs, trunk, and gynoid differed based upon COMP (Tissue_{Arms}: $H_{(2)} = 13.443$, 166 Tissue_{Legs}: $H_{(2)} = 12.097$, Tissue_{Trunk}: $H_{(2)} = 13.107$, Tissue_{Gynoid}: $H_{(2)} = 13.298$, all p≤0.05), with 167 total tissue for android differing based upon age and COMP (age: Tissue_{Android}: $H_{(2)} = 6.406$, 168 COMP: Tissue_{Android}: $H_{(2)}$ = 12.428, both p≤0.05). LM for arms, legs and trunk did not differ 169 based upon age (LM_{Arms}: F_(2,47) = 2.405, LM_{Legs}: F_(2,47) = 0.298, LM_{Trunk}: LM: F_(2,47) = 0.147, all 170 $p \ge 0.05$), running discipline (LM_{Arms}: $F_{(3,47)} = 0.423$, LM_{Legs}: $F_{(3,47)} = 0.256$, LM_{Trunk}: $F_{(3,47)} = 1.300$, 171 all p≥0.05) or COMP (LM_{Arms}: F_(2,47) = 2.957, LM_{Legs}: F_(2,47) = 0.856, LM_{Trunk}: LM: F_(2,47) = 0.296, 172 all p≥0.05). LM_{Android} or LM_{Gynoid} did not differ based age (LM_{Android}: H₍₂₎ = 1.470, LM_{Gynoid} H₍₂₎ = 0.131, both p \ge 0.05), COMP (LM_{Android}: H₍₂₎ = 1.182, LM_{Gynoid} H₍₂₎ = 1.184, both p \ge 0.05) or 173 174 running distance (LM_{Android}: $H_{(2)} = 3.934$, LM_{Gynoid} $H_{(2)} = 1.340$, both p ≥ 0.05). Stepwise multiple 175 regression demonstrated that age and COMP modestly predicted total android tissue and total fat in arms and total android tissue, respectively (Fat_{Arms}: R²_{adj} = 0.179, F_(1,47) = 5.778, p≤0.05, 176 177 VIF = 1.00, Tissue_{Anrdoid}: R^{2}_{adj} = 0.286, $F_{(1,47)}$ = 19.823, p≤0.0001, VIF = 1.00; Table 2).

178 **Regional Analysis – Bone Health Markers:**

179 Results of regional bone health marker analysis via a whole body DXA scan be seen in Table 180 3. BMC for arms and legs did not differ by age (BMC_{Arms}: $F_{(2,47)}$ = 1.605, BMC_{Legs}: $F_{(2,47)}$ = 181 0.781, both p≥0.05) or running distance (BMC_{Arms}: F_(2,47) = 0.158, BMC_{Legs}: F_(2,47) = 0.567, both 182 p≥0.05), however did differ by COMP (BMC_{Arms}: F_(2,47) = 5.911, BMC_{Legs}: F_(2,47) = 4.158, both 183 p≤0.05). BMC for trunk, and roid or gynoid did not differ based upon age (BMC_{Trunk}: $H_{(2)} = 2.477$, BMC_{Android}: H₍₂₎ = 1.896, BMC_{Gynoid}: H₍₂₎ = 2.342, all p≥0.05) or running distance (BMC_{Trunk}: H₍₂₎ 184 185 = 1.492, BMC_{Android}: H₍₂₎ = 1.108, BMC_{Gynoid}: H₍₂₎ = 3.927, all p≥0.05), however did differ based 186 upon COMP (BMC_{Trunk}: H₍₂₎ = 10.832, BMC_{Android}: H₍₂₎ = 9.696, BMC_{Gynoid}: H₍₂₎ = 7.834, all 187 p≤0.05).

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- 189 ****INSERT TABLE 1 ABOUT HERE****
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- 191 ****INSERT TABLE 2 ABOUT HERE****

192 **DISCUSSION:**

193 The primary aims of this study were to profile body composition and bone health markers via 194 DXA in female middle-distance and endurance runners within the United Kingdom across 195 differing age, running discipline and COMP, to determine potential differences between body 196 composition and bone health markers within these cohorts. Additionally this study aimed to 197 determine whether running discipline, age or COMP are predictors of body composition and 198 bone health markers within these cohorts. For body composition, our whole body findings 199 suggest BF% differs based upon COMP in female athletes, and that COMP is a predictor of 200 BF% and FM (~30%; Table 2). Similarly, differences in BM between female athletes differs 201 based upon age and COMP, with these variables being modest predictors (~30%; Table 2) of 202 BM. LM appears to be unaffected by age, running discipline and COMP. From a regional 203 analysis perspective total android tissue appears to be influenced by both age and COMP. 204 Regarding whole body bone health markers, both BMD and BMD_Z differ based upon athlete 205 age and running discipline, and these variables being a modest predictor (17 - 18%) of both 206 BMD and BMD_Z. Our findings show that COMP_{COMP} athletes have lower BMC when compared 207 to recreational counterparts (p≤0.05), and both age and COMP were a modest predictor of 208 BMC (~26%; Table 2). Regional analysis of BMC indicates that trunk, android and gynoid are 209 influenced by COMP.

210

211 Despite emphasis often placed on the importance of body composition from a sports 212 performance and health perspective (2,39,44), data pertaining to body composition and bone 213 health markers in female athletes is still relatively scarce. Further female-specific research is 214 needed in relation to these markers, specifically as the number of women participating in sport 215 and exercise is increasing in many countries (10). In relation to body composition and bone 216 health markers, Santos et al. (36) developed body composition reference values for male and 217 female athletes from both SF and DXA-derived measurements. However, despite a range of 218 sports being represented in the Santos et al. (36) study, runners were grouped with athletes 219 from other track and field disciplines (including sprinting, hurdling, and field-events), therefore

any interpretation from these findings against the findings in the present study may not be suitable comparisons. Whilst further research in females across a range of sports, age categories and COMP is still required, the present study offers novel information in relation to body composition and bone health of female middle-distance and endurance runners. To the best of the authors' knowledge, this is the first study to identify independent variables of age, running discipline and COMP as a predictor of body composition and bone health markers within these cohorts.

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228 Body Composition Markers:

229 Our findings indicate that both whole body and regional body composition markers of body fat 230 and total tissue (i.e. whole body: BF% and FM, regional: RF%_{Arms}, RF%_{Trunk}, Tissue_{Arms}, 231 Tissue_{Legs}, Tissue_{Trunk} and Tissue_{Gynoid}) were primarily influenced by COMP (~11% - 30%; 232 Table 2) for female runners included within this study. The mean FM values for runners 233 observed in the present study were comparable with those observed by both Carbuhn et al. 234 (4) and Herbert et al. (13) (FM: 11.9 ± 0.9 vs. 12.2 ± 3.2 kg vs. 12.6 ± 7.1 kg). The values 235 presented by Herbert et al. (13) were measured within high-level runners, and further support 236 our findings and subsequent post-hoc comparisons, that COMP may be influencing factors of 237 FM variables within female running cohorts. More specifically to BF%, our post-hoc testing 238 indicated that COMP is a factor of difference between BF%. The differences in BF% observed 239 within the present study are comparable to those observed by Carbuhn et al. (4) in female 240 collegiate runners. Similarly, our LM findings are higher than those observed by Herbert et al. 241 (13) (LM: 38.7 ± 3.6 vs. 41.8 ± 3.7 kg). The exact reasons underpinning these similarities (in 242 the case of BF%) and discrepancies (in the case of LM) require further investigation, however, 243 speculatively, higher training load, training volumes and seasonal training phases in 244 increasing levels of COMP_{PRO} vs. COMP_{COMP} vs. COMP_{REC} may explain these findings (14). 245 Within a sporting context, where performance outcomes are often based upon physiological 246 determinants, such as maximal power or speed for a given duration, relative to BM (12,13) 247 and time to complete a specific distance (i.e. middle or endurance running) is often viewed as

a desirable objective, aiming to optimize LM and BF%, with body fat often viewed as 'dead
weight' (18) may be viewed as favorable training optimization for athletes.

250

Bone Health Markers:

252 The total BMD values presented in the current study are lower than those observed by 253 Klomsten Andersen *et al.* (19) in both runners and cyclists (Runners: 1.283 ± 0.09 g·cm² vs. 1.174 ± 0.1 g·cm², Cyclists: 1.195 ± 0.1 g·cm² vs. 1.156 ± 0.1 g·cm²), and those observed by 254 255 Herbert *et al.* (13) for both BMD and BMD_z in female runners (BMD: 1.203 ± 0.08 g·cm²; BMD_z: 256 1.05 ± 0.9) and their non-athlete controls (BMD: $1.191 \pm 0.1 \text{ g} \cdot \text{cm}^2$; BMD_z: 1.02 ± 1.2). It should 257 be noted that within the Klomsten Andersen et al. (19) study, a mix of male and female athletes 258 were included within the study design, with DXA-derived indices not presented by sex, 259 meaning that any direct comparison must be treated with caution. Exercise can be considered 260 osteogenic, with athletes that have higher BMD exhibiting favorable adaptations of bone 261 microarchitecture, particularly at weight-bearing sites, and greater bone strength than their 262 sedentary counterparts (1,32,37). These findings are supported from our regional analysis that 263 indicates that COMP is a modest predictor of BMC across both android and gynoid measures. 264 It has previously been suggested that athletes involved in sports involving lower-impact, 265 repetitive loading cycles (e.g. endurance running) or non-weight-bearing sports (e.g. cycling 266 and swimming) do not typically elicit any exercise-induced skeletal benefits (33) when 267 compared to athletes from sports that generate higher intensity loading forces which enhance 268 bone mineral accretion (17,20). This may explain the findings in the present study in relation 269 to BMD. Negative bone health outcomes are associated with LEA and are well identified in 270 physically active women and are discussed in relation to conditions such as the female athlete 271 triad (7,30) or relative energy deficiency in sport (25). More specifically, female athletes 272 experiencing LEA are likely to develop low BMD (30) and alterations in bone microarchitecture 273 and bone strength (1), which may increase risk of osteoporosis and fractures (25). Research 274 in female athletes has suggested that 18 – 24 year olds have a higher risk of LEA and disordered eating than their older counterparts (8,38). Whilst LEA was not assessed within the 275

276 present study, our findings indicate that 18 – 24 year olds have the lowest total BMD within 277 the tested age categories of the present study, and speculatively, may suggest that medical 278 professionals and practitioners may wish to consider LEA screening or targeted interventions 279 within these cohorts in relation to low BMD and associated health implications alongside body 280 composition monitoring, and presents an area of future research development. Additionally, 281 our regional analysis indicates differences were observed between COMP groups, and COMP 282 was a predictor of BMC. These findings suggest that practitioners may wish to consider 283 competitive level as a potential factor in conjunction with the potential of LEA when screening 284 for bone health markers in female runners.

285

286 Limitations and Future Directions:

287 The present study is not without limitations. Firstly, the study aimed to recruit female runners 288 from a range of differing age categories (between 18 - 40 years) and competitive levels, 289 however, 18 – 24 year olds and professional-level athletes are under-represented in the 290 current study (Table 1). Therefore, whilst these data provide some insight into body 291 composition and bone health markers of these cohorts, translating these findings to all athletes 292 within these under-represented categories must be done so with caution. Due to the low 293 sample size of professional endurance runners recruited to this study, further sub-group 294 analysis could not be undertaken (e.g. effects of age and running distance with COMP as the 295 ranking or dominant factor). As a result, this in itself presents an area of direction for future 296 research within higher levels of competitive female endurance runners. Secondly, despite 297 DXA being widely deemed the gold standard method for body composition analyses due to its 298 accuracy and repeatability (37), the method is not without its limitations. Our findings suggest 299 the possible role of age and COMP on FM within these cohorts. Research indicates that with 300 increasing fat mass comes increased risk of error via DXA (3). Additionally, the potential 301 effects of the menstrual cycle on indices of body composition were not considered within this 302 study. Although the effects of such changes on the accuracy of body composition measures 303 via DXA scan are not fully understood (28), the influence of menses on the reliability of body 304 composition estimates appears minimal in a cohort of pre-menopausal females (15,31,41), 305 and within accepted measurement error of a DXA scan; whether this is true for female runners, 306 remains to be investigated. Within these limitations, is the fact that DXA manufacturers' body 307 composition estimation algorithms are not developed from athletic populations - meaning that 308 values for more competitive and professional level athletes are compared against 'general 309 population' reference values (29). Therefore, refining algorithms to better reflect body 310 composition and bone health characteristics of athletic cohorts (both male and female) may 311 increase the resolution and accuracy of future research. Lastly, additional screening for low 312 energy availability (via validated tools such as the Low Energy Availability in Females 313 Questionnaire; LEAF-Q; devised by Melin et al. (24)) or the inclusion of biochemical and/or 314 exercise testing within female runners alongside body composition and bone health screening 315 would further support findings from present study, and in itself, remains an opportunity for 316 future research.

317 **PRACTICAL APPLICATION:**

318 Our findings suggest that running discipline undertaken has minimal influence on differences 319 in body composition and bone health markers in female runners, with age and competitive 320 level appearing to exercise greater influence on certain whole body and regional DXA 321 variables, namely FM, BF%, BMD, BMDz, BMC (whole body) and TissueAndroid, BMCTrunk, 322 BMC_{Android} and BMC_{Gvnoid} (regional). Such findings may be of interest to medical, sport science 323 and nutrition practitioners to tailor suitable educational resources and interventions, based 324 upon age and competitive level of the athlete/s and advice relating to the realization of training 325 adaptations, performance and health.

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332 Ethical approval: All procedures performed in studies involving human participants were in 333 accordance with the ethical standards of the institutional and/or national research committee 334 and with the 1964 Helsinki declaration and its later amendments or comparable ethical 335 standards.

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514 **LEGENDS**:

515 **Table 1**. Absolute values for all dependent variables derived from dual energy X-ray 516 absorptiometry (DXA) scans.

517 **Table 2.** Results from regression analysis of independent predictors on dependent variables

518 of whole body and regional analysis from a dual energy X-ray absorptiometry (DXA) scan of

519 female, middle-distance and endurance runners

- 520 Figure 1. Grouped scatterplot depicting Body Fat Percentage plotted against Running
- 521 discipline. A; < 3,000 m, B; 3,000 10,000 m, C; 10 miles Half Marathon, D; Marathon to
- 522 Ultradistance.
- 523 Figure 2. Grouped scatterplot depicting Fat Mass plotted against Running discipline. A; <
- 524 3,000 m, B; 3,000 10,000 m, C; 10 miles Half Marathon, D; Marathon to Ultradistance.

		Body Composition Marker				Bone Health Marker			
	DXA variable	BM (kg)	BF%	FM (kg)	LM (kg)	BMD (g·cm²)	BMDz	BMC (kg)	
	Total (<i>n</i> =51)	57.1 ± 8.1	21.3 ± 8.2	12.6 ± 7.1	41.8 ± 3.7	1.174 ± 0.1	0.6 ± 0.9	2.490 ± 0.4	
Running Discipline	< 3000m (<i>n</i> =8)	54.6 ± 3.6	18.7 ± 3.6	10.2 ± 2.5	41.6 ± 2.4	1.219 ± 0.1	1.2 ± 0.7	2.595 ± 0.3	
	3000m – 10,000m (<i>n</i> =10)	56.3 ± 6.9	19.2 ± 8.5	11.1 ± 6.3	42.5 ± 3.3	1.168 ± 0.1	0.5 ± 1.0	2.424 ± 0.4	
	10 miles – Half-marathon (<i>n</i> =21)	57.3 ± 8.3	24.2 ± 6.6	14.2 ± 5.9	40.5 ± 3.9	1.158 ± 0.1	0.4 ± 1.0	2.456 ± 0.4	
	Marathon/Ultra (<i>n</i> =12)	58.9 ± 11.0	19.8 ± 11.5	12.6 ± 10.9	43.6 ± 3.7	1.178 ± 0.1	0.7 ± 1.0	2.536 ± 0.4	
Age (years)	18–24 (<i>n</i> =5)	50.6 ± 3.8	15.7 ± 1.4	7.9 ± 0.7	40.3 ± 3.5	1.136 ± 0.1	0.2 ± 0.7	2.224 ± 0.2	
	25 – 30 (<i>n</i> =18)	55.3 ± 5.5**	20.3 ± 6.2	11.4 ± 4.3	41.4 ± 3.5	1.149 ± 0.1	0.3 ± 0.9	2.357 ± 0.3	
	31–40 (<i>n</i> =28)	59.4 ± 9.3**	23.0 ± 9.5	14.2 ± 8.6	42.3 ± 3.9	1.197 ± 0.1	0.9 ± 1.0	2.624 ± 0.4	
COMP	Recreational (<i>n</i> =18)	63.0 ± 10.0	27.6 ± 8.8	18.0 ± 9.0	42.1 ± 3.8	1.199 ± 0.1	0.9 ± 1.0	2.712 ± 0.4	
	Competitive (<i>n</i> =31)	53.9 ± 4.5	18.1 ± 5.5∆	9.8 ± 3.3	41.6 ± 3.8	1.158 ± 0.1	0.5 ± 0.9	2.369 ± 0.3	
	Professional (<i>n</i> =2)	51.9 ± 2.5	14.7 ± 4.3	7.7 ± 2.6	41.8 ± 0.0	1.201 ± 0.1	1.1 ± 0.8	2.490 ± 0.3	

525 **Table 1**. Absolute values for all dependent variables derived from dual energy X-ray absorptiometry (DXA) scans.

526 BF% = body fat percentage; BM = body mass (kg); BMC = bone mineral content (kg); BMD = bone mineral density (g·cm⁻²); BMD_Z = bone mineral density Z-scores; COMP =

527 competitive level. FM = Fat Mass; LM = Lean Mass. All date are presented as mean ± SD. ** = denotes significance from 18 – 24 years, ^Δ = denotes significance from COMP_{Rec}

528 **Table 2.** Results from regression analysis of independent predictors on dependent variables of whole body and regional analysis from a dual

529	energy X-ray absorptiometry	y (DXA) scan of female,	middle-distance and endurance runners
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Whole Body					Regional Analysis			
В	SE (<i>B</i>)	β	R ²		В	SE (<i>B</i>)	β	R ²
				Predictor - Tissue _{Android}	0.419	0.174	0.334*	0.112
3.128	1.456	.257*	.340	Age (years)				
				Predictor - Fat _{Arms}				
-8.487	1.783	562**	.316	COMP	-0.569	0.128	-0.549**	0.301
-7.188	1.567	548**	.300					
025	.011	307*	.179					
317	.140	.320*	.173					
.183	.074	.313*	.256					
	B 3.128 -8.487 -7.188 025 317 .183	Whole B SE (B) 3.128 1.456 -8.487 1.783 -7.188 1.567 025 .011 317 .140 .183 .074	Whole Body B SE (B) β 3.128 1.456 .257* -8.487 1.783 562** -7.188 1.567 .548** 025 .011 307* 317 .140 .320* .183 .074 .313*	Whole Body B SE (B) β R ² 3.128 1.456 .257* .340 -8.487 1.783 562** .316 -7.188 1.567 .548** .300 025 .011 307* .179 317 .140 .320* .173 .183 .074 .313* .256	Whole Body β R ² B SE (B) β R ² Predictor - TissueAndroid 3.128 1.456 .257* .340 Age (years) Predictor - FatArms Predictor - FatArms Predictor - FatArms -8.487 1.783 562** .316 COMP -7.188 1.567 548** .300 Predictor - FatArms 025 .011 307* .179 Predictor Predictor - FatArms 317 .140 .320* .173 Predictor - FatArms .183 .074 .313* .256 Predictor - FatArms	Whole Body β R ² B 3.128 1.456 .257* .340 Age (years) 0.419 3.128 1.456 .257* .340 Age (years) Predictor - Fat _{Arms} -8.487 1.783 562** .316 COMP -0.569 -7.188 1.567 548** .300 -0.569 025 .011 307* .179 -0.569 317 .140 .320* .173 -173	Whole Body Regional B SE (B) β R^2 B SE (B) 3.128 1.456 .257* .340 Age (years) 0.419 0.174 3.128 1.456 .257* .340 Age (years) Predictor - Fat _{Arms} 0.419 0.174 -8.487 1.783 562** .316 COMP -0.569 0.128 -7.188 1.567 548** .300 -0.569 0.128 025 .011 307* .179 - - - - - - - .183 .074 .313* .256 - - - - - -	Whole Body Regional Analysis B SE (B) β R^2 B SE (B) β 3.128 1.456 .257* .340 Age (years) 0.419 0.174 0.334* 3.128 1.456 .257* .340 Age (years) Predictor - Fat _{Arms} 0.174 0.334* -8.487 1.783 562** .316 COMP -0.569 0.128 -0.549** -7.188 1.567 548** .300 -0.569 0.128 -0.549** 025 .011 307* .179 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

530 BF% = body fat percentage; BM = body mass (kg); BMC = bone mineral content (kg); BMD = bone mineral density (g·cm⁻²); BMD_Z = bone mineral density Z-scores; COMP =

531 competitive level, Fat_{Arms} = Total Arm Fat, Tissue_{Android} = Total Android Tissue; * indicates statistical differences at p≤0.05 level; ** indicates statistical differences at p≤0.005 level



Figure 1. Grouped scatterplot depicting Body Fat Percentage plotted against Running discipline. A; < 3,000 m, B; 3,000 – 10,000 m, C; 10 miles

535 – Half Marathon, D; Marathon to Ultradistance.



Figure 2. Grouped scatterplot depicting Fat Mass plotted against Running discipline. A; < 3,000 m, B; 3,000 – 10,000 m, C; 10 miles – Half
Marathon, D; Marathon to Ultradistance.