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Bone mineral density in vocational and professional ballet dancers

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39 **SUMMARY**

40 According to existing literature, bone health in ballet dancers is controversial. We have verified that, compared to  
41 controls, young female and male vocational ballet dancers have lower bone mineral density (BMD) at both impact  
42 and non-impact sites, whereas female professional ballet dancers have lower BMD only at non-impact sites.

43

44 **ABSTRACT**

45 *Purpose:* The aims of this study were to a) assess bone mineral density (BMD) in vocational (VBD) and professional  
46 (PBD) ballet dancers, and b) investigate its association with body mass (BM), fat mass (FM), lean mass (LM),  
47 maturation and menarche.

48 *Methods:* The total of 152 VBD (13±2.3yrs; 112 girls, 40 boys) and 96 controls (14±2.1yrs; 56 girls, 40 boys), and  
49 184 PBD (28±8.5yrs; 129 females, 55 males) and 160 controls (27±9.5yrs; 110 female, 50 males) were assessed at  
50 the lumbar spine (LS), femoral neck (FN), forearm and total body by Dual-energy X-ray absorptiometry. Maturation  
51 and menarche were assessed via questionnaires.

52 *Results:* VBD revealed lower unadjusted BMD at all anatomical sites compared to controls ( $p<0.001$ ); following  
53 adjustments for Tanner stage and gynaecological age, female VBD showed similar BMD values at impact sites.  
54 However, no factors were found to explain the lower adjusted BMD values in VBD (female and male) at the forearm  
55 (non-impact site), nor the lower adjusted BMD values in male VBD at the FN. Compared to controls, female PBD  
56 showed higher unadjusted and adjusted BMD for potential associated factors at the FN (impact site) ( $p<0.001$ ) and  
57 lower adjusted at the forearm ( $p<0.001$ ). Male PBD did not reveal lower BMD than controls at any site.

58 *Conclusions:* Both females and males VBD have lower BMD at impact and non-impact sites compared to control,  
59 whereas this is only the case at non-impact site in female PBD. Maturation seems to explain the lower BMD at  
60 impact sites in female VBD.

61

62 **KEYWORDS:** bone mass; prevalence; associated factors; elite dance; ballerinas

63

64 **INTRODUCTION**

65 Osteoporosis and osteopenia [i.e. low bone mineral density (BMD)] are recognised as the most frequent bone  
66 disorders, linked to high treatment costs and limited quality of life due to osteoporotic fractures [1, 2]. Hence, the  
67 identification of those at high-risk is crucial for planning appropriate prevention programmes. The diagnosis of low  
68 BMD in premenopausal women and children is based on the International Society of Clinical Densitometry (ISCD)  
69 guideline, whereas a diagnosis is confirmed when BMD values lie within 2.0 standard deviations (SD) or more  
70 below the average value [3]. The American College of Sports Medicine (ACSM) has proposed different guidelines  
71 for the diagnosis in athletes. The term “low BMD” is used for BMD values between -1.0 and -2.0 SD, and the term  
72 “osteoporotic” for BMD equal or less than -2.0 SD (along with secondary risk factors for stress fractures) [4].

73 Low BMD has been traditionally associated with elderly and postmenopausal women [5], though some  
74 athletic populations, as endurance athletes, might also be at increased risk [6, 7]. In ballet dancers, however, aspects  
75 regarding low BMD remain ambiguous [8]. While some authors underline the negative effects of professional dance  
76 training on bone metabolism (e.g. lean body type required for performance) [9-11], others suggest that the  
77 mechanical impact from dancing may provide a protection against low BMD, particularly at impact sites [12-14]. For  
78 instance, the high levels of muscular strength required for technical performance and weight-bearing activity  
79 associated with jumping may stimulate bone-forming cells [12-14]. Nevertheless, most of the relevant publications  
80 on ballet dancers have been categorised average of low quality [8]. Therefore, the aims of the present study were a)  
81 to assess BMD in vocational (VBD) and professional ballet dancers (PBD), and b) to investigate the association  
82 between BMD with body mass (BM), fat mass (FM), lean mass (LM), menarche and maturation.

83  
84 **METHODS**

85 **Study population**

86 This study was conducted by inviting active students from vocational dance schools (children undergoing 4-8 hours a  
87 day dance training in order to prepare for the profession) and active dancers from professional ballet companies.  
88 Pilot studies were administrated at a vocational dance school and a professional ballet company in order to calculate  
89 the sample size needed for prevalence estimate; sex and aged matched controls were also included in both cases. In a  
90 sample of 36 female VBD and 36 matched-controls, low BMD (Z-score of <-2.0) at the lumbar spine (LS) was found  
91 in 36% and 6%, respectively. Based on this finding, we estimated that 37 participants were needed in each group to  
92 obtain 90% power, with  $\alpha=0.05$ . Similarly, in a sample of 22 female PBD (22 matched-controls) and 10 male PBD  
93 (10 matched-controls), the prevalence of low BMD (Z-score of -1.0) at the LS was found to be 32% (vs. 5%) in  
94 female PBD and 20% (v. 0%) in male PBD. We subsequently estimated that 42 female participants and 46 male  
95 participants in each group were needed to reach significance (90% power,  $\alpha=0.05$ ). Assuming participants’ non-  
96 response and possible dropouts, we approached two vocational dance schools and four professional ballet companies.

97 To recruit participants, an introductory letter briefly explaining the purposes of the study was initially  
98 forwarded to the executive boards of the dance schools and companies. Following boards’ agreement, the research  
99 team contacted the VBD (their guardians too) and PBD to present them with the studies aims and methodologies.  
100 From the total of 595 participants (360 VBD and 235 PBD), 158 VBD and 206 PBD volunteered. From this cohort,  
101 those who had received or were receiving medications known to affect bone metabolism were excluded (one PD),  
102 together with those receiving calcium supplements (two VD and one PD). Given the differences in bone mass values  
103 between individuals from different races [15], only participants referring themselves as white European-Caucasian  
104 dancers were included. Based on these criteria, the total of 152 VBD (13±2.3yrs; 112 girls, 40 boys) and 184 PBD

105 (28±8.5yrs; 129 females, 55 males) were finally included in this study. Participants provided details on physical  
106 exercise (hours per week). Female and male VBD reported to perform 18.2±7.0 and 19.5±7.2 hours per week of  
107 dance training, respectively. Female and male PBD reported 32.9±8.4 and 32.5±9.6 hours per week of dance  
108 training, respectively. Details of the recruited dance population and its participation rate appear in Figure I.

109 Controls were also included in this study. Controls for the VBD were recruited from two local state schools,  
110 while controls for PBD were recruited from two local state universities. Eligibility criteria for controls were set  
111 according to dancers' characteristics, i.e. controls were only considered eligible if they were of the same sex, age  
112 (defined as decimal age; 12-months difference of a dancer) and race (white European-Caucasian). Exclusion criteria  
113 included current and previous participation in regular and organised physical activities. This rule did not apply to  
114 children participants involved in physical education sessions at their school. Control participation was also restricted  
115 to those who had received or were receiving medications known to affect bone metabolism. All participation criteria  
116 explaining the purpose for the recruitment was advertised via email and letters, following consent from the respective  
117 boards of directors. Out of the 282 responses (105 pupils, 177 university students), 256 fulfilled the current criteria  
118 and were included in the study [controls for VBD: 96 (14±2.1yrs), 56 girls, 40 boys; controls for PBD: 160  
119 (27±9.5yrs), 110 female, 50 males]. Female and male controls for VBD were involved in 2.4±0.5 and 2.1±0.4 hours  
120 per week of physical exercise, consisting mainly of school physical education. Female and male PBD controls did  
121 not report extra physical exercise apart from daily life routines. Details of the recruited controls and its participation  
122 rate appear in Figure II.

123 All participants provided signed informed consent. Following that, they underwent anthropometric  
124 measures, completed a menstrual questionnaire and participated in bone/body composition measurements (Figure 1).  
125 All procedures were approved by the NHS Health Research Authority, UK (Proc.14/WM/0008 and 14/WM/0009)  
126 and by the ethics committee of the Regional Administration of Health of Lisbon, Portugal (Proc.063/CES/INV/2012)  
127 in accordance with the Helsinki Declaration.

### 128 **Anthropometry measurements, menstruation, smoking, nutrition intake, hormonal analysis and pubertal** 129 **assessment**

131 Chronological age was obtained as decimal age (date of birth minus measurement date). Participants' height (m),  
132 sitting height (m) and BM (kg) were measured using standard stadiometers (Seca) and digital scales (Tanita),  
133 respectively. BM index (BMI) was calculated as kilograms per square meter ( $\text{kg.m}^{-2}$ ). Female participants completed  
134 a questionnaire to determine age at menarche. Total lifetime menses (number of menses since menarche to current  
135 age) were calculated as previously described [16]. Primary amenorrhea was defined as the absence of menarche by  
136 the age of 15 [17]. Gynaecological age (years) was calculated from the year of menarche to the age at which data  
137 were collected – current age [18].

138 Participants were asked to report their smoking history habits. Nutrient intakes were recorded via a  
139 validated 3-day food diary (two weekdays and one during weekend) [19]; this information was only assessed in VBD  
140 and their controls. The Food Processor SQL Edition, version 9.8.1 was used to estimate average energy, calcium and  
141 vitamin D intakes.

142 Blood samples were collected in early morning after an 8-hour fasting. Serum insulin-like growth factor-1  
143 (IGF-1) was measured by immunoradiometric assay kit (IRMA, IMMUNOTECH SAS, Marseille, France), in an  
144 automated analyser (Wallac Wizard 1470, Finland). The assay ranges were from 2 to 1.200ng/mL). The intra-assay  
145 and inter-assay CV's were below or equal to 6.3% and 6.8%, respectively. Blood samples were centrifuged at 2500g

146 for 10 min and serum stored at -80°C until analyses. Finally, pubertal development in VBD and their controls were  
147 self-reported using the Tanner sexual staging questionnaire [20].

148

### 149 **Body composition and bone measurements**

150 BMD at the LS, femoral neck (FN) and forearm (1/3 distal radius) were measured using Dual-energy X-ray  
151 absorptiometry (DXA). Body composition was assessed through a DXA whole-body scan [FM and LM (Kg)]. As  
152 participants were from different regions, two different DXA devices were used [Hologic (Discovery Wi) and Lunar  
153 (GE Lunar Prodigy)]. The total of 68 (44.7%) VBD and 178 (96.7%) PBD were subjected to Lunar scans device  
154 while the remaining 84 (55.3%) VBD and 6 (3.3%) PBD were scanned using Hologic. In addition, 20 (27.1%)  
155 children controls and 110 (68.8%) adult controls were assessed on a Lunar device vs. 70 (72.9%) and 50 (31.2%) on  
156 Hologic, respectively.

157 It is known that Lunar and Hologic BMD measurements demonstrate high correlation values between them  
158 [21, 22]. It is also known that there is a tendency for Lunar model to inflate BMD values compared to Hologic [22].  
159 Therefore, besides the daily calibration required from each DXA manufacturer, cross-calibration of the two scanners  
160 was also conducted on a group of 20 men and women; the age of these 20 participants covered the age-range of the  
161 entire sample (both dancers and controls) used for the purpose of the present study. The 20 participants were  
162 measured with both Lunar and Hologic within a period of 5 days. Subsequently, regression equations using BMD  
163 from Lunar as dependent variable and BMD from Hologic as independent variable were performed taking into  
164 account cross-calibration. The correlation between the two DXA models were high (forearm BMD:  $r=0.96$ , adjusted  
165  $r^2=0.93$ , std. error of estimate=0.03; LS BMD:  $r=0.96$ , adjusted  $r^2=0.92$ , std. error of estimate=0.05; FN BMD:  
166  $r=0.97$ , adjusted  $r^2=0.93$ , std. error of estimate=0.05). The Hologic BMD data were further converted to the Lunar  
167 data using the following equations: Forearm BMD Lunar =  $-0,085263 + 1,356535 * \text{Hologic}$ ; LS BMD Lunar =  
168  $0,030762 + 1,161805 * \text{Hologic}$ ; FN BMD Lunar =  $0,084782 + 1,116509 * \text{Hologic}$ . Following the BMD adjustments,  
169 Z-scores at each anatomical site were further calculated for VBD considering standard data reference ranges for  
170 gender and age provided by the Lunar manufacture (BMDCS data reference for children adjusted for height).

171

### 172 **Statistical analyses**

173 Independent t-tests were used to compare general characteristics between dance population and controls. Chi-square  
174 test was adopted to determine whether there is a significant difference in the distribution of Tanner stages between  
175 VBD and controls. Chi-square analyses were further employed to examine prevalence differences of low BMD  
176 between VBD (stratified by sex) and their controls. Analysis of covariance (ANCOVA) was conducted in VBD and  
177 PBD (also stratified by sex) in order to identify potential associated factors that might explain differences in BMD  
178 between groups (i.e. VBD\*matched controls, and PBD\*matched controls). Consequently, BMD at each anatomical  
179 site (dependent variable) was adjusted for: BM, FM, LM, Tanner stage, age at menarche, gynaecological age, and  
180 energy intake (covariates were entered as separate constituents). However, prior to the aforementioned analysis, all  
181 BMD data were controlled for school/company and/or DXA effect, since our dancers were recruited from a) different  
182 ballet schools/companies and b) were scanned using two DXA devices. Missing data were identified as “system  
183 missing” using the SPSS software - version 20.0. We had missing data for FM (7.9% and 8.2% in VBD and PBD,  
184 respectively) and nutrition intake (15.1% and 18.8% in VBD and controls, respectively). Statistical significance was  
185 set at  $p<0.05$ .

186

187 **RESULTS**

188 Table I depicts the general characteristics of all participants. Table I indicates that maturity differences between  
189 dancers and controls are more pronounced in female VBD than their male counterparts. Compared to controls,  
190 female and male VBD revealed significantly lower BM (by 10.8kg and 11.1kg, respectively;  $p<0.001$ ), BMI (by  
191  $4.4\text{kg/m}^2$  and  $3.6\text{kg/m}^2$ , respectively;  $p<0.001$ ) and FM (by 9.0kg and 8.0kg, respectively;  $p<0.001$ ). In female VBD,  
192 age of menarche was ~18 months later than controls ( $p<0.001$ ). Similarly, female and male PBD revealed  
193 significantly lower BM (by 9.2kg and 6.0kg, respectively;  $p<0.001$ ) and BMI (by  $3.9\text{kg/m}^2$  and  $2.0\text{kg/m}^2$ ,  
194 respectively;  $p<0.001$ ) compared to controls. Female PBD also demonstrated significantly lower FM (by 10.3kg,  
195  $p<0.001$ ) and higher LM (by 2kg,  $p<0.01$ ) compared to controls, and had their menarche approximately two years  
196 later than controls ( $p<0.001$ ). There was no significant difference between VBD and controls for calcium and  
197 vitamin D intake, but both female and male VBD consumed significantly less calories per day compared to controls  
198 (by 215.1kcal/day or 13.2% and 278.0kcal/day or 17.4%, respectively,  $p<0.05$ ). Serum IGF-1 concentrations were  
199 not significantly different in VBD compared to controls. Table I also depicts unadjusted BMD values for potential  
200 associated factors (i.e. BMD data were only adjusted for DXA-device and school/company). Both female and male  
201 VBD show significantly lower unadjusted BMD values for potential associated factors at all measured anatomical  
202 sites compared to controls ( $p<0.001$ ). However, female PBD demonstrate significantly higher unadjusted BMD at the  
203 FN (by 11.9%,  $p<0.001$ ), and significantly lower at the forearm (by 13.9%,  $p<0.001$ ). Male PBD show significantly  
204 higher unadjusted BMD values than controls at the FN (by 15.9%,  $p<0.001$ ) and LS (by 10.3%,  $p<0.01$ ).

205 Tables II and III depict the ANCOVA results for VBD and PBD, respectively. In particular, Table II  
206 illustrates that both female and male VBD have significantly lower adjusted BMD values at all anatomical sites  
207 compared to controls. BM, LM, FM, and energy intake were positively associated with BMD in female VBD at the  
208 FN ( $p<0.001$ ,  $p<0.001$ ,  $p<0.01$  and  $p<0.05$ , respectively). However, these covariates did not explain group  
209 differences (i.e. VBD versus controls); only when controlling for Tanner stage and gynaecological age BMD  
210 differences between groups were dissipated. The factors determining BMD differences between VBD and their  
211 matched controls at the LS were Tanner stage (females and males both at  $p<0.001$ ) and body mass (only for males,  
212  $p<0.001$ ). No factors were detected to explain the lower adjusted BMD values in VBD (both in female and male) at  
213 the forearm (non-impact site) than controls, nor the lower adjusted BMD values in male VBD at the FN (impact site).  
214 Table III confirms that our female PBD have higher adjusted BMD values at the FN ( $p<0.001$ ), and lower adjusted  
215 BMD values at the forearm ( $p<0.001$ ) than controls. LM and gynaecological age were positively associated with  
216 these findings at the FN ( $p<0.05$ ,  $p<0.001$ , respectively); the fact that our female PBD had their menarche later than  
217 controls seems to explain the BMD differences between groups at the forearm ( $p<0.001$ ). FM is positively associated  
218 with BMD at the LS in female PBD ( $p<0.01$ ). Male PBD revealed higher adjusted BMD at impact sites than controls  
219 (FN and LS), and similar BMD values at the forearm; LM is positively associated with these findings at the LS  
220 ( $p<0.01$ ).

221 Table IV shows the prevalence of low BMD in VBD ( $Z\text{-score} < -2.0$ ). Significantly higher prevalence of  
222 low BMD at the forearm (9.2% vs. 0%,  $p=0.01$ ) and LS (16.4% vs. 5.5%,  $p<0.05$ ) was noted in female VBD  
223 compared to controls. Although not significant, the proportion of cases with low BMD was higher in male VBD at  
224 all anatomical sites compared to controls.

225  
226  
227

## 228 **DISCUSSION**

229 Data on BMD in dancers has been ambiguous thus far. This is supported by a recent systematic review highlighting  
230 the need for further research on the field [8]. To our knowledge, the present study is the first to compare BMD values  
231 in a relatively large cohort of both vocational and professional ballet dancers. We found that female and male VBD  
232 have lower BMD values compared to matched-controls at both impact (FN and LS) and non-impact sites (forearm).  
233 It is noteworthy that the proportion of cases with low BMD ( $Z$ -Score  $< -2.0$ ) in female VBD was significantly higher  
234 compared to controls at both impact (LS) and non-impact sites (forearm); although not significant, male VBD  
235 demonstrated higher prevalence of low BMD at all three assessed anatomical sites. Nevertheless, after adjusting  
236 BMD for maturation markers (Tanner stage and gynecological age), we found similar values at impact sites (both FN  
237 and LS) in female VBD. This means that BMD differences between groups at these sites can be explained by the fact  
238 that our female VBD dancers are late matures compared to controls. However, maturation markers did not explain  
239 the lower BMD displayed by VBD (both female and male) at non-impact sites compared to controls, nor the lower  
240 BMD in male VBD at the LS. Considering female PBD, we found significantly higher unadjusted and adjusted BMD  
241 values at impact sites (FN) and significantly lower BMD at the forearm compared to matched controls. These  
242 findings suggest that weight-bearing exercise might be able to improve BMD despite a relatively low BM, an  
243 indication that such exercise might be able to override any potential negative effect. A similar result has been  
244 obtained for male PBD who did not reveal lower BMD compared to controls at any site. The latter confirms previous  
245 data [23] and could be partly explained by the fact that males have less pronounced endocortical resorption and  
246 higher periosteal expansion compared to females [24].

247 Dancing has been considered as a weight-bearing activity [13]. Studies using weight-bearing physical  
248 activities have shown positive effects on bone mineral accrual in both adults and children [25, 26]. Indeed, it has  
249 been suggested that 60 min x 3 a week of weight-bearing exercise is sufficient to prevent low BMD in general  
250 population [27]. Since our participants were vocational and professional dancers, they were involved in daily classes  
251 of several hours of weight-bearing activity [28, 29]. Considering data on bone cell biology and function of osteocytes  
252 as mechanosensory cells [30, 31], it would be expected to find significantly higher BMD values at impact sites  
253 (particular FN) and similar BMD values at non-impact sites compared to controls. However, dancing is also an  
254 aesthetic activity whereas body size is essential for performance. This requirement might place dancers at risk for  
255 low BM, a well-known risk factor for low bone mass phenotypes. Indeed, in our study, both VBD and PBD had  
256 significantly lower BM values compared to their controls. Further, compared to matched controls, female PBD also  
257 revealed higher prevalence of primary amenorrhea (and latter age at menarche), another well-known osteoporosis  
258 risk factor. Nevertheless, the fact that female PBD showed higher BMD at impact sites compared to controls  
259 suggests that dance training is able to stimulate BMD gains, even in the presence of osteoporosis risk factors. Indeed,  
260 female PBD only revealed lower BMD values compared to non-exercising controls at the forearm (non-impact site),  
261 which might indicate that exercise (dance training) can counterbalance the potential negative effects of osteoporosis  
262 risk factors at loading sites. However, it seems such a compensatory effect could not be seen in VBD since they  
263 demonstrated significantly lower bone mass at all studied anatomical sites. Actually, the prevalence of low BMD at  
264 the forearm and LS was also significantly higher in female VBD compared to controls. As LS is mainly constituted  
265 by trabecular bone (known to be more sensitive to mechanical stress from exercise [32]), and as ballet dancing  
266 requires high levels of muscular strength (placing considerable mechanical stress on lower back [28, 33]), it would  
267 not be expected to find a significantly higher number of cases with low BMD at this anatomical site compared to  
268 controls. It seems logical to suggest maturation as the reason for these findings in female VBD. Indeed, a



269 disproportionately high number of VBD were at Tanner stage I compared to controls, which might indicate that  
270 dancers are late matures. Delayed puberty has been linked with low BMD in children and adolescents [34]. Further,  
271 maturation markers (i.e. Tanner stage and gynaecological age) seem also to explain the differences in BMD at the  
272 FN in female VBD. This finding is not surprising due to selection criteria for professional dance training; children  
273 have to go through auditions for a place in a vocational dance school, where specific body stereotypes (small body  
274 size; ecto-mesomorphic body type) are essential for acceptance [35]. However, although maturation seems to explain  
275 the group differences in BMD at impact sites, this is not the case when the forearm (non-impact site) is considered.  
276 Indeed, in line with available data [10, 18, 36, 37, 38, 39], age at menarche, together with BM, LM FM, and energy  
277 intake, were significantly associated with BMD at the forearm; nevertheless, these factors seem not to explain BMD  
278 differences between female VBD and controls at this anatomical site. Considering male VBD, the present study did  
279 not find factors to explain the lower BMD values compared to controls at both impact (FN) and non-impact sites.  
280 Previous studies usually focus in female dancers as it is generally accepted that females have increased odds for low  
281 BMD. However, the present study suggests that young male dancers may also be at risk for low BMD. Future studies  
282 should also consider young male dancers in relation to BMD in different settings. Further, factors such as low  
283 energy availability, genetics and/or hormonal levels should be considered in future studies, given their association  
284 with low bone mass phenotypes [4, 40].

285         The current results regarding BMD in VBD might be of concern, as young dancers may enter adulthood  
286 with relatively low BMD, which may further impair the peak bone mass attainment [41]. Delayed puberty has been  
287 reported to be associated with lower IGF-1 levels and low bone mass in children and adolescents [34]; interestingly  
288 though, serum IGF-1 was not significantly different between VBD and controls (both in female and male), despite  
289 the difference seen in Tanner staging. Nevertheless, findings in children should be interpreted with caution due to  
290 biological changes which occur during growth [41]. Longitudinal studies should be conducted in VBD to ascertain  
291 how bone mass changes throughout growing.

292         The clinical significance of low BMD lies on the increased risk of fracture [3, 4]. We did not record  
293 fractures or injuries among our studied population. Nevertheless, recent data have shown that over one year period  
294 the incidence of injury in VBD was 1.42 per student and the risk of injury 76% [42]. Also, in PBD, a total of 355  
295 injuries were recorded during a year, with an overall incidence of 6.8 injuries per dancer [43]. However, to our  
296 knowledge, there are no available data on the association between dance injuries and low BMD [8]. Notwithstanding,  
297 the prevalence of Z-scores below -1.0 is significantly higher among our dance population compared with controls.  
298 Indeed, since athletes in weight-bearing sports usually have 5-15% higher BMD than non-athletes [4], the ACSM  
299 emphasizes that a BMD Z-score of < -1.0 in athletic populations should be further investigated, even in the absence  
300 of fractures [4]. However, to the best of our knowledge, there are no preventative/screening measures in dance  
301 population regarding overall dancers' bone health yet.

302         It is reasonable to assume that the present study might have been influenced by methodological limitations  
303 such as the use of a self-reported questionnaire to assess age at menarche, gynaecological age and Tanner stage. We  
304 also acknowledge the lack of injury and fracture records for our participants as well as alcohol intake. Another  
305 limitation may be that the current data incorporate dancers born and raised in north or south Europe, but performing  
306 at the same company. We further recognise the potential selection bias of the current participants since they were  
307 recruited from specific geographic regions. Finally, the assessment of bone geometry, a known determinant of bone  
308 strength, should also be considered in future studies to further substantiate the findings of this study.  
309

310

311 **CONCLUSIONS**

312 Compared to controls, female and male vocational ballet dancers demonstrated lower bone mineral density at impact  
313 and non-impact sites; maturation markers in the young female vocational dancers seem to explain these findings only  
314 at impact sites. In contrast, unlike male professional dancers who demonstrated a healthy bone mineral density  
315 profile, their female counterparts revealed lower bone mass at the studied non-impact site compared to controls, but  
316 higher values at impact sites. Future studies should explore how bone mass changes as vocational dancers grow and  
317 progress to professional level.

318

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327

328 **REFERENCES**

- 329 1. Harvey N, Dennison E, Cooper C (2010) Osteoporosis: impact on health and economics. *Nat Rev*  
330 *Rheumatol* 6(2):99-105.
- 331 2. Barrett-Connor E (1995) The economic and human costs of osteoporotic fracture. *Am J Med*  
332 98(suppl2A):S3-8.
- 333 3. International Society for clinical Densitometry. Updated 2013 official positions for adult and pediatric.  
334 <http://www.iscd.org/documents/2014/02/2013-iscd-official-position-brochure.pdf> (Accessed May 2, 2016).
- 335 4. Nattiv A, Loucks AB, Manore MM, et al. (2007) American College of sports Medicine position stand: the  
336 female athlete triad. *Med Sci Sports Exerc* 39(10):1867-82.
- 337 5. World Health Organization. WHO scientific group on the assessment of osteoporosis at primary health care  
338 level. WHO Summary Meeting Report, Brussels, WHO, 2004.
- 339 6. Scofield KL, Hecht S (2012) Bone health in endurance athletes: runners, cyclists, and swimmers. *Curr*  
340 *Sports Med Rep* 11(6):328-34.
- 341 7. Pollock N, Grogan C, Perry M, Pedlar C, Cooke K, Morrissey D, et al. (2010) Bone Mineral Density and  
342 other Features of the Female Athlete Triad in Elite Endurance Runners. *Int J Sport Nutr Exerc Metab*  
343 20:418-26.
- 344 8. Amorim T, Wyon M, Maia J, et al. (2015) Prevalence of low bone mineral density in female dancers. *Sports*  
345 *Med* 45:257-68.
- 346 9. Burckhardt P, Wynn E, Krieg, MA, et al. (2011) The effects of nutrition, puberty and dancing on bone  
347 density in adolescent ballet dancers. *J Dance Med* 15(2):51-60.
- 348 10. Keay N, Fogelman I, Blake G. (1997) Bone mineral density in professional female dancers. *Br J Sports Med*  
349 31:143-47.
- 350 11. Dolye-Lucas AF, Akers JD, Davy BM (2010) Energetic efficiency, menstrual irregularity, and bone  
351 mineral density in elite professional female ballet dancers. *J Dance Med Sci* 14(4):146-54.
- 352 12. Lichtenbelt WD, Fogelholm M, Otteenheim R, et al. (1995) Physical activity, body composition and bone  
353 density in ballet dancers. *Br J Nutr* 74:439-51.
- 354 13. Khan KM, Green RM, Saul, A, et al. (1996) Retired elite female ballet dancers and nonathletic controls  
355 have similar bone mineral density at weightbearing sites. *J Bone Miner Res* 11(10):1566-74.
- 356 14. To W, Wong M (2011) Does oligomenorrhea/ amenorrhea and underweight imply athlete female triad  
357 syndrome in young female dancers? *Eur J Sport Sci* 11(5):335-40.
- 358 15. Bachrach LK, Hastie T, Wang MC, et al. (1999) Bone mineral acquisition in healthy Asian, Hispanic, black,  
359 and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab* 84:4702-12.
- 360 16. Cobb KL, Bachrach LK, Greendale G, et al. (2003) Disordered eating, menstrual irregularity and bone  
361 mineral density in female runners. *Med Sci Sports Exerc* 35:711-19.
- 362 17. Practice Committee of the American Society for Reproductive Medicine (2004) Current evaluation of  
363 amenorrhea. *Fertil Steril* 82:266-72.
- 364 18. Dimitriou L, Weiler R, Lloyd-Smith R, Turner A, Heath L, James Nic, Reid A (2014) Bone mineral density,  
365 rib pain and other features of the female athlete triad in elite light weight rowers. *BMJ Open* 4(2):1-9.
- 366 19. Crawford PB, Obarzaner E, Morrison J, Sabry ZI (1994) Comparative advantage of 3-day food records over  
367 24-hour recall and 5-day food frequency validated by observation of 9- and 10-year-old girls. *J AM Diet*  
368 *Assoc* 94(6):626-30.

- 369 20. Duke PM, Litt IG, Gross RT (1980) Adolescent' self-assessment of sexual maturation. *Pediatrics* 66(6):918-  
370 20.
- 371 21. Pocock NA, Sambrook PN, Nguyen T, et al. (1992) Assessment of spinal and femoral bone density by Dual  
372 X-Ray absorptiometry: Comparison of lunar and hologic instruments. *J Bone Miner Res* 7(9):1081-84.
- 373 22. Hagiwara S, Engelke K, Yang S, et al. (1994) Dual X-ray absorptiometry forearm software: Accuracy and  
374 intermachine relationship. *J Bone Miner Res* 9(9):1425-27.
- 375 23. Fredericson M, Chew K, Ngo J, Cleek T, Kiratli J, Cobb K (2007) Regional bone mineral density in male  
376 athletes: a comparison of soccer players, runners and controls. *Br J Sports Med* 41(10):664-8.
- 377 24. Holroyd C, Cooper C (2008) Dennison E. Epidemiology of osteoporosis. *Best Pract Res Clin Endocrinol*  
378 *Metab* 22(5):671-85.
- 379 25. Guadalupe-Grau A, Fuentes T, Guerra B, et al. (2009) Exercise and bone mass in adults. *Sports Med*  
380 39(6):439-68.
- 381 26. Greene DA, Naughton GA (2006) Adaptive skeletal responses to mechanical loading during adolescence.  
382 *Sports Med* 36(9):723-732.
- 383 27. Vainionpää A, Korpelainen R, Leppäluoto J, Jämsä T (2005) Effects of high-impact exercise on bone  
384 mineral density: a randomized controlled trial in premenopausal women. *Osteoporosis Int* 16(2):191-7.
- 385 28. Koutedakis Y, Sharp NC (2004) Thigh-muscles strength training, dance exercise, dynamometry, and  
386 anthropometry in professional ballerinas. *J Strength Cond Res* 18(4):714-18.
- 387 29. Twitchett T, Angioi M, Koutedakis Y, et al. (2009) Video analysis of classical ballet performance. *J Dance*  
388 *Med Sci* 13(4):124-28.
- 389 30. Bonewald LF (2011) The amazing osteocyte. *J Bone Miner Res* 26(2):229-38.
- 390 31. Bonewald LF, Johnson ML (2008) Osteocytes, mechanosensing and Wnt signaling. *Bone* 42:606-15.
- 391 32. Heinonen A, Sievänen H, Kannus P, Oja P, Pasanen M, Vuori I (2000) High-Impact Exercise and Bones of  
392 Growing Girls: A 9-Month Controlled Trial. *Osteoporosis Int* 11:1010-17.
- 393 33. Koutedakis Y, Jamurtas AZ (2004) The dancer as a performing athlete: physiological considerations. *Sports*  
394 *Med* 34(10): 651-61.
- 395 34. Bounjour J, Chevalley T (2014) Pubertal timing, bone acquisition, and risk of fracture throughout life.  
396 *Endocr Rev* 35(5):820-47.
- 397 35. Claessens ALM, Beunen GP, Nuyts MM et al. (1987) Body structure, somatotype, maturation and motor  
398 performance of girls in ballet schooling. *J. Sports Med* 27:310-17.
- 399 36. Peel N (2014) Disorders of bone metabolism. *Surgery* 33(1):15-20.
- 400 37. Ma N, Gordon C (2012) Pediatric osteoporosis: where are we now. *J Pediatr* 161(6):983-90.
- 401 38. Hage RPE, Courteix D, Benhamou CL, Jacob C, Jaffré C (2009) Relative importance of lean and fat mass  
402 on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol* 105(5):759-64.
- 403 39. Ilich JZ, Kerstetter JE (2000) Nutrition in Bone Health Revisited: A Story Beyond Calcium. *J Am Coll Nutr*  
404 19(6):715-37
- 405 40. Eisman JA (1999) Genetics of osteoporosis. *Endocr Rev* 20(6):788-804.
- 406 41. Heaney RP, Abrams S, Dawson-Hughes B, et al (2000) Peak bone mass. *Osteoporosis Int* 11:985-1009.
- 407 42. Ekegren CL, Quested R, Brodrick A (2014) Injuries in pre-professional ballet dancers: incidence,  
408 characteristics and consequences. *J Sci Med Sport* 17(3):271-5.

- 409 43. Allen N, Nevill AM, Brooks JH, et al (2012) Ballet injuries: injury incidence and severity over 1 year. J  
410 Orthop Sports Phys Ther 42(9):780–90.  
411