

SUMMARY

- 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.
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ABSTRACT

- *Purpose:* The aims of this study were to a) assess bone mineral density (BMD) in vocational (VBD) and professional
- (PBD) ballet dancers, and b) investigate its association with body mass (BM), fat mass (FM), lean mass (LM), maturation and menarche.
- *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
- the lumbar spine (LS), femoral neck (FN), forearm and total body by Dual-energy X-ray absorptiometry. Maturation
- and menarche were assessed via questionnaires.
- *Results:* VBD revealed lower unadjusted BMD at all anatomical sites compared to controls (*p*<0.001); following
- adjustments for Tanner stage and gynaecological age, female VBD showed similar BMD values at impact sites.
- However, no factors were found to explain the lower adjusted BMD values in VBD (female and male) at the forearm
- (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.
- *Conclusions:* Both females and males VBD have lower BMD at impact and non-impact sites compared to control,
- whereas this is only the case at non-impact site in female PBD. Maturation seems to explain the lower BMD at impact sites in female VBD.
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- KEYWORDS: bone mass; prevalence; associated factors; elite dance; ballerinas
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INTRODUCTION

 Osteoporosis and osteopenia [i.e. low bone mineral density (BMD)] are recognised as the most frequent bone disorders, linked to high treatment costs and limited quality of life due to osteoporotic fractures [1, 2]. Hence, the identification of those at high-risk is crucial for planning appropriate prevention programmes. The diagnosis of low BMD in premenopausal women and children is based on the International Society of Clinical Densitometry (ISCD) guideline, whereas a diagnosis is confirmed when BMD values lie within 2.0 standard deviations (SD) or more 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 "osteoporotic" for BMD equal or less than -2.0 SD (along with secondary risk factors for stress fractures) [4]. Low BMD has been traditionally associated with elderly and postmenopausal women [5], though some

 athletic populations, as endurance athletes, might also be at increased risk [6, 7]. In ballet dancers, however, aspects regarding low BMD remain ambiguous [8]. While some authors underline the negative effects of professional dance training on bone metabolism (e.g. lean body type required for performance) [9-11], others suggest that the mechanical impact from dancing may provide a protection against low BMD, particularly at impact sites [12-14]. For instance, the high levels of muscular strength required for technical performance and weight-bearing activity 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.

METHODS

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 sample of 36 female VBD and 36 matched-controls, low BMD (*Z*-score of <-2.0) at the lumbar spine (LS) was found 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 α =0.05. Similarly, in a sample of 22 female PBD (22 matched-controls) and 10 male PBD (10 matched-controls), the prevalence of low BMD (*Z*-score of -1.0) at the LS was found to be 32% (vs. 5%) in 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, α =0.05). Assuming participants^{ϵ} non-response and possible dropouts, we approached two vocational dance schools and four professional ballet companies.

 To recruit participants, an introductory letter briefly explaining the purposes of the study was initially forwarded to the executive boards of the dance schools and companies. Following boards' agreement, the research team contacted the VBD (their guardians too) and PBD to present them with the studies aims and methodologies. 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 \pm 9.5yrs), 110 female, 50 males]. Female and male controls for VBD were involved in 2.4 \pm 0.5 and 2.1 \pm 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.
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129 **Anthropometry measurements, menstruation, smoking, nutrition intake, hormonal analysis and pubertal** 130 **assessment**

 Chronological age was obtained as decimal age (date of birth minus measurement date). Participants' height (m), 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 (kg.m⁻²). Female participants completed a questionnaire to determine age at menarche. Total lifetime menses (number of menses since menarche to current age) were calculated as previously described [16]. Primary amenorrhea was defined as the absence of menarche by 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 (IGF-1) was measured by immunoradiometric assay kit (IRMA, IMMUNOTECH SAS, Marseille, France), in an automated analyser (Wallac Wizard 1470, Finland). The assay ranges were from 2 to 1.200ng/mL). The intra-assay 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].

Body composition and bone measurements

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

157 It is known that Lunar and Hologic BMD measurements demonstrate high correlation values between them [21, 22]. It is also known that there is a tendency for Lunar model to inflate BMD values compared to Hologic [22]. Therefore, besides the daily calibration required from each DXA manufacturer, cross-calibration of the two scanners was also conducted on a group of 20 men and women; the age of these 20 participants covered the age-range of the entire sample (both dancers and controls) used for the purpose of the present study. The 20 participants were measured with both Lunar and Hologic within a period of 5 days. Subsequently, regression equations using BMD from Lunar as dependent variable and BMD from Hologic as independent variable were performed taking into account cross-calibration. The correlation between the two DXA models were high (forearm BMD: r=0.96, adjusted $r^2=0.93$, std. error of estimate=0.03; LS BMD: r=0.96, adjusted $r^2=0.92$, std. error of estimate=0.05; FN BMD: $r=0.97$, adjusted $r^2=0.93$, std. error of estimate=0.05). The Hologic BMD data were further converted to the Lunar data using the following equations: Forearm BMD Lunar = -0,085263 + 1,356535*Hologic; LS BMD Lunar = $0.030762 + 1.161805*$ Hologic; FN BMD Lunar = 0,084782 + 1,116509*Hologic. Following the BMD adjustments, *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).

Statistical analyses

 Independent t-tests were used to compare general characteristics between dance population and controls. Chi-square test was adopted to determine whether there is a significant difference in the distribution of Tanner stages between 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 between groups (i.e. VBD*matched controls, and PBD*matched controls). Consequently, BMD at each anatomical site (dependent variable) was adjusted for: BM, FM, LM, Tanner stage, age at menarche, gynaecological age, and 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" missing" using the SPSS software - version 20.0. We had missing data for FM (7.9% and 8.2% in VBD and PBD, respectively) and nutrition intake (15.1% and 18.8% in VBD and controls, respectively). Statistical significance was set at *p<*0.05.

RESULTS

 Table I depicts the general characteristics of all participants. Table I indicates that maturity differences between dancers and controls are more pronounced in female VBD than their male counterparts. Compared to controls, female and male VBD revealed significantly lower BM (by 10.8kg and 11.1kg, respectively; *p*<0.001), BMI (by μ 4.4kg/m² and 3.6kg/m², respectively; $p<0.001$) and FM (by 9.0kg and 8.0kg, respectively; $p<0.001$). In female VBD, 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 3.9kg/m² and 2.0kg/m², respectively; *p*<0.001) compared to controls. Female PBD also demonstrated significantly lower FM (by 10.3kg, *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 vitamin D intake, but both female and male VBD consumed significantly less calories per day compared to controls (by 215.1kcal/day or 13.2% and 278.0kcal/day or 17.4%, respectively, *p*<0.05). Serum IGF-1 concentrations were 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 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 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).

 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 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 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, *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). Table III confirms that our female PBD have higher adjusted BMD values at the FN (*p*<0.001), and lower adjusted 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 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 (FN and LS), and similar BMD values at the forearm; LM is positively associated with these findings at the LS (*p*<0.01).

 Table IV shows the prevalence of low BMD in VBD (*Z*-score < -2.0). Significantly higher prevalence of 222 low BMD at the forearm $(9.2\% \text{ vs. } 0\%, p=0.01)$ and LS $(16.4\% \text{ vs. } 5.5\%, p<0.05)$ 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.

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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

 disproportionally 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, maturation markers (i.e. Tanner stage and gynaecological age) seem also to explain the differences in BMD at the FN in female VBD. This finding is not surprising due to selection criteria for professional dance training; children 273 have to go through audience 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 the group differences in BMD at impact sites, this is not the case when the forearm (non-impact site) is considered. 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 differences between female VBD and controls at this anatomical site. Considering male VBD, the present study did 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 considerer young male dancers in relation to BMD in different settings. Further, factors such as low energy availability, genetics and/or hormonal levels should be considered in future studies, given their association 284 with low bone mass phenotypes [4, 40].

 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 biological changes which occur during growth [41]. Longitudinal studies should be conducted in VBD to ascertain how bone mass changes throughout growing.

 The clinical significance of low BMD lies on the increased risk of fracture [3, 4]. We did not record 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 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, the prevalence of *Z*-scores below -1.0 is significantly higher among our dance population compared with controls. Indeed, since athletes in weight-bearing sports usually have 5-15% higher BMD than non-athletes [4], the ACSM emphasizes that a BMD *Z*-score of < -1.0 in athletic populations should be further investigated, even in the absence of fractures [4]. However, to the best of our knowledge, there are no preventative/screening measures in dance population regarding overall dancers' bone health yet.

 It is reasonable to assume that the present study might have been influenced by methodological limitations such as the use of a self-reported questionnaire to assess age at menarche, gynaecological age and Tanner stage. We also acknowledge the lack of injury and fracture records for our participants as well as alcohol intake. Another limitation may be that the current data incorporate dancers born and raised in north or south Europe, but performing 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 strength, should also be considered in future studies to further substantiate the findings of this study.

CONCLUSIONS

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