1	Bone mineral density in vocational and professional ballet dancers			
2				
3	Tânia Amorim, MSc <sup>1,2</sup> , Yiannis Koutedakis, PhD <sup>2,3</sup> , Alan Nevill, PhD <sup>2</sup> , Matthew Wyon, PhD <sup>2,4</sup> , José Maia, PhD <sup>1</sup> ,			
4	José C. Machado, PhD <sup>5</sup> , Franklim Marques, PhD <sup>6</sup> , George S. Metsios, PhD <sup>2,3</sup> , Andreas D. Flouris, PhD <sup>3</sup> , Nuno			
5	Adubeiro, MSc <sup>7</sup> , Luísa Nogueira, PhD <sup>7</sup> , Lygeri Dimitriou, PhD <sup>8</sup>			
6				
7	<sup>1</sup> Centre of Research, Education, Innovation and Intervention in Sport, Faculty of Sports, University of Porto, Porto,			
8	Portugal			
9	<sup>2</sup> Faculty of Education, Health and Wellbeing, University of Wolverhampton, Walsall, UK			
10	<sup>3</sup> School of Sports and Exercise Sciences, University of Thessaly, Trikala, Greece			
11	<sup>4</sup> National Institute of Dance Medicine and Science, UK			
12	<sup>5</sup> i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal			
13	<sup>6</sup> Faculty of Pharmacy, University of Porto, Porto, Portugal			
14	<sup>7</sup> School of Health Technology of Porto, Polytechnic Institute of Porto, Porto, Portugal			
15	<sup>8</sup> London Sport Institute, Middlesex University, London, UK			
16				
17				
18	Corresponding author:			
19	Tânia Amorim			
20	Centre of Research, Education, Innovation and Intervention in Sport.			
21	Faculty of Sports, University of Porto			
22	Rua Dr. Plácido Costa 91			
23	4200 – 450 Porto, Portugal			
24	tania_amorim@hotmail.com			
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				

#### 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
- 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
- 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,
- whereas this is only the case at non-impact site in female PBD. Maturation seems to explain the lower BMD atimpact 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 (28±8.5yrs; 129 females, 55 males) were finally included in this study. Participants provided details on physical
exercise (hours per week). Female and male VBD reported to perform 18.2±7.0 and 19.5±7.2 hours per week of
dance training, respectively. Female and male PBD reported 32.9±8.4 and 32.5±9.6 hours per week of dance
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.

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

128

# Anthropometry measurements, menstruation, smoking, nutrition intake, hormonal analysis and pubertalassessment

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 (kg.m<sup>-2</sup>). 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].

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

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].
- 148

#### 149 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 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\*Hologic; LS BMD Lunar = 168 0,030762 + 1,161805\*Hologic; FN BMD Lunar = 0,084782 + 1,116509\*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 4.4kg/m<sup>2</sup> and 3.6kg/m<sup>2</sup>, 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 3.9kg/m<sup>2</sup> and 2.0kg/m<sup>2</sup>, 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).

Table IV shows the prevalence of low BMD in VBD (*Z*-score < -2.0). Significantly higher prevalence of 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 compared to controls. Although not significant, the proportion of cases with low BMD was higher in male VBD at 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 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, 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 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 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 considerer 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].

The current results regarding BMD in VBD might be of concern, as young dancers may enter adulthood with relatively low BMD, which may further impair the peak bone mass attainment [41]. Delayed puberty has been reported to be associated with lower IGF-1 levels and low bone mass in children and adolescents [34]; interestingly though, serum IGF-1 was not significantly different between VBD and controls (both in female and male), despite 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.

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.

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 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.

309

310

### 311 CONCLUSIONS

- 312 Compared to controls, female and male vocational ballet dancers demonstrated lower bone mineral density at impact313 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

# 319 ACKNOWLEDGEMENTS

- This work was supported by the Portuguese Foundation for Science and Technology [PhD grant number
   SFRH/BD/88585/2012]. Thanks are expressed to the radiology services of the Beatriz Angelo Hospital. A very
   special thanks goes to all participants who volunteered.
- 323
- 324 Tânia Amorim, Yiannis Koutedakis, Alan Nevill, Matthew Wyon, José Maia, José C. Machado, Franklim Marques,
- 325 George S. Metsios, Andreas D. Flouris, Nuno Adubeiro, Luísa Nogueira, and Lygeri Dimitriou declare that they 326 have no conflict of interest.
- 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.	Dolyle-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.	Litchtenbelt WD, Fogelholm M, Otteenheijm 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 trial	
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, Sieva nen 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
- Orthop Sports Phys Ther 42(9):780–90.