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SUMMARY

Less is known on bone mass gains in dancers involved in vocational dance training. The present study found that, as young vocational dancers progress on their professional training, their bone health remains consistently lower compared to non-exercising controls. Endocrine mechanisms do not seem to explain these findings.

ABSTRACT

Purpose Little is known on bone mass development in dancers involved in vocational training. The aim of the present study was to model bone mineral content (BMC) accruals and to determine whether circulating levels of oestrogens, growth hormone (GH) and insulin-like growth factor I (IGF-1) explain differences in bone mass gains between vocational dance students and matched-controls.

Methods The total of 67 vocational female dancers (VFD) and 68 aged-matched controls (12.1±1.9yrs and 12.7±2.0yrs at baseline, respectively) were followed for two consecutive years (34 VFD and 31 controls remained in the study for the full duration). BMC was evaluated annually at impact [femoral neck (FN); lumbar spine (LS)], and non-impact sites (forearm) using DXA. Anthropometry, age at menarche (questionnaire) and hormone serum concentrations (immunoradiometric assays) were also assessed for the same period.

Results VFD demonstrated consistently reduced body weight ($p<0.001$) and BMC at all three anatomical sites ($p<0.001$) compared to controls throughout the study period. Menarche, body weight, GH and IGF-1 were significantly associated with bone mass changes over time ($p<0.05$) but did not explain group differences in BMC gains at impact sites ($p>0.05$). However, body weight did explain the differences between groups in terms of BMC gains at the forearm (non-impact site).

Conclusion Two consecutive years of vocational dance training revealed that young female dancers demonstrate consistently lower bone mass compared to controls at both impact and non-impact sites. The studied endocrine parameters do not seem to explain group differences in terms of bone mass gains at impact sites.

KEYWORDS bone health, children, endocrinology, performance, training, BMC

INTRODUCTION

Bone tissue is constantly renewed by the coordinated activity of osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells) in a process known as bone remodelling [1]. This process is regulated by both local factors and systemic hormones [2,3]. The release of growth hormone-releasing hormone in the hypothalamus stimulates the production of growth hormone (GH) from the pituitary gland [2]. GH acts on its primary target - the liver - where it stimulates the production of insulin-like growth factor I (IGF-1) [3]. GH and IGF-1 regulate bone cells by enhancing osteoblast activity [4], reducing osteoblast apoptosis and promoting osteoblastogenesis through stimulation of Wnt/β-catenin activity [3]. Relatively low GH and IGF-1 levels are associated with low bone mineral density (BMD) [5,6].

GH and IGF-1 activities decrease with age [7], but they are particularly up-regulated during the growing years [6,8]. Rising levels of gonadal steroids (specifically oestrogens) during growing (particularly during early puberty) are followed by rising activity of GH and IGF-1 [8]. This means that endocrine mechanisms during adolescence and puberty constitute important determinants of bone mass acquisition [9]. Delayed puberty has been reported to be associated with low bone mineral content (BMC) in children and adolescents [6]. Considering young athletes in aesthetic sports, cross-sectional studies have shown that these participants have delayed puberty [10,11], while low bone mass values at both impact and non-impact sites have also been reported in young athletes [12–14]. Dancing, for instance, is a demanding activity [15] where appropriate physical fitness is required for optimal performance [16]. At professional level, dance is characterised by incidents of burnout, high injury rates [17–19], and has been linked to reduced BMD levels even in young female vocational dancers (pre-professional) with an average age of 13 years [20]. Interestingly, a recent cross sectional study has shown that prior to vocational dance training, young female vocational dancers already revealed low bone mass parameters [21]. However, to our knowledge, no longitudinal study thus far has examined how bone mass changes as young vocational dancers progress on their vocational dance training. Further, less is known on the factors associated with BMD in young dancers [22]. Therefore, we conducted a 2-years longitudinal study aiming at: 1) measuring BMC and BMD accruals in two groups consisting of female adolescent dancers and matched-controls, and 2) determining whether circulating levels of oestrogens, GH and IGF-1 could explain differences in bone mass gains between the two groups.

METHODS

Participants' recruitment

The present participants consisted of vocational female dance students (VFD). Since all dancers are subjected to auditions with an emphasis on leanness and low body weight [15], the study of these individuals could be insightful in order to understand different bone anabolic responses. Further, due to its nature, dancing also offers a great model of mechanical loading since it may differently affect the peripheral and axial skeleton. Dance training is characterised by numerous jumps and static technical skills that require high levels of muscular strength, inducing direct impacts on femoral neck (FN) and lumbar spine (LS) – impact sites - and no stimuli at the forearm (non-impact site) [15].

Projected power to detect differences between dance students and controls was based on a prior study from our group that has measured cross-sectionally first year female vocational dance students (n=34,

10.9±0.7yr) and matched controls (n=30, 11.1±0.5yr) (unpublished data); BMC at the FN (dancers: 2.95±0.69g/cm^2; controls: 3.67±0.7169g/cm^2) was selected as the main outcome given that literature suggests that in paediatric populations analysis on BMC outcomes should take precedence over BMD [23]. Assuming a 5% error and 90% power, calculations indicated that a sample of 40 volunteers was required for the present longitudinal study (20 dance students and 20 controls). To recruit the necessary VFD, an introductory letter explaining the purposes of the study was sent to the executive boards of a vocational dance school which offered 4-8 hours of dance training a day to professional dance aspirants. From the 106 female students that were enrolled at this vocational school for the academic year 2012/2013, 67 (63.2%) volunteered (no exclusion criteria for age were applied). All volunteers completed a questionnaire concerning their ethnicity (due to differences in bone mass according to ethnicities, only Caucasians were eligible for the study), physical activity, medical history and past/current calcium/vitamin D supplementation. None reported any medications/supplementation known to influence bone metabolism, nor reported illnesses/treatments that might affect bone metabolism; they described themselves as Caucasians and were involved in 16.3±6.5 hours of dance training per week.

Female children and adolescent non-dancers were also recruited from two random local state schools to act as controls. Eligibility criteria for controls were set according to dancers' characteristics (i.e. age and race). Exclusion criteria included current or past participation in sport activities outside their school curriculum, as well as those who had received/were receiving medications known to affect bone metabolism and to who reported illnesses/treatments that might affect bone metabolism. The total of 68 (17.4%) female students met the inclusion/exclusion criteria and were enrolled (participants had 2.4±0.5 hours/week of exercise physical education classes). Details on the participants' recruitment appear in Figure 1.

All participants provided signed informed consent according to the Declaration of Helsinki. The study was approved by the ethics committee of the Regional Administration of Health of Lisbon, Portugal (Proc.063/CES/INV/201

Participants' measurements

Data were collected annually for three consecutive years, started at January 2013 and finished at March 2015. Annual collection occurred within the same period as the baseline measuring session. Specifically, information on bone mass, anthropometry, menarche and biological maturation were collected each January for VFD and each March for controls. Bloods were collected each January for both groups.

All 67 (100%) VFD available for assessment at baseline (January 2013) underwent anthropometric and bone assessments and reported past/current menstrual status, but only 51 (76.1%) provided blood samples. From 2013 to 2014, 12 new VFD were recruited, while 17 withdraw from the study due to dropout or injuries. In 2014, therefore, a total of 62 VFD underwent anthropometric measures, participated in bone measurements and reported past/current menstrual; 59 (95.2%) donated blood. From 2014 to 2015, an additional of 16 VFD withdraw the study for the reasons previously mentioned; a total of 46 VFD were assessed in 2015 [all underwent anthropometric and bone measurements, menarche, and 40 (87.0%) donated blood].

Considering controls, at baseline (March 2012), 68 (100%) controls underwent anthropometric measures, participated in bone measurements and reported menarche; 38 (55.9%) donated blood. From 2013 to

2014, 24 controls withdraw the study due to family relocation or lost of interest; 44 were available for assessment: all 44 (100%) participated in anthropometric, bone measurements and reported menarche; 32 (72.7%) donated blood. In 2015 an additional of 13 controls withdraw the study due to family relocation. From the 31 available for assessment in 2015, all underwent anthropometric, bone measurements and reported menarche; 23 (74.2%) donated blood. Details on the participants' measurements appear in Figure 1.

Anthropometry, menarche and maturation assessment

Chronological age (obtained as decimal age) and anthropometric measurements were collected at one-year interval. Height, sitting height and body weight were measured in t-shirt, shorts and bare feet using a stadiometer (Seca217 portable stadiometer, Hamburg, Germany) with accuracy of 0.1 cm and an electric scale (TANITA BC-418 MA Segmental Body Composition Analyser; Tanita, Tokyo, Japan) with an accuracy of 0.1 kg.

At one-year interval, age of menarche was determined by questionnaire or email during the follow-up. Biological maturity was assessed using the offset equation [24]. Based on this equation, the year(s) to/from peak height velocity (PHV) and an estimation of the age at PHV were predicted in all participants at one-year interval.

Hormonal analyses

Blood samples were collected early morning after an 8-hour fasting. In menstruating subjects, samples were collected during the follicular phase of the menstrual cycle (fifth and ten days after the onset of menstrual bleeding). Plasma oestrogens concentrations were assessed by electrochemiluminescence immunoassay (ECLIA) kit (06656021190 Estradiol G3 Elecsys cobas and 100, Roche Diagnostic Systems); the intra-assay and inter-assay coefficient of variation (CV) were below or equal to 2.4% and 2.7%, respectively. Serum GH were evaluated by an immunoradiometric assay kit (IRMA GH, ref. IM1397) from IMMUNOTECH SAS, (Prague, Czech Republic); the intra-assay and inter-assay CV's were below or equal to 2.7% and 7.1%, respectively. Serum IGF-1 concentrations were measured by an immunoradiometric assay kit (IRMA IGF-I, ref. A15729) from IMMUNOTECH SAS, (Marseille, France); the intra-assay and inter-assay CV's were below or equal to 6.3% and 6.8%, respectively. Blood samples were submitted to centrifugation at 2500g for 10 min; plasma and serum samples were stored at -80ºC for future analyses.

Bone measurements

BMC (g) and BMD (g/cm^2) were determined for non-dominant forearm (33% radius), lumbar spine (L1-L4) (LS) and femoral neck (FN). Participants were assessed by the same experienced technician in two different centres, using the Lunar (GE Lunar Prodigy) and Hologic (Discovery Wi) dual-energy X-ray absorptiometry (DXA).

Although a high correlation between Lunar and Hologic DXA BMD measurements has been previously established [25], there is a tendency for Lunar model to inflate BMD values by 15% compared to Hologic [26]. Therefore, a cross-calibration of the scanners was conducted using a group of 20 participants; the age of these 20 participants covered the age-range of the current sample (both dancers and controls). These participants were measured with both Lunar and Hologic within a period of 5 days. Regression equations were then conducted using BMC and BMD values from Lunar as dependent variables and BMC and BMD from Hologic as the

independent ones. The correlation between the two DXA models were high (forearm BMD: r=0.96, adjusted r^2=0.93; LS BMD: r=0.96, adjusted r^2=0.92; FN BMD: r=0.97, adjusted r^2=0.93; forearm BMC: r=0.98, adjusted r^2=0.96; LS BMC: r=0.96, adjusted r^2=0.92; FN BMC: r=0.94, adjusted r^2=0.88). The Hologic BMC and 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; forearm BMC Lunar=0,148564+1,117715*Hologic; LS BMC Lunar = 7,143123 + 0,923483*Hologic; FN BMC Lunar = 0,079107+ 1,106219*Hologic.

Statistical analyses

Exploratory analyses were conducted using the SPSS 20.0 software (IBM SPSS, Chicago, IL) to check for the presence of outliers (via the Kolmogorov-Smirnov test); 2 dance students and 3 controls lied in abnormal distances from other values and were excluded. Independent t-tests were employed to compare general characteristics between dance population and controls at each measured occasion. Nonparametric tests (Mann–Whitney test) were applied if the data were not normally distributed; this was the case for the GH, IGF-1 and oestrogens. Repeated measures ANOVA were utilised to compare characteristics between the two groups across the measurement occasions. Based on a multilevel approach (hierarchical linear models) applied to longitudinal data, SuperMix software (SSI - Scientific Software International, Inc.) was used to investigate the predictors (i.e. age, body weight, height, menarche, oestrogen, GH and IGF-1) of bone mass accrual over time in each anatomical site, and to determine if the aforementioned factors could explain differences between our two groups. These analyses are appropriate for study designs where data are organised in more than one level (in this case, participants are organized into two groups); multilevel models can be used without the assumption of homogeneity that is required by ANCOVA. Chronological age was used as the metric of time: time 0 corresponds to mean value (on average around 12 years of age); negative values at X axis represents the number of years before mean chronological age, whereas positive values represent number of years after mean chronological age. The level of significance was set at $p<0.05$.

RESULTS

Table 1 shows the general characteristics of the current participants. At the onset of the study, participants had a mean chronological age of about 12 years (VFD: 12.1±1.9; controls: 12.7±2.0, $p>0.05$). Over time VFD always revealed a significantly lower body weight and BMC/ BMD values at all anatomical sites compared to controls. VFD had their menarche approximately one year later than controls ($p<0.001$), but the estimated age at PHV did not significantly differ between groups (~12 years old for both groups). Serum IGF-1 concentrations were significantly higher in dance students than controls at the 2yr follow-up year ($p<0.001$). When participants were aligned according to their age at menarche (Graph 1), VFD continued to display lower BMC at all anatomical sites compared to controls with the same age at menarche. In both groups (and at all anatomical sites), BMC gains decrease after menarche.

Table 2 summarises the coefficients that a) predict bone mass changes over time, and b) identify potential factors that might explain differences in BMC/ BMD between groups (variable*group). The interaction between groups for BMC gains (i.e. chronological age*group) was not significant at impact sites; VFD always

revealed lower BMC values at these anatomical sites than controls throughout the 2-years study period. In contrast, the interaction chronological age*group revealed significantly positive values for BMC at the forearm (non-impact site) and BMD at all the other anatomical sites; baseline differences between groups in terms of bone mass values have been accentuated during the follow-up. Menarche and body weight were found to be significant predictors of bone mass accruals throughout the follow-up at all anatomical sites, whereas serum concentrations of IGF-1 were significant predictor only at the forearm (Table 2). GH also had a significant predictive effect on BMD at the FN and forearm. Nevertheless, when a group interaction were analysed, it was not found a significant interaction at impact sites (both FN and LS) between menarche*group and weight*group; these variables (menarche and body weight) did not explain group differences in bone mass gains at the FN and LS. However, a significant group effect was found at the non-impacted site (forearm), indicating that BMC and BMD accruals differ between groups according to body weight. We found no significant interaction between GH and IGF-1 with group (GH*Group; IGH-1*Group), meaning that GH and IGF-1 concentrations did not explain group differences in bone mass gains at the forearm when comparing VFD with controls.

DISCUSSION

It is generally accepted that both professional and VFD have increased odds for low BMD compared to non-dancers [22,27]. However, the associated factors are not completely clear [22]. To the best of our knowledge, this is the first longitudinal study aiming to investigate bone mass accruals and its association with circulating levels of oestrogens, GH and IGF-1 in VFD. The main finding was the low BMC and BMD values displayed by dance students in relation to controls at baseline as well as the absence of a "catch-up" accrual throughout the 2-years follow-up. Interestingly, endocrine mechanisms do not seem to explain differences between VFD and non-exercising controls in terms of bone mass gains at impact sites.

Bone mass accruals significantly increased during the follow-up in both groups, as well as circulating levels of oestrogens, GH and IGF-1. This was expected since our population was in a growing phase [28]. Clinical work revealed that growing bone is more responsive to mechanical loading than mature bone [29]; during puberty osteogenic hormones are likely to interact with physical exercise to positively affect bone mass accruals [29]. Considering our results, at 2-yr follow-up, IGF-1 serum concentrations were significantly increased in our VFD. These increments might reflect a dance training effect since higher circulating levels of serum IGF-1 has been documented in young athletes via GH-independent mechanism [3,30], leading to greater bone mass accruals than their non-exercising counterparts. However, in our dancers the higher increments in IGF-1 serum levels seem not to be translated into higher bone mass gains. Indeed, it would have been expected that bone mass differences between groups have lessen during the follow-up (particularly at impact sites), not only due to increasing levels of circulating IGF-1, but also due to the effects of dance training [31,32]; this was not the case. It seems unlikely that group differences were caused by delayed growth/maturity, as a) age at PHV and GH/oestrogens concentrations did not differ between groups, b) body weight was a significant predictor of bone mass differences between groups only at the forearm, and c) by comparing groups according to their age at menarche, VFD continued to display lower BMC at all anatomical sites compared to controls. Instead, we suggest that skeletal biological determinants might be involved as the dynamic actions of liver-derived IGF-1 in bone involve complex signalling pathways that might directly affect both osteoblasts and osteoclasts [4]. IGF-1

can either act on the commitment of mesenchymal stem cells to oesteoprogenitor cells [33], or induce RANKL synthesis in osteoclasts, leading to osteoclastogenesis [3]. Whether the aforementioned factors explain the low BMC and BMD values seen in our dancers compared to controls warrant further investigation.

Bone mass formation and development are influenced by genetic and endocrine mechanisms that are modulated by environmental factors such as physical exercise [34,35]. Therefore the degree to which physical exercise influences bone formation depends on individual's genetic background [35,36], and bone specific characteristics (e.g., cortical or trabecular bone) [37]. The fact that GH and IGF-1 serum concentrations were significantly associated with bone mass values only at non-impact sites (forearm), might indicate that gene-environment factors are interacting differently in determining dancers' bone mass phenotypes across skeletal sites. Moreover, the fact that body weight, a well-known risk factor for low bone mineralization, was found to be a significant predictor of group differences in bone mass gains at forearm, but not at impact sites, further supports the aforementioned hypothesis. Essentially, the absence of a "catch-up" bone mass accrual by dancers in relation to controls at the FN and LS was rather surprising. Other studies on athletic populations also found that adolescent runners with low bone mass values at baseline continued to display low bone parameters after 2-yr follow-up [38]. The determinants of bone mass accruals in athletic populations may be influenced by specific sport adaptations and body characteristics used for selection purposes. Therefore, future studies on dancers (and on other elite athletes) should consider genetic markers as well as gene-environment interactions to further understand the pathogenesis of low bone mass parameters, particularly at weight-bearing sites. Poor diet and menstrual disturbances are also known to impair bone mass acquisition. We found that our sample of dancers had their menarche a year later than controls; interestingly though, this factor did not explain group differences in bone mass gains at impact sites (FN and LS). In fact, when VFD were compared to controls according to their age at menarche (and not by chronological age), dancers continued to display lower bone mass gains throughout the length of the study.

Although this is the first study which longitudinally investigated bone mass accruals and bone osteogenic hormones in vocational dance students, our findings should be interpreted with caution. Firstly, the present data are observational and cause-effect cannot be determined. Also, although our sample of vocational dancers is large and well-defined considering the entire population of elite dance students performing at a national level (response rate was 63.2%), we nevertheless acknowledge that this sample size was relatively small for a longitudinal design. Further, our sample was going through a period of significant biological changes. This might explain the high variance of hormonal values in both groups, which reinforces the need for future studies with larger sample sizes. The clinical significance of low BMD lies on fractures. Another point might be that although the incidence of injuries in elite dancers is high [39,40], the relationship between injuries and bone mass phenotypes in has not yet been investigated. Future studies should establish the relationship among injuries and bone mass outcomes. Furthermore, the relationship between vitamin D and bone mass acquisition is well established. As our population of VFD trained indoors (meaning no vitamin D synthesis from sun exposure), future studies should also examine vitamin D serum levels in determining dancers' bone health. Finally, the use of two different DXA scans to assess participants and the need to adjust the data for potential bias is a limitation. However, this approach has been previously used [41,42].

CONCLUSION

Young female vocational dance students have lower bone mass values compared to matched-controls at both impact and non-impact sites throughout the 2-year follow up. Endocrine mechanisms do not seem to explain the differences in terms of bone mass gains between groups at impact sites.

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