ORIGINAL ARTICLE

Associations between nutrition, energy expenditure and energy availability with bone mass acquisition in dance students: a 3-year longitudinal study

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Abstract

Summary Three years of study showed that female and male vocational dancers displayed lower bone mass compared to controls, at forearm, lumbar spine and femoral neck. Energy intake was found to positively predict bone mass accruals only in female dancers at femoral neck. Vocational dancers can be a risk population to develop osteoporosis.

Purpose To determine whether risk factors normally associated with low bone mass in athletic populations (i.e. nutrition intake, energy expenditure and energy availability) are significant predictors of bone mass changes in vocational dance students.

Methods The total of 101 vocational dancers (63 females, 12.8 ± 2.2 years; 38 males, 12.7 ± 2.2 years) and 115 age-matched controls (68 females, 13.0 ± 2.1 years; 47 males, 13.0 ± 1.8 years) were monitored for 3 consecutive years. Bone mass parameters were measured annually at impact sites (femoral neck, FN; lumber spine, LS) and non-impact site (forearm) using DXA. Nutrition (3-day record), energy expenditure (accelerometer), energy availability and IGF-1 serum concentration (immunoradiometric assays) were also assessed.

Results Female and male vocational dancers had consistently reduced bone mass at all anatomical sites ($p < 0.001$) than controls. IGF-1 did not differ between male vocational dancers and controls, but female dancers showed it higher than controls. At baseline, calcium intake was significantly greater in female vocational dancers than controls ($p < 0.05$). Male vocational dancers' fat and carbohydrate intakes were significantly lower than matched controls ($p < 0.001$ and $p < 0.05$, respectively). Energy availability of both female and male vocational dancers was within the normal range. A significant group effect was found at the FN regarding energy intake ($p < 0.05$) in female dancers. No significant predictors were found to explain bone mass differences in males.

Conclusion Our 3-year study revealed that both female and male vocational dancers displayed lower bone mass compared to controls, at both impact and non-impact sites. The aetiology of these findings may be grounded on factors different than those usually considered in athletic populations.

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Introduction

Osteoporosis occurs when bone resorption exceeds bone formation [1]. This imbalance leads to reduced bone mass and strength, microarchitecture changes of bone tissue and increased fracture risk [1]. Bone mineral density (BMD) is frequently used to diagnose osteoporosis and predict bone fracture risk [2, 3]. Osteoporosis is traditionally associated with the elderly and postmenopausal women [4]. In other populations, such as elite dancers, this clinical condition, along with low BMD, has been recognized as a significant health concern [5, 6].

It has been emphasized that professional and vocational dance students have low bone mass values compared to controls or normative values at several skeletal sites [7–10]. The main risk factors include low body weight, menstrual disturbances and low energy availability, the inter-relationship of which is known as the "female athlete triad" [11] or "relative energy deficiency in sport" (RED-S) [12]. Indeed, as an aesthetic activity, dancers are exposed to high levels of artistic and physical fitness demands, whereas low body weight is strongly emphasized [13]. As a result, elite dancers may restrict their energy intake, leading to a negative energy balance [6–8].

This energy imbalance can affect the hypothalamus, decreasing the circulating levels of IGF-1; this hormone is sensitive to changes in nutrition intake, particularly caloric intake, hindering further bone mineralization [14]. However, to the best of our knowledge, the question of whether the aforementioned factors are critical in determining dancer's bone health warrants further investigations, given that the mechanisms and risk factors associated with low BMD in dancers are not fully understood [15]. Therefore, the aims of the present study were to determine (a) whether nutrition, energy expenditure and energy availability are significant predictors of bone mineral content (BMC) and BMD changes throughout growth in female and male vocational dance students and (b) whether these factors are able to explain difference in bone mass accruals between vocational dancers and matched controls.

Methods

Study duration and design

The study lasted for 3 consecutive years. Information on bone mass, anthropometry, menstruation, biological maturation (estimation of the age at peak height velocity (PHV)), nutrition (energy intake, calcium, fat, carbohydrates) and energy availability were collected each January for vocational dance students, whereas the same information was collected each March for controls. Blood samples were collected each January in both groups. Information on physical exercise frequency and bone-specific loading was collected at the third year using a validated questionnaire.

Participants

The participants' recruitment process was conducted as previously described [16], and the relevant flow chart appears in Fig. 1. Calculations were performed based on a sample of female dance students ($n = 34$, 10.9 ± 0.7 years) and matched controls ($n = 30$, 11.1 ± 0.5 years); BMC at the FN (dancers: 2.95 ± 0.69 g/cm^2; controls: 3.67 ± 0.72 g/cm^2) was
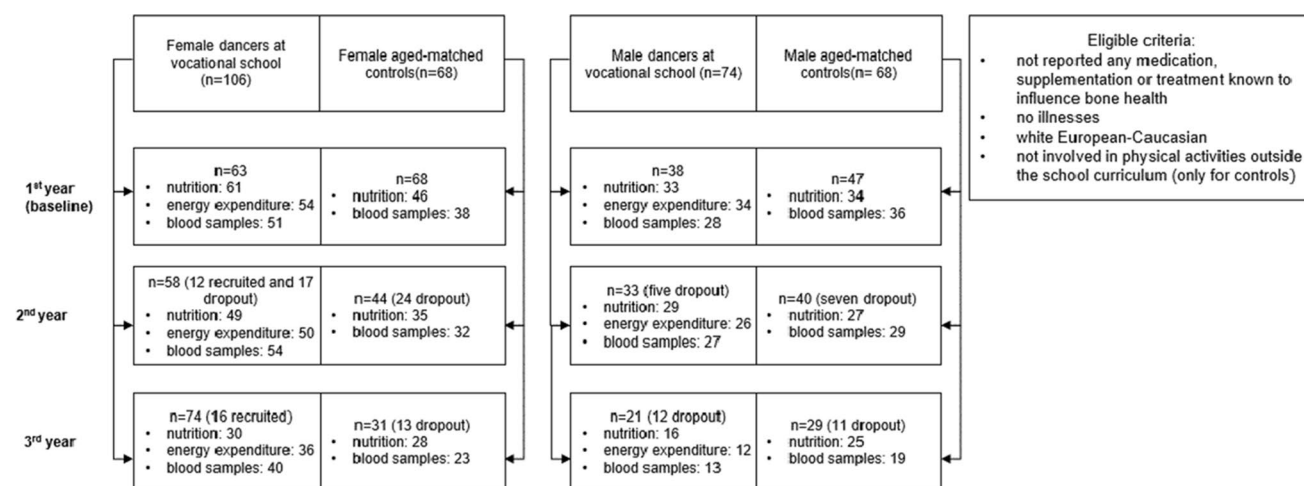

Fig. 1 Flow chart of study population to investigate if normal risk factors (nutrition intake, energy expenditure and energy availability) are significant predictors of bone mass in vocational dance students

All sample for each year participated in anthropometric, bone and past/current menstruation assessments

considered. Assuming a 5% error and 90% power, calculations indicated that a minimum sample of 40 volunteers (20 dance students and 20 controls) was required for the present longitudinal study. From a total of 106 female students enrolled at the vocational school (school that offers professional dance training) in the academic year of study, 63 (59.4%) volunteered; none reported any medication, supplementation or treatment known to affect bone metabolism. All 63 described themselves as white European-Caucasian. Also, out of the total of 74 male dance students enrolled at the vocational school for the same academic year, 38 (51.4%) volunteered. As none reported any medication, supplementation or treatment known to affect bone metabolism, all 38 were included. They also described themselves as white European-Caucasian.

Regarding female controls, out of the 68 females recruited on for a previous study [16], 50 agreed to provide information on nutrition for the purposes of the present study. The recruitment process adopted for the male controls has been previously described in detail [16]; 68 (8.0%) male students met the inclusion/exclusion criteria and were further enrolled in the study.

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/2012).

Data collection

All dance students underwent baseline anthropometric measures, participated in bone measurements and reported past/current menstrual (only female). Sixty-one females and 33 males (96.8% and 86.8, respectively) provided nutrition data. Moreover, 54 females and 34 males (85.7% and 89.5, respectively) were assessed for energy expenditure, while 51 females and 28 males (80.9% and 73.7%, respectively) provided blood samples for analyses. From baseline to year one, 12 female dance students were additionally recruited, while 17 females and 5 males withdrew from the study due to professional dance training dropout or illness. For year one, a total of 58 female and 33 male dance students underwent anthropometric measures, participated in bone assessments and reported past/current menstruation. Also, 49 female (vs. 29 male) (84.5% and 87.9%, respectively) reported nutrition intake, 50 females and 26 males (86.2% and 78.8%, respectively) were assessed for energy expenditure, and 54 females and 27 males (93.1% and 81.8%, respectively) provided blood samples. From year one to year two, 16 additional female dance students were recruited, and 12 male vocational dancers withdrew for the reasons previously reported. A total of 74 female and 21 male dance students were assessed in year 2. They all underwent anthropometric and bone measurements, as well as reported past/current

menstruation, 30 females and 16 males (71.4% and 76.2%, respectively) reported nutritional intakes, 36 females and 12 males (85.7% and 57.1%, respectively) were assessed for energy expenditure, and 40 females and 13 males (95.2% and 61.9%, respectively) provided blood samples.

All age-matched controls underwent baseline anthropometric measures, participated in bone measurements and reported past/current menstruation (females), while 46 females and 34 males (92.0% and 72.3%, respectively) reported nutrition information, and 38 females and 36 males (76.0% and 76.6%, respectively) provided blood samples. From baseline to year one, only 44 female and 40 male controls were available for assessment (24 females and 7 males withdrew the due to family relocation or loss of interest). They participated in anthropometric and bone measurements and reported past/current menstrual status. Thirty-five females and 27 males (79.5% and 67.5%, respectively) provided nutrition information, while 32 females and 29 males (79.5% and 72.5%, respectively) provided blood samples. In year two, 13 female and 11 male controls withdrew from the study due to family relocation. From the 31 and 29 controls available for assessment in year two, all underwent anthropometric, bone measurements and reported past/current menstruation. A total of 28 females and 25 males (90.3% and 86.2%, respectively) reported nutrition information, while 23 females and 19 males (74.3% and 65.5%, respectively) provided blood samples.

Anthropometry, menarche and biological maturation assessment

Height, sitting height and body weight were measured in t-shirt, shorts and bare feet using a stadiometer (Seca, Seca217 portable stadiometer, Hamburg, Germany) with accuracy of 0.1 cm and an electric scale (TANITA BC-418 MA Segmental Body Composition Analyser; Tanita Corporation, Tokyo, Japan) with an accuracy of 0.1 kg. All measurements were administered by the same investigator every year.

Age of menarche was determined by questionnaire or email during the follow-up. Biological maturity was assessed using the offset equation [17]. Based on this equation, the year(s) to/from PHV and an estimation of the age at PHV were predicted in all participants at 1-year interval.

Nutritional intake and energy availability

Nutrient intakes were recorded via a validated 3-day food diary [18]. Participants were asked to record all food and beverages consumed during two school days and one weekend day following suitable instructions. The software Food Processor SQL Edition, version 9.8.1., was used to estimate average energy and nutrition intakes. During the same week

that nutrition information was collected, energy expenditure was also estimated using an accelerometer, SenseWear [19]; each participant used the device for 7 consecutive days. Energy availability was further estimated using standard formula [energy availability = energy intake (kJ) − energy expenditure during exercise (kJ))/fat-free mass (kg)]; information on dietary energy intake (provided by the food diary), exercise energy expenditure (information retrieved from the accelerometer) and body fat percentage (obtained from DXA) was used for the estimation of energy availability.

Hormonal analyses

Blood samples were collected in early morning after an 8-h fast. Blood samples were submitted to centrifugation at 2500 g for 10 min; plasma and serum samples were stored at − 80ºC until they were analysed. In males (both dancers and controls), 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 coefficients of variation were below or equal to 6.3% and 6.8%, respectively. The IGF-1 data for female vocational dancers and their controls were retrieved from one of our previous studies but assessed via immunoradiometric assays [16].

Physical exercise in terms of bone-specific loading

Participants were asked to record all regular physical exercise activities performed throughout their lives, including years and frequency (times per week) of participation using the bone-specific physical activity questionnaire—BPAQ [20] (considering their age, this questionnaire was filled in by their parents on the third year of data collection). This questionnaire has been originally validated for young adults, and several reports have appeared using it in children and adolescents to predict bone-relevant weight-bearing exercise [21–23]. We did not apply this test to our controls given that they have been selected on the basis that they participated or had previously participated in no structured physical exercise outside school curriculum. To account for the school physical education lessons (twice a week), the following activities for control participants were considered: walking/hiking, running/jogging, soccer and jump rope.

Bone measurements

Bone mineral content (BMC) (g) and BMD (g/cm^2) were measured at the non-dominant forearm (33% radius), lumbar spine (L1–L4) (LS) and femoral neck (FN). Participants were assessed in two different centres using dual-energy X-ray absorptiometry (DXA): Lunar (GE Lunar Prodigy) and Hologic (Discovery Wi). The same certified technician performed all scans and analyses at both centres. Besides the daily calibration required from each DXA manufacturer, cross-calibration of the scanners was conducted using a group of 20 participants, the age of whom covered to the age range of the entire sample (both dancers and controls) we used. These 20 individuals were measured with both Lunar and Hologic within a period of 5 days. Regression equations using BMC and BMD from Lunar as dependent and Hologic as independent variable were performed based on the participants' data through cross-calibration of DXA; the Hologic BMC and BMD data were further converted to the Lunar data using previously described equations [16, 24, 25].

Statistical analyses

Exploratory analyses were conducted using SPSS 20.0 (IBM SPSS, Chicago, IL) to check for the presence of outliers (boxplots, histograms and scatterplots were used to identify the outliers). Independent *t*-tests were employed to compare general characteristics (age, weight, height, age at menarche and nutritional parameters) between vocational dancers and controls at each measured occasion (stratified by sex). Based on a multilevel approach applied to longitudinal data, SuperMix software (SSI—Scientific Software International, Inc.) was used to investigate the predictors of bone mass accrual over time in each anatomical site. These analyses considered to be appropriate for study designs where data are organized in more than one level (in this case, participants were organized into groups); multilevel models can be used without the assumption of homogeneity that is required by ANCOVA. Chronological age (Fig. 2) was used as the metric of time: time 0 corresponds to mean chronological age (on average around 12 years of age); negative values at X axis represent the number of years before mean chronological age, whereas positive values signify the number of years after mean chronological age. The level of significance was set at $p < 0.05$.

Results

General characteristics of all participants appear in Table 1. At the start of the study, both female and male participants had a mean chronological age of approximately 12 years old (female dancers: 12.8 ± 2.2, female controls: 13.0 ± 2.1, $p > 0.05$; male dancers: 12.7 ± 2.2, male controls: 13.0 ± 1.8, $p > 0.05$). For the study's duration, female and male vocational dancers constantly demonstrated significantly lower body weight ($p < 0.001$) compared to matched controls. Female vocational dancers had their menarche approximately 1 year later than controls ($p < 0.001$), but the estimated age at PHV did not differ between groups (~ 12 years
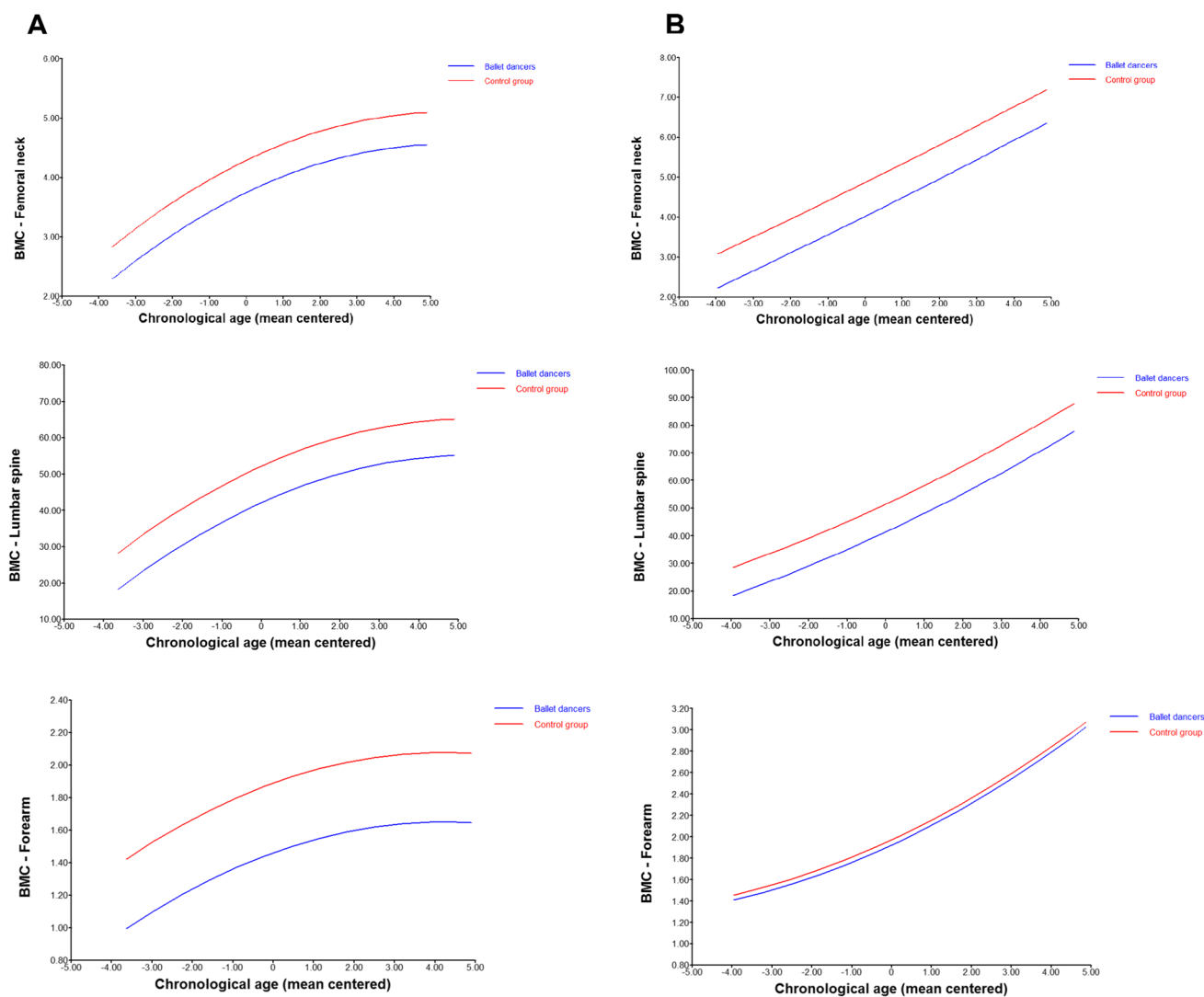
Fig. 2 Bone mineral accrual in female (**A**) and male (**B**) vocational dance students and their aged- sex-matched controls. Chronological age is represented as years from/to the mean chronological age.

BMC-FN, bone mineral content femoral neck; BMC-LS, bone mineral content lumbar spine; BMC-FA, bone mineral content forearm. Units for bone mineral content (BMC) are in grams (g)

old for both groups); the age at PHV in male vocational dancers was ~6 months later than their controls ($p < 0.001$). At baseline, calcium intake was significantly increased in female dancers than controls ($p < 0.05$), but there was no difference between groups throughout the study's duration. Male dancers' fat and carbohydrate intakes were significantly lower than matched controls ($p < 0.001$ and $p < 0.05$, respectively). Energy availability of both female and male dancers was within the normal range (i.e. > 30 kcal/kgFFM/day). Serum IGF-1 concentrations were significantly higher in female dancers compared to controls at 2-year follow-up ($p < 0.001$); their male counterparts reported no significant difference ($p > 0.05$). The amount of current and past physical exercise relevant to the skeleton was significantly higher in female dancers compared to controls: score of 67.7 ± 11.7

vs. 3.8 ± 3.7, $p < 0.001$ (current exercise), and 20.7 ± 11.4 vs. 1.3 ± 1.5, $p < 0.001$ (past exercise), respectively. The same was also the case in male dancers compared to controls: score of 74.1 ± 3.8 vs. 4.3 ± 2.3, $p < 0.001$ (current exercise), and 22.3 ± 5.0 vs. 2.1 ± 0.9, $p < 0.001$ (past exercise), respectively.

Bone mass accruals over time are displayed in Fig. 2. Regarding the BMC gains in females, the interaction between group and age (i.e. chronological age*group) was not significant ($p > 0.05$), meaning that female vocational dancers always revealed lower BMC values at all anatomical sites than controls. This was also the case for BMD at the forearm ($p > 0.05$). In contrast, the interaction chronological age*group for BMD at the FN and LS was significantly positive (BMD FN: 0.02, $p < 0.01$; BMD LS: 0.02, $p < 0.01$).

Table 1 General characteristics of the female and male vocational dance students and aged- and sex-matched controls

Female vocational dance students

	1st year (baseline)		2nd year		3rd year	
	VD ($n=63$)	Controls ($n=50$)	VD	Controls	VD	Controls
Age (yrs.)	12.8 ± 2.2	13.0 ± 2.1	$13.6 \pm 2.0^{+++}$	$14.0 \pm 2.1^{+++}$	$14.9 \pm 1.9^{+++}$	$15.0 \pm 2.0^{+++}$
Weight (kg)	$39.3 \pm 9.0^{***}$	52.7 ± 12.8	$43.1 \pm 8.1^{***\ +++}$	$55.2 \pm 10.7^{+++}$	$44.5 \pm 6.7^{***\ +++}$	$58.6 \pm 12.4^{++}$
Height (cm)	151.7 ± 10.5	154.6 ± 8.8	$154.5 \pm 8.1^{+++}$	$156.8 \pm 8.1^{+++}$	$156.9 \pm 6.6^{+++}$	158.4 ± 6.9
Age menarche (yrs.)[(1)]	12.5 ± 1.4	11.5 ± 1.2	-	-	-	-
Age at PHV (yrs.)[(1)]	12.6 ± 1.2	12.6 ± 0.8	-	-	-	-
Energy intake (Kcal/day)	1724.5 ± 508.0	1855.3 ± 479.2	1787.75 ± 444.98	1824.53 ± 674.76	1722.5 ± 412.0	1673.4 ± 460.0
Calcium (mg)	$827.2 \pm 360.2^*$	652.33 ± 332.0	871.8 ± 487.1	718.9 ± 316.1	758.71 ± 297.3	756.0 ± 234.9
Carbohydrates (g)	224.6 ± 69.9	238.8 ± 85.5	222.77 ± 58.59	229.23 ± 99.95	$206.7 \pm 48.49^+$	274.4 ± 346.1
Fat (g)	$59.1 \pm 83.7^*$	83.7 ± 46.9	19.68 ± 2.81	25.75 ± 4.70	61.8 ± 20.4	$55.5 \pm 19.3^+$
Energy availability (kcal/kgFFM/day)	46.9 ± 22.9	-	$39.3 \pm 18.8^{+++}$	-	31.0 ± 13.1	-
IGF-1 (ng/mL)	304.4 ± 120.7	314.5 ± 115.7	329.21 ± 152.05	298.00 ± 111.09	$334.5 \pm 104.7^*$	261.7 ± 63.3
Bone-specific loading (current)	-	-	-	-	$67.7 \pm 11.7^{***}$	3.8 ± 3.7
Bone-specific loading (past)	-	-	-	-	$20.7 \pm 11.4^{***}$	1.3 ± 1.5
BMC FN (g)	$3.54 \pm 0.95^{**}$	3.97 ± 0.89	$3.73 \pm 0.90^{***\ +++}$	$4.51 \pm 0.71^{+++}$	$3.76 \pm 0.77^{***\ ++}$	$4.65 \pm 0.68^+$
BMC LS (g)	$38.53 \pm 13.8^{***}$	48.77 ± 12.85	$41.67 \pm 13.21^{***\ +++}$	$54.38 \pm 10.38^{+++}$	$43.76 \pm 11.94^{***\ +++}$	$57.23 \pm 9.31^{++}$
BMC FA (g)	$1.37 \pm 0.31^{***}$	1.84 ± 0.33	$1.46 \pm 0.31^{***\ +++}$	$1.92 \pm 0.28^{+++}$	$1.52 \pm 0.29^{***\ +++}$	1.95 ± 0.27
BMD FN (g/cm^2)	$0.93 \pm 0.17^{***}$	1.08 ± 0.14	$0.94 \pm 0.19^{**\ +++}$	1.06 ± 0.13	$0.93 \pm 0.20^{***}$	$1.13 \pm 0.21^+$
BMD LS (g/cm^2)	$0.90 \pm 0.17^{***}$	1.07 ± 0.18	$0.91 \pm 0.19^{***\ +++}$	$1.14 \pm 0.14^{+++}$	$0.93 \pm 0.17^{***}$	$1.20 \pm 0.15^{++}$
BMD FA (g/cm^2)	$0.60 \pm 0.09^{***}$	0.76 ± 0.10	$0.63 \pm 0.10^{***\ +++}$	$0.81 \pm 0.08^{+++}$	$0.66 \pm 0.10^{***\ +++}$	$0.84 \pm 0.08^{+++}$

Male vocational dance students

	1st year (baseline)		2nd year		3rd year	
	VD ($n=38$)	Controls ($n=68$)	VD	Controls	VD	Controls
Age (yrs.)	12.7 ± 2.2	13.0 ± 1.8	$13.7 \pm 2.1^{+++}$	$13.9 \pm 1.7^{+++}$	$14.8 \pm 2.2^{+++}$	$14.7 \pm 1.6^{+++}$
Weight (kg)	$44.6 \pm 13.5^{**}$	54.8 ± 13.2	$45.7 \pm 9.2^{***\ +++}$	$59.7 \pm 13.5^{+++}$	$49.2 \pm 11.0^{*\ ++}$	$59.9 \pm 12.5^{+++}$
Height (cm)	156.6 ± 15.8	159.8 ± 10.3	$160.5 \pm 11.3^{++}$	$163.5 \pm 9.7^{+++}$	$163.2 \pm 9.7^{++}$	$165.7 \pm 8.2^{+++}$
Age at PHV (yrs.)[(1)]	$12.7 \pm 1.0^{***}$	12.2 ± 0.8	-	-	-	-
Energy intake (Kcal/day)	1599.3 ± 463.9	1900.0 ± 451.8	1698.7 ± 396.6	2080.5 ± 467.6	1900.3 ± 280.2	1880.3 ± 455.3
Calcium (mg)	725.3 ± 370.0	645.82 ± 214.59	808.3 ± 334.6	$866.6 \pm 284.0^+$	714.8 ± 212.9	$850.0 \pm 405.5^+$
Carbohydrates (g)	$211.2 \pm 68.5^*$	232.8 ± 84.2	213.0 ± 54.2	253.4 ± 81.9	232.9 ± 27.8	224.5 ± 74.76
Fat (g)	$54.60 \pm 19.23^{***}$	82.39 ± 22.77	60.2 ± 17.4	70.6 ± 19.3	71.1 ± 10.9	61.3 ± 17.5
Energy availability (kcal/kgFFM/day)	36.2 ± 18.1	-	29.2 ± 11.7	-	26.9 ± 13.0	-
IGF-1 (ng/mL)	257.8 ± 71.5	241.8 ± 73.7	$272.7 \pm 97.2^+$	266.0 ± 106.7	281.5 ± 96.9	321.5 ± 125.5
Bone-specific loading (current)	-	-	-	-	$74.1 \pm 3.8^{***}$	4.3 ± 2.3
Bone-specific loading (past)	-	-	-	-	$22.3 \pm 5.0^{***}$	2.1 ± 0.9
BMC FN (g)	$3.93 \pm 1.32^*$	4.55 ± 0.98	$4.06 \pm 1.01^{***\ +++}$	$5.14 \pm 1.20^{+++}$	$4.56 \pm 1.39^{++}$	$5.33 \pm 1.28^{+++}$
BMC LS (g)	$39.97 \pm 19.16^*$	47.07 ± 12.43	$41.75 \pm 17.76^{**\ +++}$	$54.34 \pm 15.38^{+++}$	$46.24 \pm 10.06^+$	$56.75 \pm 15.87^{+++}$
BMC FA (g)	$2.25 \pm 0.98^*$	1.83 ± 0.33	$1.53 \pm 0.36^{***\ +++}$	$2.06 \pm 0.40^{+++}$	1.78 ± 0.60	$2.11 \pm 0.44^{+++}$
BMD FN (g/cm^2)	$0.90 \pm 0.23^{***}$	1.13 ± 0.18	$0.91 \pm 0.20^{**\ +++}$	$1.07 \pm 0.25^+$	1.10 ± 0.23	$1.14 \pm 0.18^+$
BMD LS (g/cm^2)	$0.82 \pm 0.21^{***}$	0.98 ± 0.18	$0.84 \pm 0.20^{***\ +++}$	$1.05 \pm 0.21^{+++}$	0.95 ± 0.24	$1.04 \pm 0.21^{+++}$
BMD FA (g/cm^2)	$0.59 \pm 0.10^{***}$	0.72 ± 0.10	$0.62 \pm 0.09^{***\ +++}$	$0.80 \pm 0.12^{+++}$	$0.67 \pm 0.12^{**+++}$	0.81 ± 0.11

Values are means $\pm$ SD

$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; statistical significant differences between groups

$^+p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; statistical significant differences within groups

VD vocational dance students, *PHV* peak height velocity (estimation), *BMC* bone mineral content, *BMD* bone mineral density, *FN* femoral neck, *LS* lumbar spine, *FA* forearm in 33% radius, *GH* growth hormone, *IGF-1* insulin-like growth factor 1

[(1)]Means calculated considering all pre-professional ballet dancers and controls assessed throughout the 3 years

The only significant interaction found in male participants (chronological age*group) was at the FN (BMC: 0.24, $p < 0.05$).

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) are summarized in Table 2. A significant group effect was found at the FN regarding energy intake in female dancers (i.e. energy intake*group): female dance students will have higher bone mass values at this site (0.00004 higher, $p < 0.05$), providing that their energy is higher compared to controls of the same age and height. No predictors were found that could explain the lower BMC values displayed by female dancers compared to controls at the LS and forearm. In males, body weight was a significant predictor of bone mass accruals throughout the study at the FN and LS (BMC FN: $\beta = 0.04$, $p < 0.05$; BMC LS: $\beta = 0.44$, $p < 0.05$; BMD). However, no significant predictors were found able to explain bone mass differences between groups (i.e. male dancer vs. controls).

Discussion

Vocational dance students and professional dancers are thought to be at risk of developing low BMD and osteoporosis later in life. This is supported by several studies [8, 26–28], while international bodies consider dancers' bone health as a topic of major concern [6]. However, unlike dance injuries and their aetiology [29, 30], little is known in relation to vocational dancers' bone health (both in female and male young dancers) [15]. To our knowledge, the present study is the first to longitudinally investigate the association between bone mass accruals and nutrition energy availability in adolescent male vocational dance students. We found longitudinal evidence that young vocational dancers' BMC and BMD (both female and male) are lower than controls and that nutrition (calcium, fat, carbohydrates) were not significant predictors of bone mass gains. Energy intake was found to positively predict bone mass accruals only at the FN in female dancers.

It was not surprising to find that body weight was significantly lower in all occasions compared to controls in both female and male dancers. This is probably because professional dancing emphasizes leanness and an aesthetic silhouette, putting young dancers at increased risk for a relative low energy availability due to restricted diets (to achieve ideal body shapes) [6–8]. The latter could place them closer to the female athlete triad/RED-S and, consequently, low BMD. However, as our dancers' energy availability were within the normal range, it is rather unlikely that this parameter would trigger the female athlete triad and/or RED-S. Interestingly, energy availability was a predictor for FN

BMC only in female dancers. In fact, although all participants revealed the same chronological age, pubertal development and, hence, bone status [31], between sexes could be completely different. Besides, post-pubertal period is more favourable to improve bone characteristics than pre-puberty [32]. Indeed, energy deficiency and low energy intake have been linked to low BMD phenotypes through the hormone IGF-1 [14]. Nevertheless, IGF-1 serum concentrations in our female vocational dancers were similar and even higher (at 2-year follow-up) compared to controls. Likewise, IGF-1 serum concentrations did not differ between our male dancers and their matched controls. These findings increase the improbability for our dancers to develop RED-S. However, since energy availability was positively associated with bone mass gains in our female dancers at FN (impact site), it is tempting to suggest that dancers would have even lower BMC and BMD values compared to controls, if their energy availability was below the normal range. Future studies should assign dancers into normal BMD and low BMD to investigate whether energy intake and energy availability predict low bone mass phenotypes.

The few studies which examined male dancers failed to agree whether BMD levels are higher or lower than controls [33, 34]. Nevertheless, it has been suggested that young male dance students may too be at risk for low BMD as it was found that adjusted BMD values for body weight and maturation were significantly lower compared to controls at both impact and non-impact sites [24]. This is in line with the present findings where the absence of a "catch-up" bone accrual has been noticed during the 3-year study period; male dancers' bone mass values remained consistently lower compared to non-exercising controls. Interestingly though, risk factors such as body weight, maturation, menarche, energy availability and nutrition intake seem not to support the aforementioned results. It is also interesting to stress that the amount of exercise found herein relevant to bone was significantly higher in both female and male dancers compared to controls. Consequently, it would be expected to find pointedly higher bone mass accruals compared to controls [35], which clearly was not the case. It might be that genetic mechanisms are mediating the degree of bone mass gains from exercise stimuli [36–39], which should be properly explored in future studies.

Our findings should be interpreted in light of their limitations. Firstly, our results have been based on a mixed longitudinal design with a relatively small sample size. Secondly, the main outcome—BMC, measured via DXA—does not account for changes in bone microarchitecture. However, DXA (as well as BMC as main outcome) is considered the gold standard to longitudinally assess children [40]. Also, the energy availability could have changed during the academic year, and the three evaluation points could not be a representation of the true energy availability throughout the

Table 2 Predictors of bone mass changes throughout the follow-up in vocational dance students

Estimates for female vocational dance students and controls

Predictors	BMC FN	BMC LS	BMC FA	BMD FN	BMD LS	BMD FA
Intercept	3.77 ± 0.10	44.77 ± 1.21***	1.52 ± 0.04	0.65 ± 0.01***	0.92 ± 0.02***	0.95 ± 0.03***
Chronological age	0.28 ± 0.03***	2.00 ± 0.44***	0.01 ± 0.01***	0.01 ± 0.004**	0.02 ± 0.01***	NS
Chronological age^2	−0.03 ± 0.001***	NS	NS	−0.02 ± 0.001**	−0.002 ± 0.001*	NS
Group	0.25 ± 0.13*	0.99 ± 1.70***	0.26 ± 0.06***	0.13 ± 0.02***	0.12 ± 0.03***	0.10 ± 0.03**
Energy intake	NS	NS	NS	−0.0001 ± 0.00001**	NS	NS
Calcium	NS	NS	NS	NS	NS	NS
Carbohydrates	NS	NS	NS	NS	NS	NS
Fat	NS	NS	NS	NS	NS	NS
Energy availability	0.01 ± 0.003*	NS	NS	NS	NS	NS
Chronological age*group	NS	NS	NS	0.02 ± 0.001**	0.02 ± 0.01**	NS
Energy intake*group	-	-	-	0.00004 ± 0.0002*	-	-
Calcium*group	-	-	-	-	-	-
Carbohydrates*group	-	-	-	-	-	-
Fat*group	-	-	-	-	-	-

Estimates for male vocational dance students and controls

Predictors	BMC FN	BMC LS	BMC FA	BMD FN	BMD LS	BMD FA
Intercept	4.13 ± 0.18	42.06 ± 1.98***	1.97 ± 0.09***	0.89 ± 0.04***	0.85 ± 0.03***	0.60 ± 0.01**
Chronological age	NS	2.28 ± 0.95*	NS	NS	NS	0.02 ± 0.01**
Chronological age^2	0.05 ± 0.01***	0.95 ± 0.16***	NS	0.01 ± 0.004**	0.01 ± 0.002***	0.05 ± 0.01**
Group	0.48 ± 0.20*	5.52 ± 2.24*	NS	0.18 ± 0.05***	0.12 ± 0.03***	0.14 ± 0.02***
Weight	0.04 ± 0.02*	0.44 ± 0.22*	NS	NS	NS	NS
Height	NS	0.41 ± 0.19*	NS	NS	NS	NS
Energy intake	NS	NS	NS	NS	NS	NS
Calcium	NS	NS	NS	NS	NS	NS
Carbohydrates	NS	NS	NS	NS	NS	NS
Fat	NS	NS	NS	NS	NS	NS
Energy availability	NS	NS	NS	NS	NS	NS
IGF-1	NS	NS	NS	NS	NS	NS
Chronological age*group	0.24 ± 0.09*	NS	NS	NS	NS	NS
Weight*group	NS	NS	-	-	-	-
Height*group	-	NS	-	-	-	-
Energy intake*group	-	-	-	-	-	-
Calcium*group	-	-	-	-	-	-
Carbohydrates*group	-	-	-	-	-	-
Fat*group	-	-	-	-	-	-
IGF-1*group	-	-	-	-	-	-

Values are estimates ± standard errors

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; significant predictor

Energy availability prediction was estimated considering only data from vocational dance students

NS not significant, *BMC* bone mineral content, *BMD* bone mineral density, *FN* femoral neck, *LS* lumbar spine, *FA* forearm, *GH* growth hormone, *IGF-1* insulin-like growth factor 1

year. The lack of data on energy availability in controls is a further limitation; however, the present controls did not perform any extracurricular exercise, and their energy intakes were not below recommended. Finally, the use of a self-reported questionnaire to assess bone-specific loading and nutrition intake should be considered.

Conclusion

During the present 3-year follow-up study, female and male vocational dancers demonstrated consistently lower bone mass values compared to controls, at both impact and non-impact sites. The aetiology of these findings may be

grounded on factors different than those considered by the female athlete triad/RED-S.

Data availability All data and material are real and available if it is requested.

Code availability Not applicable.

Declarations

Ethics approval Study design protocol and consent forms involving human participants were in accordance with the Helsinki Declaration (1964) and approved by the ethics committee of the Regional Administration of Health of Lisbon, Portugal (Proc.063/CES/INV/2012).

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest None.

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