

# Interval Changes in Bone Mineral Density in Exercising Young Women with or without Menstrual Dysfunction—an 18-month Longitudinal Study

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## Objectives:

To evaluate the bone mineral density changes of the axial and appendicular skeleton in a group of collegiate dance students undergoing intensive physical training over an 18-month interval and to assess any differences between those with or without menstrual dysfunction during their training.

## Methods:

Full-time collegiate dance students were recruited from a tertiary performing arts institute. All subjects had basic anthropometric assessment, dual energy X-ray absorptiometry, and quantitative peripheral computed tomographic scans to determine bone mineral density. The measurements were then repeated around 18 months after the initial assessment. Subjects who had suffered from oligomenorrhoea and amenorrhoea during the 18-month training were compared to those who had remained eumenorrhoeic. All subjects were also compared to a group of non-exercising eumenorrhoeic controls of comparable age.

## Results:

There was no significant difference in the initial bone mineral density measurements of the axial and appendicular skeleton between the two groups. The exercising group showed larger interval increments over the 18-month interval in lumbar spine bone mineral density as well as hip bone mineral density values as compared to non-exercising controls (n=19), which had minimal increments ( $p<0.001$ ). Within the exercising group, those who developed oligo/amenorrhoea (n=10) showed marginally lower core trabecular bone mineral density increments as compared to those that remained eumenorrhoeic (n=26).

## Conclusion:

Young women undergoing regular intensive weight-bearing exercises as in the collegiate dancers here studied have higher bone mineral density accrual as compared to non-exercising females of the same age-group. Oligo/amenorrhoea during training could have a small negative impact on such bone accrual.

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## Introduction

In adults the mass of bony tissue present and bone mineral density (BMD) at any time is a net result of the amount achieved at maturity and that lost with ageing. The bone mass acquired at the end of the growth period is a major determinant of the BMD in later life, and is thus pivotal in dictating the risks of osteoporosis

and fractures. Puberty and late adolescence is a critical period in the acquisition of bone mass, and has a direct

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impact on the final peak bone mass achieved for an individual. Many factors independently affect bone mass accrual, notably genetics, sex, dietary components of calcium and proteins, endocrine factors, and mechanical forces. The responsiveness of bone to either an increase or decrease in mechanical strain is probably greater in growing bones in adolescents than in adults<sup>1</sup>, so that weight-bearing exercises should have an important impact on stimulating bone accrual in strategic weight-bearing sites<sup>2,3</sup>. While most cross-sectional data support the benefits of exercise in children and adolescents, these findings were not consistent in all longitudinal studies relating bone mass to physical activity<sup>4,5</sup>. There is also controversy as to whether areal BMD gains in response to exercise could be translated into a commensurate increase in volumetric BMD increase or an increase in bone strength<sup>6</sup>.

In dancers, intensive dance training is often associated with under-nutrition or genuine eating disorders due to the need for stringent body weight control. These two elements are frequently associated with oligomenorrhoea and amenorrhoea to form the well-described athlete triad syndrome<sup>7</sup>, which in turn is well-known for its association with osteoporosis<sup>8</sup> and an increased risk of stress fractures. Thus, the occurrence of this syndrome can obviously decimate any benefits that dance exercises have on BMD. The objective of this study aims at investigating the effect of intensive dance training on axial and appendicular BMD in a longitudinal cohort of young female dance students in their late teens, and the differences in interval BMD changes between those that remained eumenorrhoeic during the training period and those that developed oligo/amenorrhoea, using a group of non-exercising young women of comparable age as controls.

## Methods

The subjects analysed in this study were recruited from two sources. The young dancers were recruited on a voluntary basis from full-time degree or diploma students from a dance school in a collegiate academy of performing arts. All were of Chinese ethnic origin, and all had completed at least 12 months of training in the institution in either ballet, Chinese dance, or modern dance. All subjects were aged between 17 and 22 years, and all were assessed to be healthy and free of significant medical disorders as part of their school

admission medical requirement. Each dancer trained for a minimum of 18 hours per week, either in the form of class exercise, skills training, or rehearsals. The research on these dance students was approved by the review board of their institution. The non-exercising young female controls were recruited from an adolescent gynaecology clinic of a general district hospital. All subjects were assessed to be free from significant medical disorders and were not on hormones or oral contraceptives in the past 6 months prior to recruitment. Only those who were of comparable age to the dancers, were eumenorrhoeic, and who were not engaged in full-time competitive sports, nor had regular weight-bearing exercise of over 3 hours per week were included in the subsequent analysis and comparison. The study of control subjects was approved by the local ethics committee of the hospital. Written consent was obtained for all participants.

The evaluation of both groups of subjects consisted of a detailed structured questionnaire that collected data on basic epidemiological aspects, including age of menarche, training history and current training pattern if applicable, as well as other sports activities taken in leisure hours. For dance students, particular details of the amount of exercise undertaken by these subjects, including the years of previous training, the total number of hours of training per week, and the distribution of the time spent in basic exercises, foot work, and rehearsals were obtained. All subjects were asked about their menstrual patterns in the past 12 months and factors that might influence menstrual function. Subjects were asked of particular question on the regular use of oral contraceptives or other therapeutic hormones for over 3 months in the past year, so that they would be excluded from recruitment.

A physical evaluation was performed, which included basic weight and height, measurements, and estimation of their body fat composition using bioelectrical impedance analysis method by means of a Tanita Body Fat Analyser TBF 501 (Tanita, Japan). The BMD of the anterior-posterior lumbar spine (L2 to L4) and proximal femur including the femoral neck, greater trochanter, and the Ward's triangle were measured using dual energy X-ray absorptiometry (DXA) utilising the Norland XR26 Mark II system (Norland Medical System Inc, WI, USA). Absolute BMD values were used in the subsequent analysis. Quality control scans of

the system were performed daily with a manufacturer-supplied anthropometric spine phantom. Values were expressed in  $\text{g}/\text{cm}^2$ . The non-dominant distal radius and bilateral distal tibia were measured with the Densiscan 2000, a high precision multislice peripheral quantitative computerised tomography (pQCT) system (Scano Medical, Zurich, Switzerland) with an effective X-ray energy of 40 keV and a local radiation dose of less than 50 $\mu\text{Sv}$  for a four-slice screening program. A standard Institute for Biomedical Engineering (ETH/UNI, Zurich) phantom measurement was performed daily for quality control. The forearm or lower legs were positioned in a radiolucent anatomically fitting splint during the pQCT scanning. After displaying an anterior-posterior projection scout view, a reference line was set perpendicular to the long axis of the limb and fixed on the middle point of the end plate of the distal radius or tibia. For the four-slice program, the first distal slice started at 7 mm from the reference line, with three further slices made proximal to the first slice at an interval of 4.5 mm between each slice, with a slice thickness of 1 mm. The average volumetric BMD of the trabecular bone in a core volume (central 50% of the total bone cross-sectional area) and total BMD of both the cortical and trabecular bone (total cross-sectional area) within the bone volume were measured for statistical analysis. All values were expressed in  $\text{mg}/\text{cm}^3$ . Cross-sectional area and mean cortical thickness of the radius or tibia were calculated from the mean of the slices taken using manual graphics together with a computer program.

Both groups of participants were asked to repeat the questionnaire, the basic anthropometric measurements, and the DXA and pQCT scans for BMD after an 18-month interval from the first assessment, with a range of 16 to 20 months. Based on their reported menstrual pattern in the 18 months between the two assessments, they were stratified into the oligo/amenorrhoeic group or eumenorrhoeic group. Amenorrhoea was defined as no menstruation for over 90 days for any period of time over the past 18 months, oligomenorrhoea was defined as mean cycle lengths of 43 to 90 days irrespective of the amount of flow, and eumenorrhoea was defined as cycles between 25 and 42 days. None of the subjects reported cycle lengths shorter than 25 days. No particular temporal pattern was noted within the 18 months in the development of menstrual dysfunction in the study group. As it was intended that

the control group should consist of only eumenorrhoeic subjects, those with oligo/amenorrhoeic patterns within the study interval were excluded from further analysis. In the dancers with oligo/amenorrhoea, a full hormonal profile, including prolactin levels, thyroid function tests, follicular stimulating hormone levels, and testosterone levels were taken. Pelvic ultrasound was performed to exclude polycystic ovarian syndrome.

Statistical analysis was performed using the student's *t* test and Mann Whitney *U* tests for continuous variables when appropriate. A *p* value of  $<0.05$  was considered significant. Data were analysed using the Statistical Package for the Social Sciences version 14.0 (SPSS, Chicago, IL, USA).

## Results

The final analysis consisted of 36 dancers, of whom 26 (72.2%) were eumenorrhoeic and 10 (27.7%) were categorised as oligo/amenorrhoea between the two assessments, and 19 eumenorrhoeic controls. The mean age of the dancers and the controls were comparable, and their mean age of menarche did not differ. Prolactin levels and thyroid function tests were normal in all the study and control subjects. Based on pelvic ultrasound findings and hormonal profile results, none of the dancers with oligo/amenorrhoea satisfied the clinical criteria for the diagnosis of polycystic ovarian diseases. Follicular stimulating hormone levels were all within reproductive age ranges.

The dancers had significantly lower body mass index and lower body fat percentage as compared to the controls, as well as a significantly later menarche age (Table 1). There was no significant difference in the BMD values of the lumbar spine or hip between the two groups, nor were there any differences between the appendicular skeleton as measured at the distal radius or tibia (Table 1). Comparing the interval changes of the various parameters at the 18-month reassessment, the dancers showed significant increments in their lumbar spine and hip BMD values, but the increments in the control group were minimal. The differences were statistically significant at the lumbar spine, neck of femur and Ward's triangle (Table 2). There were however no demonstrable differences in the changes in the distal radius or tibial volumetric BMD values. When the dancers were divided into the oligo/amenorrhoeic

**Table 1. Comparison between basic characteristics of exercising and non-exercising subjects**

	Exercising (n=36) <sup>†</sup>	Non-exercising (n=19) <sup>†</sup>	p value; mean difference (95% confidence interval)
Age (years)	18.36 (1.46)	18.63 (0.76)	0.45; -0.27 (-0.99 to 0.45)
Height (cm)	159.25 (3.64)	158.21 (5.68)	0.41; 1.04 (-1.48 to 3.56)
Weight (kg)	46.3 (3.54)	48.1 (6.79)	0.20; -1.79 (-4.58 to 0.98)
Body mass index (kg/m <sup>2</sup> )	18.24 (1.2)	19.13 (1.89)	0.03; -0.88 (-1.72 to -0.05)
Body fat (%)	19.33 (3.63)	23.15 (3.51)	<0.001; -3.82 (-5.86 to -1.78)
Age of menarche (years)	12.64 (0.93)	11.84 (1.11)	0.007; 0.80 (0.23 to 1.36)
Lumbar spine L2-L4 (g/cm <sup>2</sup> )	0.924 (0.089)	0.971 (0.147)	0.14; -0.047 (-0.11 to 0.016)
Mean neck of femur (g/cm <sup>2</sup> )	0.909 (0.074)	0.867 (0.138)	0.14; 0.042 (-0.014 to 0.099)
Mean Ward's triangle (g/cm <sup>2</sup> )	0.75 (0.092)	0.766 (0.131)	0.58; -0.016 (-0.078 to 0.044)
Mean trochanter (g/cm <sup>2</sup> )	0.754 (0.079)	0.711 (0.117)	0.11; 0.042 (-0.011 to 0.096)
Distal radius core BMD* (mg/cm <sup>3</sup> )	264.55 (66.8)	277 (76.6)	0.53; -12.4 (-52.4 to 27.5)
Distal radius total BMD (mg/cm <sup>3</sup> )	545.5 (52.9)	564.9 (62)	0.23; -19.36 (-51.3 to 12.6)
Distal tibia core BMD (mg/cm <sup>3</sup> )	304.8 (39.7)	315.6 (40.8)	0.34; -10.8 (-33.6 to 12)
Distal tibia total BMD (mg/cm <sup>3</sup> )	599.3 (112)	625.7 (111)	0.40; -26.4 (-90.2 to 37.2)

\* BMD denotes bone mineral density

<sup>†</sup> Standard deviation is shown in brackets

and eumenorrhoeic subgroups, comparison of the oligo/amenorrhoeic group with eumenorrhoeic non-dancing controls showed the same differences as before (Table 3). On the other hand, when comparing the oligo/amenorrhoeic dancers with the eumenorrhoeic dancers, no significant differences in the BMD increments of the

lumbar spine and hip sites could be shown between the two groups, indicating that menstrual status did not have any significant impact on the BMD increments during the study interval. However, the oligo/amenorrhoeic dancers showed significantly lower increments in the core (trabecular) radial (p=0.007) and tibial (p=0.01)

**Table 3. Comparison of interval change after 18 months in exercising subjects with or without oligo/amenorrhoea**

	Oligo/amenorrhoeic dancers (I) [n=10] <sup>†</sup>	Eumenorrhoeic dancers (II) [n=26] <sup>†</sup>
Weight change (kg)	-0.10 (0.81)	-0.48 (1.15)
Body mass index change (kg/m <sup>2</sup> )	-0.064 (0.33)	-0.18 (0.46)
Body fat change (%)	0.51 (1.66)	-0.45 (2.66)
Lumbar spine BMD* change (g/cm <sup>2</sup> )	0.0921 (0.0543)	0.0666 (0.0486)
Neck of femur BMD change (g/cm <sup>2</sup> )	0.0384 (0.0266)	0.0489 (0.033)
Ward's triangle BMD change (g/cm <sup>2</sup> )	0.0415 (0.0293)	0.0332 (0.0213)
Trochanter BMD change (g/cm <sup>2</sup> )	0.0161 (0.0515)	0.0281 (0.0159)
Distal radius (core) BMD change (mg/cm <sup>3</sup> )	7.0 (8.09)	16.8 (9.56)
Distal radius (total) BMD change (mg/cm <sup>3</sup> )	13.1 (9.51)	21.0 (17.85)
Mean tibia (core) BMD change (mg/cm <sup>3</sup> )	6.8 (7.8)	14.4 (7.44)
Mean tibia (total) BMD change (mg/cm <sup>3</sup> )	14.0 (11.66)	21.1 (12.32)

\* BMD denotes bone mineral density

<sup>†</sup> Standard deviation is shown in brackets

**Table 2. Comparison of interval change after 18 months between exercising and non-exercising subjects**

	<b>Exercising (n=36)<sup>†</sup></b>	<b>Non-exercising (n=19)<sup>†</sup></b>	<b>p value; mean difference (95% confidence interval)</b>
Weight change (kg)	-0.38 (1.07)	-0.10 (0.99)	0.35; -0.27 (-0.87 to 0.32)
Body mass index change (kg/m <sup>2</sup> )	-0.15 (0.43)	-0.009 (0.39)	0.23; -0.141 (-0.87 to 0.32)
Body fat change (%)	-0.18 (2.44)	-1.68 (2.33)	0.03; 1.49 (0.127 to 2.86)
Lumbar spine BMD* change (g/cm <sup>2</sup> )	0.0736 (0.0508)	0.0155 (0.0218)	<0.001; 0.0581 (0.0335 to 0.0826)
Neck of femur BMD change (g/cm <sup>2</sup> )	0.046 (0.0313)	0.0082 (0.0103)	<0.001; 0.0377 (0.028 to 0.0526)
Ward's triangle BMD change (g/cm <sup>2</sup> )	0.0355 (0.0236)	0.0101 (0.0132)	<0.001; 0.0254 (0.0136 to 0.0372)
Trochanter BMD change (g/cm <sup>2</sup> )	0.0248 (0.0299)	0.0152 (0.0112)	0.18; 0.009 (-0.0047 to 0.023)
Distal radius core BMD change (mg/cm <sup>3</sup> )	14.11 (10.11)	13.26 (7.82)	0.75; 0.84 (-4.49 to 6.19)
Distal radius total BMD change (mg/cm <sup>3</sup> )	18.8 (16.24)	28.05 (22.17)	0.08; -9.24 (-19.7 to 1.26)
Distal tibial core BMD change (mg/cm <sup>3</sup> )	12.34 (8.21)	13.78 (5.81)	0.50; -1.44 (-5.69 to 2.81)
Distal tibial total BMD change (mg/cm <sup>3</sup> )	19.16 (12.4)	25.5 (14.22)	0.09; -6.33 (-13.75 to 1.09)

\* BMD denotes bone mineral density

<sup>†</sup> Standard deviation is shown in brackets

volumetric BMD as compared to eumenorrhoeic dancers, though the total (trabecular+cortical) distal radial or tibial BMD values did not differ significantly. There were also marginally significant differences in these parameters when compared with eumenorrhoeic controls (Table 3).

## Discussion

Our findings supported the beneficial effects of

physical exercise and physical training on BMD, with significantly higher BMD increments being shown in dancers in the lumbar spine, hip sites as well as the distal tibia as compared to controls after an 18-month period of training. In dance exercises, the main component of such activity came from jumping up and down in support of the dancers' own body weight, with a frequency that could reach up to once every 2 to 3 seconds. It is hypothesised that such weight-bearing

<b>I versus II (p value; mean difference [95% confidence interval])</b>	<b>Eumenorrhoeic controls (III) (n=19)<sup>†</sup></b>	<b>I versus III (p value; mean difference [95% confidence interval])</b>
0.33; -0.38 (-1.20 to 0.42)	-0.10 (0.99)	0.98; -0.005 (-0.72 to 0.71)
0.46; -0.11 (-0.44 to 0.20)	-0.009 (0.39)	0.71; 0.054 (-0.24 to 0.35)
0.29; -0.96 (-2.81 to 0.88)	-1.68 (2.33)	0.01; -2.19 (-3.90 to -0.48)
0.18; -0.025 (-0.063 to 0.12)	0.0155 (0.0218)	<0.001; -0.076 (-0.105 to 0.047)
0.37; 0.01 (-0.013 to 0.034)	0.0082 (0.0103)	<0.001; -0.0302 (-0.044 to -0.016)
0.35; -0.008 (-0.026 to 0.009)	0.0101 (0.0132)	<0.001; 0.0313 (-0.047 to 0.015)
0.28; 0.012 (-0.011 to 0.034)	0.0152 (0.0112)	0.002; -0.0008 (-0.025 to 0.024)
0.007; 9.84 (2.88-16.8)	13.26 (7.82)	0.05; 6.26 (-0.082 to 12.6)
0.19; 7.90 (-4.25 to 20)	28.05 (22.17)	0.053; 14.95 (-0.21 to 30.12)
0.01; 7.68 (1.97-13.38)	13.78 (5.81)	0.011; 6.98 (1.74-12.23)
0.12; 7.08 (-2.1 to 16.27)	25.5 (14.22)	0.68; 22.21 (1.23-21.66)

exercises provide an osteogenic stimulus to bones<sup>9</sup>, and the effects could be seen in a variety of sports, such as athletics<sup>10</sup>, weight-lifting<sup>11</sup>, high-impact sports such as basketball or volleyball, and medium-impact sports such as soccer or track<sup>12</sup>. On the contrary, this effect was not observed in sports activities that involved extensive energy expenditure and muscle strength but no true weight bearing, such as in swimming<sup>12,13</sup>. Our findings are consistent with the hypothesis that repetitive stress applied to weight-bearing sites over extended periods of time as in dance training served as an important strengthening agent for bones in these young females.

We were unable to detect any baseline differences in the BMD values between the study and control groups at the time of recruitment. In a previous study in which we compared BMD values between exercising and non-exercising young women<sup>14</sup>, we were able to show that eumenorrhoeic exercising young women had significantly higher mean BMD values as compared to eumenorrhoeic non-exercising controls, but that this difference was lost in exercising women who had menstrual dysfunction. In this series, however, as the focus of the study was on interval BMD changes, the presence or absence of menstrual dysfunction before recruitment was not used for sub-categorisation. Thus, it would be expected that within the dancers recruited, some would have had a history of menstrual dysfunction, so that their mean BMD values would not be significantly different from eumenorrhoeic controls<sup>14</sup>. In addition, the small sample size of the control group in the present series would make the data set underpowered to detect a significant difference in baseline BMD values.

The strength of our data lies in its longitudinal nature. Longitudinal studies have shown that during pubertal maturation, the accumulation rate of areal BMD as measured by DXA at major sites of the axial skeleton such as the lumbar spine or the hip, increases by 4- to 6-fold over a 3-year period from 11 to 14 years in females<sup>15</sup>. The increase in BMD in the appendicular skeleton tends to be less marked, with only a 2-fold increase measured in the forearm<sup>16</sup> or midshaft of the femur<sup>17</sup>. From menarche onwards, the rate of increase in BMD declines as peak bone mass is about to be achieved any time before the end of the second decade to the early third decade<sup>18</sup>. Our findings of minimal BMD increments over the 18-month interval in the group of eumenorrhoeic controls

were consistent with previously reported data that these females could have almost reached their peak bone mass before the end of the second decade<sup>15</sup>.

While the benefits of exercise was not uniformly demonstrated in all longitudinal studies<sup>4,5</sup>, similar studies in different age-groups have often been able to show higher BMD bone accrual in association with exercise and training<sup>3,19</sup>. Our findings of higher BMD accrual for the dancers over the study period as compared to non-exercising controls would suggest that the stimulus of exercise could result in further bone accrual even in this age period of 17-22 years. The possible implications of this effect on peak bone mass and on BMD in later life have also been described in several studies<sup>2,20</sup>, though further more extensive long-term follow-up studies will be needed to evaluate the impact on postmenopausal osteoporosis.

Our data comparing eumenorrhoeic and oligo/amenorrhoeic dancers showed equal increments of BMD in the axial skeletal sites, but lower trabecular bone increments in the appendicular skeleton in the distal radius and tibia. Due to the small sample size of this series, it is difficult to conclude whether such differences are the results of hypo-estrogenism associated with the athlete triad syndrome. In postmenopausal hypo-estrogenic states, trabecular bone loss had been shown to occur before cortical bone loss due to higher bone turnover<sup>21</sup>. Thus, in this group of young dancers we have described, the lower trabecular bone volumetric BMD in the distal radius and tibia could reflect bone loss of such a nature. Indeed, studies in distance runners have shown lower BMD in those with menstrual problems not only in the axial skeleton, but also the appendicular skeleton as well<sup>22</sup>. A recent study in ballet dancers have shown that weight-bearing exercise may offset the effects of hypogonadism at predominantly cortical weight-bearing sites, but not sites that contained substantial amounts of trabecular bone<sup>23</sup>. Our findings could probably be explained by the same mechanism.

In our data, possible bone loss in the axial skeleton of oligo/amenorrhoeic dancers could have been masked by the effects of intensive exercise, so that the negative impact of menstrual dysfunction on BMD accrual only showed up in trabecular sites in the appendicular non-major weight-bearing sites. When only axial skeletal

sites such as the lumbar spine or hip are considered, a recent study on sub-elite female adolescent runners has shown no detrimental effect on bone accrual despite delayed pubertal development<sup>24</sup>. Similarly, a longitudinal study of a small group of adolescent gymnasts have also shown that despite lower body fat composition and weight, they were able to have enhanced BMD values over a period of 3 years as compared to controls<sup>25</sup>. It is apparent from such data that despite the potential association of osteoporosis to oligo/amenorrhoea in young women undergoing intensive physical training, the majority of these young women have been able to benefit from such activity to enhance their BMD accrual as compared to sedentary controls of the same age. True osteoporosis appears to be rare in these women, to the

extent that it has been proposed that the criteria for athlete triad syndrome should use osteopenia rather than osteoporosis<sup>7</sup>.

In summary, our longitudinal data showed that the intense physical training in the dancers in their late adolescence were conducive to persistent increments in their bone mass accrual at axial skeletal sites irrespective of their menstrual status. Those with oligo/amenorrhoea, however, apparently had lower trabecular bone BMD increments in their appendicular skeletal sites, which could signify the negative effects of hypogonadism associated with menstrual dysfunction. Studies with larger sample sizes will be needed for further evaluation.

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