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Relatively High Bone Mineral Density in Chinese Adolescent Dancers Despite Lower Energy Intake and Menstrual Disorder[1]

LI-CHEN YANG[*, 2], YAN LAN[#], JING HU[*], YAN-HUA YANG[*], QIAN ZHANG[*], ZHEN-WU HUANG[*], AND JIAN-HUA PIAO[*]

[*]Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, 100050 China; [#]Beijing Dance Academy, Beijing, 100081, China

Objective The effect of dietary restriction, intense exercise and menstrual dysfunction on bone mineral density remains controversial. The aim of this study was to assess the skeletal health status and relationship between bone mineral density and nutrient intake, menstrual status, estrogen level and other factos in Chinese adolescent dancers. **Methods** Sixty dancers and 77 healthy controls underwent measurements of bone density, body composition, and estrogen level. Nutrient intake, menstrual status and physical activity were assessed with questionnaires. The correlation between these factors were analyzed. **Results** The dancers under study had a significantly lean body mass index (18.3±1.4 kg/m^2 *vs*. 21.7±3.1 kg/m^2), lower percentage of body fat (0.25±0.05 *vs*. 0.34±0.04) and later age at menarche (14.0±0.9 y *vs*. 13.0±1.3 y), and the estrogen level, daily calorie and fat intake in them were also lower than in the controls. All the dancers undertook intensive physical activity every day and up to 69% of them suffered from irregular menarche. Yet they had relatively high BMD and BMC of the total body and legs than the controls after adjusting for BMI and age. Site-specific BMD was positively correlated to BMI, body composition and training hours per week and negatively correlated to the age at menarche and menstrual frequency. **Conclusions** The relatively high BMD and BMC of the dancers at the total body and legs were probably caused by high levels of weight-bearing physical activity. To ameliorate disordered eating, especially low energy intake might be helpful to prevent the Triad and to improve the bone health in adolescent dancers.

Key words: Adolescent dancers; Bone mineral density; Nutrient intake; Menstrual status; Estrogen level

INTRODUCTION

Osteoporosis has become one of the major public health problems[1]. Recent studies have suggested that accretion of peak bone mass during adolescence and young adulthood is essential because at least 40% of bone mass is formed at this stage, and it may be difficult for a woman to accrue additional bone mass in her later life[2], so optimizing bone health and maximizing the peak bone mass (PBM) during adolescent years are important for the prevention of osteoporosis in late maturity time.

As we know, the loss and strength of bone mass are influenced by both the genetic and environmental factors, but the dietary nutritional status，physical activity level and hormonal disturbances also play a significant role in the accumulation of bone mass. There are many reports about three interrelated conditions that are prevalent in athletes: disordered eating, amenorrhea and osteoporosis, which are defined as the female athlete triad (the Triad). A significant issue is the failure to reach peak bone mass, particularly in athletes with delayed menarche[3]. Yet maximizing total mass accretion for young women at risk cannot be implemented without further understanding of the relationship between the above-mentioned factors and bone density.

Adolescent dancers are an interesting group for a study on factors affecting bone mineral density (BMD), since dancing is a demanding discipline involving factors that could have both positive (weight-bearing exercise) and negative (malnutrition, low body mass and fat-free weight, hormonal imbalance) effect on BMD. And these dancers are believed to be at greater risk for disordered eating and irregular menarche[4]. Yet studies in athletes show conflicting results, ranging from low BMD to relatively high BMD[5-6].

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[2]Correspondence should be addressed to Dr. Li-Chen YANG. Address: Room 103, Nanwei Road 29, Xuanwu District, Beijing, 100050. Tel: 86-10-83132906, Fax: 86-10-83132021. E-mail: Lichen_yang@yahoo.com.cn

Biographical note of the first author: Li-Chen YANG, female, Ph D, associate professor, Nationl Institute of Nutriton and Food Safety, China CDC, focus on: micronutrients and health.

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The adolescent dancers were used in this study as a model to investigate the skeletal health status and its differences from the control population. The nutrient intakes, physical activity level, body weight and composition, menstrual status, and hormonal level (estrogen) were measured in all the subjects. In particular, the correlation between these factors was also analyzed. The study was designed to screen some possible protective or risk factors of osteoporosis and offer some scientific data for the prevention of the occurrence of osteoporosis or the Triad in its early course. And since the Triad is also present in normally active females[7], the prevention of one or more of the Triad components should be geared towards all physically active girls and young women.

MATERIALS AND METHODS

Selection of Participants

Eighty girls aged 15-17 years were enrolled in this study from Beijing Secondary Dance School, attached to the Beijing Dance Academy. Exclusion criteria were as follows: (1) treatment, during the last year, with drugs able to modify BMD (thyroid and sex hormones, steroids, calcium, and vitamin D); (2) presence of additional diseases potentially involved in bone loss development (oncologic, renal and gastrointestinal diseases); and (3) nutritional treatments during the last 6 months (food supplementations by oral, external route or potential nutrition); (4) family history of osteoporosis.

Finally, 60 dancers met the inclusion criteria and were therefore considered eligible for this study. These dancers were matched for age with 77 healthy controls recruited from the Beijing Jingsong Vocational School, while the control subjects with a medical condition, or receiving treatment potentially able to influence BMD were excluded.

All the subjects gave informed consent to the study. This study was also approved by the Ethical Committee of Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Medical Examination

Weight, height, and a brief physical examination were performed by a nurse practitioner; meanwhile pubertal development was self-evaluated by use of Tanner scale for sexual maturation. Levels of hemoglobin in whole blood was measured by cyanmethemoglobin method and serum estrogen levels were assessed by radial immunodiffusion (RID) method.

Nutrient Intakes

Nutrient intake data, including energy, calcium and protein content, were obtained from a 7-day 24 hour diet records. Disordered eating practices, including dietary habits, past eating behaviors, induced vomiting or used laxatives *etc* were all involved in the questionnaire. Dietary intake was calculated according to China Food Composition Table[8] and evaluated by the same trained person.

Physical Activity

Physical activity was assessed by means of the modified activity questionnaire (according to the questionnaire of the 2002 China National Health and Nutrition Survey), including hours spent in dancing per week, intensity of physical exercise per week, duration of training and the training history (start of dancing, amount and type of training from childhood to the present time). Caloric expenditure in relation to kinds of activities was calculated according to similar energy costs in kilocalories per kilogram per minute[9].

Menstrual Dysfunction

Menstrual dysfunction and musculoskeletal injuries were assessed by a health/medical and menstrual history questionnaire. All subjects were questioned about the age of menarche, frequency of menstruations over the last 12 months, and the timing and duration of any menstrual disruption or amenorrhea. Secondary amenorrhea is the absence of at least three consecutive menstrual cycles in postmenarchal women[10].

Bone Density and Body Composition

All the subjects were subjected to bone density and body composition measurement with a dual energy X-ray absorptiometer (DXEA, NORLAND XR-36), which was performed by the professionals in Beijing No.304 Hospital. The coefficient of variation of repeated measurements was less than 1%. Bone mineral status was assessed by areal BMD (g/cm^2), BMC (g), and BMD Z-score. BMD Z-score measures bone density in terms of standard deviations above or below the mean bone density of healthy subjects matched for age, race and sex; and in children and adolescents a BMD Z-score <-1 was arbitrarily considered as BMD reduction (osteopenia)[11].

Statistical Analysis

All data were reported as $\bar{x} \pm s$ unless otherwise indicated. Student's *t*-test and analysis of covariance were used to calculate differences between means. X^2

test was used to compare prevalence of BMD reduction between dancers and controls. Pearson correlation coefficient was used to determine the relationships between supposed factors related to BMD and BMC. Statistical analyses were performed using SPSS software. Level of significance was set at $P \leqslant 0.05$.

RESULTS

Background Information of the Subjects

As shown in Table 1, there was no significant difference in the age and Hb between the two groups; the dancers were taller than the contorls ($P<0.05$), but the weight, BMI, and percentage body fat in the dancers were all significantly lower than in the controls ($P<0.05$). And the dancers had lower estrogen level ($P<0.05$).

TABLE 1

Descriptive Information of All the Subjects

Variable	Dancers (*n*=60)	Controls (*n*=77)
Age (years)	16.5±0.7	16.4±0.6
Hb (g/L)	137.8±11.7	130.5±10.9
Weight (kg)	48.9±4. 8[1]	55.0±8.8
Height (cm)	163.5±4.3[1]	159.1±5.9
Percentage body fat	0.25±0.05[2]	0.34±0.04
Estrogen level (pg/ml)	231.64±147.47[1]	315.41±81.95
BMI（kg/m^2）	18.3±1.4[1]	21.7±3.1

Note. 1: $P<0.05$ compared with control group. 2: $P<0.01$ compared with control group.

Nutrient Intakes

The daily calorie and fat intake in the dancers were significantly lower than in the control group (1792.55±430.22 kcal *vs*. 2614.73±543.79 kcal, 63.66±15.71 g *vs*. 164.41±30.32 g, $P<0.05$). The dancers had higher calcium intake than the controls (613.57±269.49 *vs*. 407.33 ±172.83, $P<0.05$), which might be related to the high consumption of milk. No significant difference was found in iron, protein, and carbohydrate intake between the two groups. Both BMI and percentage body fat had positive correlation with calorie and fat intakes (r=0.47~0.59, $P<0.01$).

Menstrual Status and Secondary Sex Characteristic Development

As shown in Table 2, the proportions in the dancers who did not experience their first menarche (primary amenorrhea) and suffered from secondary amenorrhea and irregular menarche were all significantly higher than in the controls. Their age at menarche and the breast development according to Tanner score were all significantly later than those of the controls ($P<0.05$).

TABLE 2

Prevalence of Menstrual Irregularities of All Subjects

Variable	Dancers (*n*=58)	Controls (*n*=77)
Primary Amenorrhea	11 (19.0%)[1]	0 (0%)
Irregular Menorrhea	34 (72.3%)[1]	21 (27.3%)
Secondary Amenorrhea	13 (26.5%)[2]	11 (14.3%)
Age at Menarche (years)	14.0±0.9[1]	13.0±1.3
Tanner Stage for Breast Development	3.4±0.8[2]	4.0±0.7

Note. 1: $P<0.05$ compared with control group; 2: $P<0.01$ compared with control group.

Physical Activity Level

As shown in Table 3, the sedentary activities, sleeping, and outdoor time of the dancers were all significantly shorter than those of the controls. Unlike the controls, the dancers were trained by professional dancing courses up to 5.0±1.7 years, and their mean training time and intensity were all significantly higher than those of the controls, so their total energy expenditure was higher and their available energy (= calorie intake-calorie expenditure) was significantly lower as compared with the controls.

TABLE 3

Physical Activity Assessment of All the Subjects

Variable	Dancers (*n*=60)	Controls (*n*=77)
Sedentary Activities (h/day)	2.24±1.76[2]	4.82±2.20
Training Time (hours /week)	26.5 ±5.2	/
Sleeping Time (h/day)	7.46±0.85[1]	8.44±1.58
Outdoor Time (h/day)	0.55±0.75[2]	2.97±2.07
Calorie Expenditure (kcal/24 h)	1 642.72±247.66[1]	1 287.25±219.98
Available Energy[3] (kcal/24 h)	191.38±45.34[2]	1 280.58±658.0

Note. 1: $P<0.05$ compared with control group; 2: $P<0.01$ compared with control group; 3: available energy=calorie intake-calorie expenditure.

Bone Mineral Density (BMD)

As shown in Table 4, 26.7% of dancers were found as having osteopenia (defined as bone mineral density 1-2.5 standard deviations below the average[11-12]), compared with the age matched controls (14.3%, *P*>0.05).

TABLE 4

BMC, BMD of All Measured Sites and *Z*-Score of TBMD

Variable	Controls (*n*=77)	Dancers (*n*=60)
Z Score of TBMD -2.5~ -1	11 (14.3%)	16 (26.7%)
Total Body BMD (g/cm^2)	0.872±0.006	0.92±0.007[1]
Total Body BMC (g)	2 221.37±25.39	2 396.95±30.85[2]
Left Leg BMD (g/cm^2)	0.901±0.008	0.965±0.009[1]
Left Leg BMC(g)	382.34±5.66	442.21±6.87[2]
Right Leg BMD (g/cm^2)	0.913±0.008	0.992±0.009[1]
Right Leg BMC (g)	405.57±51.1	444.11±42.95[2]
Left Arm BMD (g/cm^2)	0.519±0.007	0.511±0.007
Left Arm BMC (g)	132.95±2.42	127.97±1.99
Right Arm BMD (g/cm^2)	0.513±0.007	0.502±0.007
Right Arm BMC (g)	131.71±2.38	127.27±1.96
Distal left Forearm BMD (g/cm^2)	1.198±0.0024	1.185±0.026

Note. 1: *P*＜0.05 compared with control group，adjusting for age and BMI; 2: *P*＜0.01 compared with control group，adjusting for age and BMI.

However, the dancers had significantly higher BMD and BMC at the total body and legs than the controls, after adjusting for BMI and age (*P*<0.05). And the BMD and BMC at arms and distal left forearm did not show difference and the values were even higher in control (*P*>0.05).

Factors Affecting Bone Mineral Density

Fig. 1 shows the approximate linear relation between number of menstrual cycles per year and TBMD in the dancers. Among the girls suffering from irregular menarche, the TBMD in the dancers who had 0-3 menstrual cycles (amenorrhoeic) was significantly lower than in the dancers who had 4-10 (oligomenorrhoeic) and 11-13 menstrual cycles (eumenorrhoeic) (*P*<0.01, *P*<0.05 respectively). And the TBMC in the dancers with amenorrhorea were significantly lower than in those with eumenorrhorea (2 134.47±198.37 g *vs*. 2 379.40±285.16 g, *P*<0.01).

As shown in Table 5, the nutritional status indices, including BMI and body composition (percentage body fat), had positive correlation with all measured sites BMD after adjusting for age (*P*<0.01). Except left arm (*P*<0.05), no relationship existed between estrogen level and sites-specific BMD. TBMD and BMD of arms were significantly negatively related to the age of menarche (*P*<0.01). The dancers with age of menarche later than 15 years of age had lowest BMD at all measured sites (not shown, *P*<0.05). No relationship was found between measured-sites BMD and age of dancing, and period of training after adjusting for age and BMI (data not shown). But BMD at legs were positively related to the training hours per week (*P*<0.05).

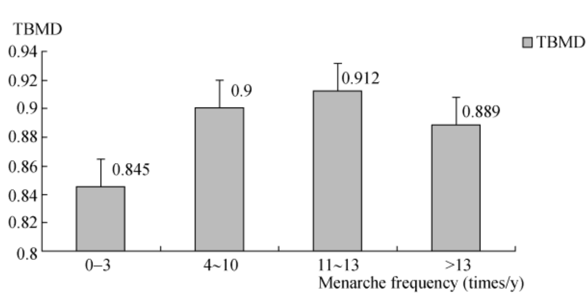

FIG. 1. Relationship of menarche frequency and total body mineral density (TBMD) of dancers. Among the dancers suffering from the irregular menarche, the TBMD of the girls who had 0-3 menstrual cycles (amenorrhoeic) was significantly lower than that of the dancers who had 4-10 (oligomenorrhoeic) and 11-13 menstrual cycles (eumenorrhoeic) (*P*<0.01, *P*<0.05, respectively). The phenomenon indicated that the extent of menstrual irregularity had correlation with BMD, the more serious the menstrual irregularity, the lower the TBMD was.

TABLE 5

Correlations between Related Factors and Sites-Specific BMD of Sixty Female Ballet Dancers

	Total Body		Left Leg		Right Leg		Left Arm		Right Arm		Distal of Left Forearm	
	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*
BMI (kg/m^2)	0.596[1]	0.000	0.574[1]	0.000	0.434[1]	0.001	0.367[1]	0.005	0.342[1]	0.009	0.414[1]	0.001
Percentage Body Fat (%)	0.493[1]	0.008	0.419[1]	0.001	0.375[1]	0.004	0.619[1]	0.000	0.477[1]	0.000	0.318[2]	0.015
Estrogen Level (pg/ml)	-0.122	0.36	-0.1	0.943	-0.7	0.604	-0.27[1]	0.04	-0.71	0.199	-0.128	0.561
Age of Menarche (years)	-0.195	0.023[1]	-0.118	0.38	-0.16	0.231	-0.217	0.012[1]	-0.254	0.003[2]	-0.226	0.009[2]
Training Hours/Week (h/w)	0.273	0.07	0.312[2]	0.037	0.321[2]	0.02	0.091	0.553	0.251	0.096	0.134	0.378

Note. 1: $P<0.05$, 2: $P<0.01$. There were no correlations between TBMD and site-BMD with age of dancing, peirod of training (data not shown).

DISCUSSION

Among the 3 conditions of “female athlete triad”, disordered eating, is often the first component to appear, and it may contribute to amenorrhea, thereby increasing the risk of bone loss and related fractures[7]. The term disordered eating was adopted, in part, to include the spectrum of unhealthy eating behaviors ranging form restricting food intake to clinical eating disorders. In this study, we found that the diet restriction was very common in the dancers, potentially driven by a need to maintain a low body mass for performance. The average energy intake of the dancers were all lower than 1 800 kcal/d, and there were 33.3% of the dancers whose daily caloric intake was less than 1 600 kcal/d. And the mean fat intake in them was also significantly lower than that in the controls ($P<0.05$). Intensive physical activity will obviously lead to insufficient energy availability. And the direct outcome is reflected in the lower weight, BMI and percentage body fat (PBF), which is alo confirmed by our data.

On the other hand, evidences suggest that disordered eating, particularly caloric restriction, causes menstrual abnormality[13-15]. In this study, the dancers with amenorrhoea (0-3 times/y) and oligomenorrhoea (4-10 times/y) took less energy than the eumenorrhoeic (11-13 times/y) dancers (1 709 kcal, 1 751 kcal, and 1 837 kcal respectivly, $P>0.05$). The implication of the work of Loucks *et al.*[16] is that menstrual irregularity can be prevented or reversed by increasing caloric intake sufficient to compensate for energy expended through exercise.

Primary amenorrhea has recently been redefined by the American Society of Reproductive Medicine as an absence of menstrual cycles in a girl who has not menstruated by age of 15, although she has experienced other normal developmental changes occurring during puberty[17]. As expected, the dancers under study had significantly later age at menarche than the controls. This is in agreement with earlier studies, where dancers started menses later as compared with the general population[18]. And our data also showed that BMD and age of menarche were inversely related, and the dancers with age at menstruation later than 15 got lowest TBMD, which was also consistent with the result of Xing-Biao CHEN[19].

The combination of amenorrhea and malnutrition associated with disordered eating results in loss of bone mineral density, which can lead to osteoporosis. Reports also pointed out that the athletes with amenorrhea and less menorrhea had lower BMD than those with normal menorrhea[20-21]. In our study, we also found the approximately linear relation between number of menstrual cycles per year and bone density as reported by Karen[12]. The TBMD in the dancers with menstrual cycles 0-3 times were significantly lower than those with 4-10 times, and 11-13 times per year. However, the dancers in general showed higher TBMD/TBMC and BMD/BMC at legs than the controls after adjusting for BMI and age, while the BMD at arms were similar between the two groups and the values were even higher in the control group.

As expected, significant negative relationships were found between measured-sites BMD and nutritional elements (BMI and percentage body fat (PBF)), and the dancers under study maintained a significant correlation between BMI, PBF, and caloric, fat intake as in the healthy population[22]. Since the dancers had a low BMI and a relatively low energy intake, a high TBMD/BMC was unexpected.

The relatively high TBMD was in contrast to Torsteveit MK *et al*.[5], who found decreased TBMD in elite athletes than in controls. Another study[23] involving 29 Chinese girls receiving regular ballet training for at least 6 years, showed no significant differences in either measured BMD or spinal BMD after adjusting for BMI. This result revealed that the negative effect of a lower body weight may neutralize the positive effect of ballet dancing on BMD, especially in the femoral neck. Similar results were found in Young N's study[24]. Yet To WW[6] and Van Markern and his colleagues[25] found a result similar to our study; the adolescent dancers showed higher TBMD and BMD at legs than non-exercising adolescent females, after controlling for body mass.

Some studies found relationships between physical activity and BMD[26], while others did not[27]. In this study there were no relationships between training history (years of receiving dancing training) and BMD. This does not mean that no relationship exists, but probably illustrates problem in quantifying the history of physical activity. In this study, the effect of physical activity, *i.e*. period of training (h/week, reflecting current physical activity), on BMD was evident in the legs. Moreover, the dancers under study showed lower BMD/BMC values at non-weight-bearing sites (arms and distal left forearm, $P>0.05$). So the higher TBMD/BMC might contribute to the higher BMD/BMC of legs, the weight-bearing sites of the dancers' body[25]. Although no relationship was found between Ca intake and BMD at the total body and legs, the greater consumption of milk products and calcium and better Ca/P seen in the dancers could help this group to avoid bone deterioration[15].

It was reported that the stress hormones produced by psychological stress may lead to a physiological alteration in the endocrinological control of the menstrual cycle, which may ultimately lead to the athlete becoming amenorrheal and the decreased production of estrogen[12]. As we know, estrogen is the well-known key hormone affecting bone metabolism, and is related to metabolism, and thus to nutritional and metabolic status. If energy availability (=calorie intake-calorie expenditure) is low over a period of time, as indicated by these hormones, the menstrual cycle is temporarily "switched off" or suppressed to conserve energy[12]. Low energy availability and decreased endogenous estrogen associated with amenorrhea can finally result in a decrease in bone mass[28]. Our data found that the dancers had significantly lower estrogen level than the controls, which might also indicate that this is a negative factor to their bone health. Yet our data only showed relationship between BMD/BMC at left arm and estrogen level.

In conclusion, this study suggests that the bone mass status is probably a combination result of nutritional status, intensive exercise, menstrual irregularity and systemic hormones decreases. And despite the factors that have a negative effect on BMD, such as low BMI, late menarche and low energy intakes *etc*, TBMD/BMC in female dancers is relatively high. These high values are probably caused by high levels of weight-bearing physical activity.

On the other hand, our data have also indicated that the disordered eating, especially low energy intake might be the pivotal negative factor of the Triad. To ameliorate the diet disorder might be helpful to prevent the Triad and to improve the bone health in adolescent dancers. Yet more prolonged experiments are needed to determine the dose-response relationships between chronic restrictions of energy availability and bone turnover.

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