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Effects of sports training & nutrition on bone mineral density in young Indian healthy females

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***Background & objectives*: Peak bone mass, a major determinant of osteoporosis is influenced by genetic, nutritional, lifestyle and hormonal factors. This study was designed to evaluate the impact of sports training on dietary intake and bone mineral and metabolic parameters in young healthy Indian females.**

***Methods*: Healthy female college going students (N=186, sportswomen, 90; controls 96) in the age group of 18-21 yr, residing in New Delhi (India) were evaluated for anthropometry, biochemistry (serum total and ionic calcium, phosphorus, total alkaline phosphatase, 25-hydroxyvitamin D & parathyroid hormone), diet, physical activity and lifestyle. Bone mineral density (BMD) at hip, forearm and lumbar spine were studied using central DXA.**

***Results*: Sports related physical activity (3 vs. 0 h/day, *P*<0.001) and direct sunlight exposure (120 vs. 30 min/day, *P*<0.001) were significantly higher in sportswomen than in controls with sedentary lifestyle. Significantly higher intake of all macronutrients (energy, protein, carbohydrates and fat) and dietary calcium was noted in the diets of sportswomen. Mean serum 25(OH)D levels were significantly higher (53.0±18.9 vs. 12.9±7.7 nmol/l; *P*<0.001) while PTH (35.3±17.6 vs. 51.7±44.9 pg/ml; *P*<0.001) and ALP levels (194.0±51.0 vs. 222.1±51.4 IU/l; *P*<0.001) were significantly lower in sportswomen when compared to controls. No significant difference was found in ionized calcium and inorganic phosphorus in the two groups. Significantly higher (*P*<0.001) total BMD and BMD at all sites except femur neck were found in sportswomen than controls (*P*<0.001).**

***Interpretation & conclusions*: Physical activity, optimal nutrition and adequate sun exposure are vital for attaining peak bone mass.**

Key words Bone mineral density - nutrition - physical activity - sun exposure - sportswomen

Adolescence, characterized by changes in height, weight and body composition, is also a crucial period for bone mineral accrual[1]. Approximately 40 per cent of peak bone mass is accumulated during adolescence which protects against post menopausal osteoporosis[2]. Therefore, optimizing peak bone mass in early adulthood is thought to reduce the risk of osteoporosis by offsetting bone losses later in life.

About 50-70 per cent of the variance in peak bone mass within a population is determined by genetic factors[3,4]. Other potential determinants which interact with genetic factors to influence bone mass include gender, diet, physical activity, sun exposure, and hormonal factors[5,6].

There is a marked variation in bone mineral density (BMD) among women from different ethnic groups. Thus, women of European origin have been observed to have lower BMD at different skeletal sites compared to their African-American counterparts[7,8] but a higher BMD than those of Far East Asian origin[9]. Among environmental factors, nutrition and vitamin D status play a crucial role in acquisition of bone mineral density[10,11]. Also, there is evidence to suggest that physical activity during adolescence and early adulthood is a key determinant of peak bone mass[12-14]. A positive association between bone mineral status and daily participation in high-impact physical activity has also been reported[15,16].

In view of limited information on Indians, we decided to study the effect of sports training and nutrition on BMD in a group of post-pubertal women, *i.e*., an age group during which bone mass is still being accumulated. To meet this objective sportswomen involved in regular moderate to high level physical activity were compared with age matched sedentary controls.

Material & Methods

Population and sample collection: The study included 186 female subjects (90-sportswomen; 96-controls), aged 18-21 yr, from different colleges affiliated to the University of Delhi (DU). A study subject was defined as a sportswoman, if she represented either her college or the State of Delhi in competitions for either individual or team sports. A subject was selected as a control if she was involved in less than 150 min of physical activity per week, including any walking involved to reach the college. Purposive sampling technique was used to select the subjects. Sportswomen from four colleges of the University – Gargi College for Women, Kamala Nehru College, Jesus and Mary College and Mata Sundri College - were contacted through the Director of Sports, University of Delhi. Initially, the sample consisted of 107 subjects, of which 17 girls dropped out. Finally, 90 subjects who gave their informed consent to participate, were recruited for detailed anthropometric, biochemical, dietary, lifestyle and BMD assessment. To formulate the control group, 117 girls were approached from a single college – Sur Homeopathic Medical College; 21 girls were eliminated since they did not provide consent. Hence, a sample of 96 controls underwent detailed assessment. The study protocol was approved by the Institutional Ethics Committee of the Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi.

The sample size was calculated with an estimated difference of 5 per cent between the two groups at lumbar spine. Lumbar spine BMD of 25 controls was initially measured and based on an anticipated 5 per cent excess BMD in sportswomen we arrived at a sample size of 99 subjects in each group (80% power).

Under anthropometric assessment height and weight recordings were done on the same day on which BMD was measured. Height was recorded without shoes; using a wall stadiometer to the nearest 1 mm. Subjects were weighed using a clinical balance to the nearest 0.1 kg, wearing light clothing and without shoes. BMI was calculated as weight (in kg) / height (in m^2). Every morning, the scale and stadiometer were calibrated with standard weight and height respectively. Subjects with systemic illness, symptoms of chronic hepatic or renal disorder, endocrine disorders and drugs affecting bone mineral health were excluded from the study.

Analytical methods: Blood samples were collected from subjects in the fasting state at 0800 h without venostasis under basal conditions for estimation of total serum calcium, serum ionic calcium phosphorus, total alkaline phosphatase (ALP), 25-hydroxyvitamin D (25-OHD) and immunoreactive parathyroid hormone (PTH). Serum was centrifuged at 4^{o}C for 15 min at 1200 *g* and divided into five aliquots, which were refrigerated. Serum Ca, ionic Ca, P and ALP were estimated on the same day, and the remaining aliquots were stored at -20^{o}C until PTH and 25(OH) D were estimated.

Serum Ca and P (Roche Diagnostics 902, Mannheim, Germany) were measured by colorimetric method (Hitachi, Automated Biochemistry Analyzer); ionic calcium by ion selective electrode method (Roche Diagnostics, AVL 9180 electrolyte analyzer) and ALP was measured by liquid kinetic method (Roche Diagnostics 902, Mannheim, Germany). PTH was assayed by electrochemiluminescence method (Elecsys 2010, Roche Diagnostics, Mannheim, Germany) and serum 25(OH)D was measured by radioimmunoassay (Diasorin, Stillwater, MN, USA).

The normal laboratory range for serum Ca was 2.20-2.55 mmol/l (8·8-10·2 mg/dl) and for serum *P* was 0.86-1.44 mmol/l (2·7-4·5 mg/dl), according to the kit manufacturers. The normal laboratory range for ionic calcium was 1.1-1.3 mmol/l and for serum alkaline phosphatase at 37^{O}C was 100-275 IU/l in adults. The normal serum concentrations of 25(OH)D and PTH were 22.4-93.6 nmol/l (9.0-37.6 ng/ml) and (10-66 pg/ml), respectively. The lowest concentration of 25 (OHD) measurable by this kit, defined as the lowest quantity differentiated from zero at 2 standard deviations (SDs) below the mean counts per min of the zero standard, is 3.74 nmol/l. Vitamin D deficiency was classified based on the measurement of serum 25(OH)D concentration, as recommended by Lips[17]. Serum 25(OH)D concentrations of 25-50 nmol/l, 12.5-25.0 nmol/l, and less than 12.5 nmol/l were classified as mild, moderate, and severe hypovitaminosis D, respectively.

Dietary information was collected using well-established 24-h dietary recall. Dietary assessment of energy, protein, carbohydrate, total fat, dietary fibre, phytate, oxalate, calcium (dairy and non-dairy) and phosphorous was calculated using Nutritive Value of Indian Foods[18]. A self-designed and structured questionnaire was prepared by the investigator to elicit information regarding physical activity and lifestyle profile which included style of dress during college and practice hours, direct sunlight exposure, surface area of the body exposed to sunlight daily, time spent outdoors, time spent in sports practice sessions (outdoor), and sunscreen usage. Direct sunlight exposure was assessed by documenting average duration of exposure and percentage of the body exposed daily[19].

The 24-h recall and questionnaire was pre-tested on five girls before finalization and administration.

Bone density expressed in g/cm^2 at anteroposterior (AP) lumbar spine (L1–L4), femur (femoral neck) and forearm (total, ultra distal and 33% radius) was measured using the Prodigy Oracle (GE Lunar Corp., Madison, WI, USA) according to standard protocol. Quality control procedures were carried out in accordance with the manufacturer's recommendations. Instrument variation was determined regularly by a daily calibration procedure using a phantom supplied by the manufacturer and mean coefficient of variation was <0.5 per cent. As per the International Society for Clinical Densitometry (ISCD) recommendations[20], short term precision study was performed by making duplicate measurements in 30 volunteers at each region of interest, repositioning the subject after each scan. The mean coefficients of variation were 0.62, 0.41, 0.65 per cent, and 0.84 per cent at femoral neck, total femur, lumbar spine, and 33 per cent radius, respectively.

Data analysis: Statistical analysis was carried out using STATA 9.0 (College Station, Texas, USA). Data were presented as mean ± SD/median (range) as appropriate. Anthropometric, dietary, biochemical and BMD parameters were compared between the groups using Student's t test or Wilcoxon rank sum test as appropriate. Analysis of covariance (ANCOVA) was used to compare the BMD values between the groups adjusting for anthropometric, dietary and biochemical parameters. The results were reported as adjusted mean (95% C.I.). $P<0.05$ was considered significant.

Results

Anthropometric and dietary parameters: There was no significant difference in the mean age, height, weight, BMI between the sportswomen and controls (Table I). The presence of underweight, overweight and obesity was 7.8, 6.6, and 3.3 per cent respectively, in sportswomen, whereas it was 16.7, 7.3 and 3.1 per cent in controls. All girls in both the groups reported normal menstruating pattern.

Diets of sportswomen showed significantly ($P<0.001$) higher intake of all nutrients *i.e*., macronutrients (energy, protein, fat, carbohydrate and fat) and other constituents (fibre, phytate and oxalate) compared to control (Table I). Forty seven per cent sportswomen met daily recommended dietary allowance (RDA) for energy as suggested by the Indian Council of Medical Research[18] as compared to only 2.1 per cent controls. The percentage of energy contribution from protein, fat, and carbohydrate was within the reference range. Although the fat intake of sports girls was high, the per cent energy contribution from fat was less than that observed in controls (Table I). Mean total calcium intake of the control subjects (409.7 ± 172.5 mg/day) was significantly less than that of sportswomen (779.1 ± 324.5 mg/day, $P<0.001$). Seventy six per cent of sportswomen met the RDA for calcium in contrast to 1 per cent of control subjects. Also the mean dietary calcium intake of the controls was far less than the WHO (2004)[21] and US (1997)[22] recommendations which lie in the range of 600-1300 and 500-1300 mg/day, respectively. However, the mean intake of sportswomen was found to be within the international recommendations.

Table I. Baseline characteristics, biochemical and dietary parameters of the sports girls and controls

Variable	Control girls (n=96)	Sports girls (n=90)	Overall (n=186)
Anthropometric:			
Age (yr)	-	18.7 ± 1.2 (18-21)	18.6 ± 1.3 (18-21)
Height (cm)	158.1 ± 5.7 (143-171)	158.5 ± 5.7 (146 - 178)	157.4 ± 5.7 (143-178.5)
Weight (kg)	52.4 ± 8.4 (37-79.3)	54.0 ± 9.1 (41.5 - 100.4)	53.2 ± 8.8 (37 - 100.4)
Body mass index (kg/m^2)	21 ± 3.5 (13.8-31.6)	21.6 ± 3.1 (16.1 - 34.7)	21.2 ± 3.3 (13.8 - 34.7)
Dietary (nutrients):			
Energy (kJ)	5975 ± 1145 (3047-8653)	8544 ± 2629* (3214-15424)	7215 ± 2257 (3047-15424)
Protein (g)	38.9 ± 11.2 (14.0-78.1)	56.2 ± 16.8* (20.4 - 136.4)	47.66 ± 17.5 (14.0 - 136.4)
Protein energy %	10.9	11.0	11.0
Carbohydrate (g)	211.5 ± 51.7 (92.7-358.2)	312.0 ± 85.6* (110.3 - 505.0)	254.45 ± 81.9 (92.7 - 505.0)
Carbohydrate energy %	59.2	61.06	59
Fat (g)	47.3 ± 9.0 (22.6-79.1)	60.7 ± 29.6* (12.0 - 141.6)	54.8 ± 21.2 (12.0 - 141.6)
Fat energy %	29.8	24.7	28.6%
Dietary fiber (g)	4.4 ± 1.2 (1.9-8.3)	8.7 ± 2.6* (2.7 - 15.9)	6.2 ± 2.7 (1.9 - 15.9)
Phytate (mg)	71.4 ± 48.4 (5.1-191.8)	129.1 ± 61.2* (4.0 - 323.8)	104.2 ± 68.1 (4.0 - 323.8)
Oxalate (mg)	37.6 ± 76.6 (2.3-347.8)	95.3 ± 178.1* (6.8 - 864.4)	66.1 ± 141.4 (2.3 - 1021.4)
Non- dairy calcium (mg)	174.4 ± 88.7 (65.6-543.8)	343.6 ± 111.3* (140.18 - 800.4)	256.8 ± 131.1 (65.6 - 800.4)
Dairy calcium	235.3 ± 150.7 (0-600)	435.5 ± 295.0* (0 - 1182)	332.7 ± 252.6 (0 - 1182)

Values are given as mean ± SD (range); *P<0.001 compared to controls

Physical activity and lifestyle parameters: Twenty five per cent of the sportswomen played volleyball, 18.7 per cent took part in athletics and 11 per cent of the subjects took part in hockey and football. Other sports played included athletics, aerobics and basketball, *etc*.

The sportswomen had participated in regular physical training sessions for last 3 to 4 yr prior to recruitment in the study. The selected sportswomen were involved in regimented sports practice for a mean of 3 h/day. In contrast, the control subjects followed a sedentary lifestyle and were not engaged in either leisure time physical activity or regular sports.

During college hours, sports subjects wore clothes wherein the body surface area exposure was limited to 15 per cent which increased to 45 per cent during practice sessions. However, the control subjects had maximum of 15 per cent body surface exposed throughout the day. A significant difference was seen in the duration of daily sun exposure between 0900-1600 h, (2 h for sportswomen and ½ h for control subjects). Around 60 per cent of sports subjects and 70 per cent of the controls did not use a sunscreen which may interfere with vitamin D synthesis.

Biochemical and hormonal parameters: Serum 25(OH) D was significantly higher while PTH and ALP levels were significantly (P<0.001) lower in sports women when compared with age matched controls (Table II). No significant difference was noted in serum total calcium and ionic calcium between the two groups. Further 2 (2.2%) sportswomen and 89 (92.7%) controls had serum 25(OH)D concentration <9 ng/ml *i.e*. below the lower limit of the normal range recommended

Table II. Baseline biochemical and hormonal parameters of sports girls and controls

Variable	Control girls (n=96)	Sports girls (n=90)	Overall (n=186)
Calcium (mmol/l)	2.4 ± 0.1 (2.2-2.8)	2.4 ± 0.1 (1.9-2.7)	2.4 ± 0.1 (1.9-2.8)
Ionic Ca (mg/dl)	1.1 ± 0.0 (1.1-1.3)	1.1 ± 0.1 (1.0-1.8)	1.1 ± 0.1 (1.0-1.8)
Phosphorus (mmol/l)	1.2 ± 0.1 (0.9-1.6)	1.2 ± 0.1 (0.9-1.6)	1.2 ± 0.1 (0.9-1.6)
ALP (IU/l)	222.1 ± 51.4 (125-366)	194.0 ± 51.0* (96-369)	208.4 ± 52.9 (96-369)
PTH (pg/ml)	51.7 ± 44.9 (8.2-212)	35.3 ± 17.6* (10.9-98)	43.7 ± 35.3 (8.2-212)
25(OH)D (nmol/l)	12.9 ± 7.7 (0.7-38.1)	53.0 ± 18.9* (18.9-99.8)	32.4 ± 24.6 (0.7-99.8)

Values are given as mean ± SD (range); ALP, alkaline phosphatase; PTH, parathyroid hormone; *P<0.001 compared to controls

Table III. Bone mineral density (BMD) parameters of the sports girls and controls

Parameter	Control girls (n=96)	Sports girls (n=90)
Total body (g/cm^2)	1.07 ± 0.087	1.13 ± 0.1*
Total femur (g/cm^2)	0.96 ± 0.12	1.08 ± 0.14*
Femur neck (g/cm^2)	1.07 ± 0.87	1.04 ± 0.13
33% radius (g/cm^2)	0.60 ± 0.09	0.65 ± 0.55*
Lumbar spine (L1-L4) (g/cm^2)	1.07 ± 0.13	1.18 ± 0.14*

Values are given as mean ± SD; *P<0.001 compared to controls

Table IV. BMD parameters of the subjects after adjusting for height, weight, serum calcium, phosphorus, alkaline phosphatase, 25(OH)D, PTH and dietary intake of energy (total calories), protein and calcium

Parameter	Control girls (n=96) Mean (CI)/95% CI	Sports girls (n=90) Mean (CI)/95% CI
Total body (g/cm^2)	1.083 (1.050, 1.117)	1.216 (1.086, 1.156)
Total femur (g/cm^2)	0.963 (0.919, 1.007)	1.075 (1.028, 1.219)*
Femur neck (g/cm^2)	1.071 (0.849, 1.293)	1.047 (0.813, 1.281)
33% radius (g/cm^2)	0.588 (0.565, 0.613)	0.664 (0.639, 0.699)**
Lumbar spine (L1-L4) (g/cm^2)	1.055 (1.007, 1.102)	1.207 (1.156, 1.257)**

*$P<0.01$; **$P< 0.001$ compared to controls

by the manufacturer. Normal, mild and moderate hypovitaminosis D was observed in 51.6, 45.1 and 3.3 per cent sportswomen, respectively. In contrast, none of the controls had a normal vitamin D while 17.3, 38.5 and 54.2 per cent had mild, moderate and severe hypovitaminosis D, respectively.

Bone mineral density (BMD) parameters: Total BMD as well as BMD at all skeletal sites except femur neck were significantly ($P<0.001$) higher in sports women in comparison to controls. BMD in sportswomen was higher than that in controls by 5, 13.1, 10.3 and 9.2 per cent at total body, total hip, lumbar spine and 33 per cent radius respectively. This difference persisted after adjustment for anthropometric, biochemical, hormonal and nutritional parameters at total hip and lumbar spine (Table IV). No significant association was found between BMD and either biochemical or hormonal parameters.

Discussion

Peak bone mass is a key determinant of skeletal health throughout life[17]. Approximately 60 per cent of the risk of osteoporosis can be explained by the amount of bone mineral achieved by early adulthood, and the subsequent bone loss accounts for the remaining risk. The attainment of peak bone mass is influenced by genetic and environmental factors[5,6]. In view of the fact that physical activity and nutrition are considered to be key determinants for acquisition of bone mass[23], the effect of these two parameters on BMD was studied by selecting physically active sportswomen and sedentary controls from different colleges of Delhi.

Physical activity during the pubertal years has been shown to positively influence adult bone health[24]. Several reports suggest that regular physical activity contributes significantly to gain in BMD, beginning in the prepubertal years. The present study clearly highlights that sportswomen who have undergone at least three years of regular physical training, had significantly higher total BMD and BMD at femur, 33 per cent radius and lumbar (L1-L4) skeletal sites when compared to controls. This is consistent with results from both cross-sectional and short-term follow up studies in past, reporting higher BMD values of physically active gymnasts or sportswomen when compared to controls[25,26]. The higher 25(OH)D levels in sportswomen can be explained by the longer duration of sun exposure. While higher 25(OH)D levels over a period of time may contribute to the higher BMD in sportswomen, the present study did not show this and a few other cross-sectional studies have also not reported an association between 25(OH)D concentration and BMD[27,28].

A significantly higher intake of energy and macronutrients was noted among the sportswomen when compared to controls. These findings are in agreement with other studies reporting nutrient intake in sportswomen as compared to their sedentary counterparts[26,28]. The higher protein intake of sportswomen as compared to controls may be a factor responsible for higher BMD in these subjects as also reported by other researchers[29]. Studies in various ethnic populations have observed a positive role of calcium in bone mass accrual and attainment of peak bone mass formation[30,31]. A higher daily calcium intake (both dairy and non-dairy) was noted in the diets of sportswomen in contrast to controls. The calcium intake of sportswomen was higher than the Indian RDA[18] (500 mg/day) but less than the WHO[21] (2004; 600-1300 mg/day) and U.S.[22] (1997; 500-1300 mg/day) recommendations. Contrary to the findings of the present study, various authors have reported no significant differences in the mean daily calcium intakes among physically active women and controls[26].

Some investigators have reported that physical activity is a more critical variable for attaining optimal BMD than dietary calcium intake[13,23]. However, the increased BMD of adult Hutterite women (a communal population involved in agriculture in America) which demonstrated a strong positive correlation between "current hours of feet" and "colony workload" with BMD, testifies to the

important interaction between nutrition and physical activity for skeletal health[32].

Ethnic and genetic factors are said to account for as much as 50-70 per cent of the variance in peak bone mass, with Asians having low peak bone mass as compared to Caucasians, while blacks having the highest bone mineral density[33]. Indians have also been reported to have low BMD compared to Caucasians[34]. Several reasons like short stature, high prevalence of hypovitaminosis D and traditional Indian vegetarian diets which are deficient in vitamin D and protein, may be responsible for lower BMD values reported in Indians.

In the current study, it was found that sportswomen not only had higher BMD than control subjects but also higher than other Indian[34], Chinese, Japanese (data provided by the densitometer manufacturer) and US white Caucasian[35] young adult populations. Interestingly, BMDs for the controls in the present study were also found to be higher than USA and Japanese subjects.

In conclusion, our results suggested that healthy Indian sportswomen with good nutrition, better bone biochemical parameters, adequate sun exposure and physical activity from younger age had higher BMD when compared to age matched sedentary controls. This suggests that consistent with other reports, lifestyle, physical activity and sun exposure are key determinants responsible for better bone mineral mass and serum vitamin D levels too[36]. Thus, it can be summarized that leading an active lifestyle which includes daily physical activity, leading to greater sun exposure along with good nutrition to attain peak bone mass.

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