

Published in final edited form as:

*Osteoporos Int.* 2010 July ; 21(7): 1155–1160. doi:10.1007/s00198-009-1040-9.

## Bone Status of Adolescent Girls in Pune (India) Compared to Age Matched South Asian & White Caucasian UK Girls

A Khadilkar<sup>1</sup>, N J Crabtree<sup>2,3</sup>, K A Ward<sup>4,5</sup>, V Khadilkar<sup>1</sup>, N J Shaw<sup>2</sup>, and M Z Mughal<sup>6</sup>

<sup>1</sup>Hirabai Cowasji Jehangir Medical Research Institute, Jehangir Hospital, Pune, India

<sup>2</sup>Department of Paediatric Endocrinology, Birmingham Children's Hospital, Birmingham, UK

<sup>3</sup>Department of Nuclear Medicine, Queen Elizabeth Hospital, Birmingham, UK

<sup>4</sup>Nutrition and Bone Health Research Group, MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

<sup>5</sup>Clinical Radiology, Imaging Sciences and Biomedical Engineering, The University of Manchester, Manchester

<sup>6</sup>Department of Paediatric Medicine, Royal Manchester Children's Hospital, Manchester, UK

### Abstract

**Purpose**—To determine whether adolescent girls from a low socioeconomic group in Pune, India, who had low dietary calcium intake (449 mg/day; range 356-538) and hypovitaminosis D (median serum 25-hydroxyvitamin D 23.4 nmol/l; range 13.5-31.9), would have lower lumbar spine (LS) bone mineral apparent density (BMAD), and total body (TB) bone mineral content (BMC) adjusted for lean body mass (LBM).

**Methods**—Dual energy X-ray absorptiometry was used to measure TB and LS BMC, bone area (BA) and TBLBM in 50 post menarcheal girls from Pune. These variables were compared with data from 34 South Asian and 82 White Caucasian age matched girls from the UK.

**Results**—Pune girls were shorter, lighter and had less LBM for height, compared to both UK groups; they had later age of menarche than UK Asians. The mean (SE) BA adjusted TB BMC and LSBMAD were reduced for Pune girls [1778g (17); 0.335g/cm<sup>3</sup>(0.006)], compared to the UK South Asians [1864g (18); 0.332 g/cm<sup>3</sup> (0.005)] and UK White Caucasians [1864g (13); 0.345 g/cm<sup>3</sup> (0.004)]. In contrast both LS and TBBMC adjusted for TBLBM were not significantly different between the groups.

**Conclusions**—Pune girls had low bone mass for projected bone area relative to UK South Asian & White Caucasian girls but had the appropriate amount of BMC for their LBM.

### Keywords

Bone Mineral Content; Bone Area; Bone Mineral Density; Lean Body Mass; Girls; India

---

**Address for correspondence:** Dr M Z Mughal, Consultant Paediatrician & Honorary Senior Lecturer in Child Health, Royal Manchester Children's Hospital, Oxford Road, Manchester, M13 9WL, Phone 44 (0)161 701 0640, Fax 44 (0) 161 276 6907, zulf.mughal@cmft.nhs.uk.

### Conflict Of Interest

None declared.

## INTRODUCTION

In developed countries, osteoporotic fractures are an important cause of morbidity and mortality in later life. Bone mineral content (BMC) and bone mineral density (BMD) are the quantifiable parameters of bone strength *in vivo*; in adults, with each standard deviation (SD) decrease in BMD risk of fracture increases by approximately 1.5-fold [1]. The peak bone mass (PBM) attained at skeletal maturity and the subsequent rate of bone loss will determine an individual's bone mass and fracture risk in later life. In women, 95% of PBM is achieved by the end of the second decade and approximately 40% of this is accumulated during the two years either side of menarche [2-4]. In some areas of India, under nutrition, and in particular calcium and vitamin D deficiency are common problems during adolescence [5]. It is plausible that the bone mass acquisition of adolescents with suboptimal dietary calcium intake and low body stores of vitamin D might be impaired. In this study we have used dual energy X-ray absorptiometry (DXA) to study bone size and mineral content of total body (TB) and lumbar spine (LS) bone in a group of socioeconomically disadvantaged adolescent girls attending a state run school in Pune, India. The data in Pune girls was compared to age matched South Asian and White Caucasian girls selected from the UK GE Lunar DXA database. We hypothesised that the total body bone mineral content (TBBMC) adjusted for height and lean body mass (LBM) and the lumbar spine bone mineral apparent density (LS-BMAD), of adolescent girls from Pune would be lower than age matched South Asian and White Caucasian girls from UK. We also measured serum concentration of bone minerals, parathyroid hormone and 25-hydroxyvitamin D (25(OH)D a measure of an individual's body stores of vitamin D), and estimated the dietary calcium and protein intake in Pune girls.

## METHODS

### The Pune Subjects

Post menarcheal girls from a state run school in Pune, India, (latitude 18.34° N) participated in this cross sectional study of bone health, in February 2006. All eligible girls in year 9 were assigned a pre-screening number (n = 84) and random numbers were generated to select 50 of these for the purpose of this pilot study. The Ethical Committee of the Hirabai Cowasji Jehangir Medical Research Institute approved the study. Twenty-seven literate parents provided a written informed consent in Marathi. Twenty-three illiterate parents provided an informed 'thumbprint consent', after the information about the study was read out in Marathi; all thumbprint consents were witnessed by the school's head teacher. The study subjects were derived from a low socio-economic group with the approximate average monthly per capita income of 745 Indian Rupees (approximately €3, US \$19). None of the subjects were receiving vitamin D or any other dietary supplements. A seven day Food Frequency Questionnaire (FFQ) and Gopalan et al's [6] tables of nutrient value of Indian foods were used to estimate daily dietary intake of calcium, vitamin D, total calories and protein. Serum concentration of total calcium (tCa, mmol/l) was measured using a Calorimetric assay and ionised calcium (iCa) was measured using an ion selective electrode (AVL 9130 ISE Analyser, Graz, Austria). Serum concentration of inorganic phosphate (iP, mmol/l) and alkaline phosphatase activity (ALP, i.u./l) were measured using an auto analyser (SEAC, Italy). Intact serum parathyroid hormone (PTH, pmol/l) was measured using an immunoassay (BioSource, Europe S.A.). The in-house reference range for the PTH assay was 1.1-6.4 pmol/l, which was established in one hundred 15 to 45 year old healthy volunteers from Pune. The sensitivity was 0.22 pmol/l and interassay variation was 10%. Serum concentration of 25-OHD (nmol/l) was measured using radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA). The sensitivity of the assay was 3.75 nmol/l and the interassay variation was <5%.

## The UK South Asian & White Caucasian Subjects

The DXA scans of 116, post menarchal, age-matched UK South Asian (34) and White Caucasian (82) controls were extracted from children recruited for the UK GE-Lunar Normative DXA study [7]. The UK GE Lunar Normative data consisted of 1508 children recruited between 1998 and 2002, from local schools in 4 urban areas within the UK namely, Birmingham, Middlesbrough, London & Leeds. Children were included in the study if they were between ages 5 to 18 years, had no history of chronic disease or metabolic bone disorders and were not taking any medication known to affect bone. Height standard deviation scores (SDS) were calculated from the 1990 UK growth standards [8]; children were excluded if their height was greater or less than 3SD's from the mean. The 1508 children were recruited from three different ethnic groups; White Caucasian European (~50%), South Asian (Indian, Pakistani & Bangladeshi) (~25%) and Afro-Caribbean (~25%) with both parents belonging to the same ethnic group. The UK GE-Lunar normative data study was approved by each of the centre's Local Research Ethics Committee. Informed written consent was obtained from the parents or guardians of each child under 16 years or participants over the age of 16 years. Verbal assent was obtained from children whose parents or guardians provided the written consent.

### Anthropometry

At both centers, standing height was measured using wall mounted stadiometers to the accuracy of 1 mm and weight was measured using electronic scales to the accuracy of 0.1kg.

### Bone Densitometry

In Pune, the GE-Lunar DPX Pro (GE Healthcare, Wisconsin, USA) pencil beam DXA scanner (software encore 2005 version 9.30.044) was used to measure lumbar spine (L2-L4), and total body (TB) bone mineral content (BMC [g]), bone area (BA [cm<sup>2</sup>]), areal bone mineral density (BMDa [g/cm<sup>2</sup>]) and lean body mass [LBM (g)] [9]. Bone mineral apparent density (BMAD [g/cm<sup>3</sup>]) was calculated for lumbar spine (L2-L4) using the method of Kroger et al [10]. This method is used to adjust for volumetric bone density and is based on bone width and uses the following equation:  $BMAD = BMD \times [4 / (\text{width} \times \pi)]$ . The precision of the DPX Pro for repeat measurements in adults are: 0.6% for LSBMDa, 0.6 % for TBBMDa and 1.1% for LBM [11].

In Birmingham, the GE-Lunar Prodigy narrow fan Beam DXA scanner (software version 5.0) was used to measure the same bone and LBM variables as in Pune subjects.

### Cross-calibration between Pune GE Lunar DPX-Pro and Birmingham GE Lunar Prodigy DXA machines

Cross-calibration was performed using the European Spine Phantom (ESP) [12]. The ESP is a semi-anthropomorphic spine phantom comprising vertebrae of 3 known areal BMDs, 0.5 g/cm<sup>2</sup>, 1.0 g/cm<sup>2</sup> and 1.5 g/cm<sup>2</sup>. The phantom was scanned 10 times without repositioning in the standard scanning mode on both scanners. Cross-calibration equations were calculated, for BMDa (g/cm<sup>2</sup>), BMC (g) and projected BA (cm<sup>2</sup>), using a linear regression model with Birmingham as the reference centre. The results demonstrated that the machines were highly correlated with  $r^2$  ranging from 0.986 to 1.000. Regression slopes were significantly different from unity for all parameters. However, intercepts were only significantly different from zero for BMD and BMC. Accordingly, to minimise centre differences due to inter machine variation, the DXA data for Pune girls' data were transformed using calculated cross calibration equations for BMD, BMC and bone area. Given that cross-calibration studies were performed using a bone phantom (the ESP) it was not possible to cross-calibrate and transform the lean body mass.

## Statistical analysis

Analyses were performed using SPSS 15.0 for Windows (SPSS Inc, Chicago, USA). Height, weight and BMI SDS (mean & 95% CI) of children in the 3 groups were calculated using the UK 1990 Growth data [8, 13]. Bone data were analyzed using a general analysis of variance model to investigate differences between the groups with age, height, bone area or LBM as covariates in the model. Post-hoc Bonferroni multiple comparison tests were used to assess group differences with an  $\alpha$ -level of  $P < 0.05$  considered to be statistically significant. Results are presented as means  $\pm$ SE unless otherwise specified. Biochemical and nutritional data are presented as median and inter quartile ranges (Pune girls only). Linear associations between TBBMC (adjusted for age, height, bone area and LBM) and LS-BMAD (adjusted for age) with serum 25OHD, PTH, dietary calcium intake and protein intake was assessed in the Pune girls

## RESULTS

Height SDS, weight SDS and LBM for height SDS of Pune girls were significantly lower in comparison to South Asian and White Caucasian girls from the UK (Table 1). Age at menarche was slightly older in the Pune Asians than UK Asians. Their areal BMD was significantly reduced at the lumbar spine ( $p < 0.05$ ) and total body ( $p < 0.05$ ) compared with their UK counterparts. After adjustment for their short stature the LSBMAD for Pune girls was still significantly reduced ( $p < 0.05$ ). The TBBA adjusted for height was not significantly different in the 3 groups, suggesting that Pune girls did not have notably narrow bones for their height. However, total body BMC (TBBMC) adjusted for TBBA of Pune girls was significantly lower than that of both UK groups ( $p < 0.05$ ), suggesting Pune girls appear to have low bone mass for projected bone area relative to UK South Asian & White Caucasian girls. In contrast, there were no significant differences between the groups for TBBMC adjusted for LBM.

The median (IQR) values for biochemical parameters for Pune girls are shown in Table 2. Thirty five (70%) girls had serum 25OHD concentrations  $< 30$  nmol/l, a level that is often associated with rickets and osteomalacia [14]. The mean serum tCa concentration was 2.09 mmol/l (SD 0.17) with 37 (74%) girls being below the normal range; 49 (98 %) of the girls had iCa below the reference range. Twenty four out of 50 (48 %) girls had PTH concentration above the upper end of the reference range.

The median (IQR) values for daily dietary intake of calcium, vitamin D, total calories and protein are shown in Table 3. The dietary intake of calcium of Pune girls was 449 (356 - 538) mg/day, which is lower than the recommended intake for girls of this age in the UK (800 mg/day) and in the USA (1200-1500 mg/day) [15-17]. The bulk (87%) of dietary calcium in Pune girls was derived from non-dairy products, such as vegetables, pulses and cereals. Approximately 30% of calcium is absorbed from milk and milk products as opposed to 10 % from non-dairy sources. The median dietary energy (calorie) intake of Pune girls was 62% and protein intake was 69% of that recommended for Indian adolescents [6]. No significant correlation was found between TBBMC (adjusted for age, height, bone area and LBM) and LS-BMAD (adjusted for age) and serum 25OHD, PTH, dietary calcium intake and protein intake in the Pune girls.

## DISCUSSION

This is, to our knowledge, the first study in which lumbar spine and total body BMC and BMD of adolescent girls from a lower socioeconomic group in India have been compared to relatively 'well off' age matched South Asian and White Caucasian girls from the UK. Pune girls were shorter, lighter and had less LBM compared to their UK counterparts. The lower

areal BMD of the spine and total body in Pune girls is likely to be part due to their smaller bones, as these parameters are confounded by body size. However, even after size correction, TBBMC adjusted for TBBA and LS-BMAD were lower in Pune girls, indicating that they had reduced amount of mineral content within their smaller 'periosteal envelopes'. To interrogate this further, bone area was adjusted for height and BMC was adjusted for bone area [18], which also showed that the bones of the Pune girls contained less mineral content in comparison to both UK groups.

It is generally agreed that the greatest physiological loads placed upon the skeleton originate from muscle forces, which play a crucial role in the development and maintenance of bone architecture and mineral content [19]. Lean body mass measured by DXA has been used as a surrogate to examine the effect of muscle on bone [20]. For their size, Pune girls had reduced lean body mass and thus muscle mass compared to UK girls. The reduced lean body mass of Pune girls is likely to have arisen as a result of chronic under-nutrition as their daily calorie and protein intakes were 62% and 69%, of the recommended nutrient intakes for Indian Adolescents, respectively [6]. However, the amount of BMC for lean body mass was appropriate in the Pune girls. Therefore, although the bones of Pune girls had low bone mass for projected bone area compared with the UK groups the amount of mineral is appropriate for their muscle mass. Other reasons for Pune girls having low bone mass include their low dietary intake of calcium, the bulk of which was obtained from poor-bioavailable sources, such as vegetables, pulses and cereals. At the time of the study we found that over 70% of Pune girls had serum 25OHD concentrations < 30 nmol/l, a level that is associated with rickets and osteomalacia [14]. Even though we did not find an association between TBBMC (adjusted for size and LBM) or LS-BMAD with serum 25OHD measured at the time of the study, it is plausible that severe chronic vitamin D deficiency might have contributed to impairment of mineralization of the bones through impaired intestinal absorption of calcium and phosphorus. At the time of the study, 48% of Pune girls had elevated serum PTH, which probably arose secondary to vitamin D and dietary calcium deficiency. Chronic hyperparathyroidism and associated skeletal demineralisation might have contributed to reduced amount of mineral content within their 'periosteal envelopes'.

The bone status of second or third generation South Asian girls in the UK, living under conditions that favour achievement of their genetic growth and bone accrual potential, was not different from that of white Caucasian girls. Therefore it is likely that with better nutrition, Pune girls might, as in their UK South Asian counterparts, achieve their optimal skeletal growth and mineralisation potential.

This study has a number of limitations. These include its cross-sectional design and the fact that UK girls were originally recruited in order to generate normative data on BMD and body composition [7, 21]. Recruitment of these children was based on an opportunistic approach rather than using a population sampling strategy, as such there may be a degree of selection bias. However, children were recruited from a mixture of local state funded, grammar and private schools, consequently, the data should be fairly representative of a wide range of social status of children from the four urban areas. Furthermore, dietary or biochemical data were not collected for these children, nor was information on socioeconomic status. Given the importance of age at menarche upon bone accrual, it is possible that the difference in postmenarcheal age between Pune and UK girls (Table 1) might have introduced differences in BMC observed in the study. However, inclusion of time since menarche in our analysis models did not significantly affect our results (data not shown). The study was performed on two different DXA machines, a narrow fan beam and a pencil beam. However, we have cross calibrated the machines using an anthropomorphic phantom to take into account these differences. Another short coming is that DXA is unable to distinguish between osteopenia and osteomalacia. As Pune girls had low body stores of



vitamin D and elevated serum PTH concentration, their low BMD might be due to a qualitative (osteomalacia) or quantitative (osteopenia) disorder of the bone, or perhaps a combination of both.

In conclusion, we have shown that underprivileged girls from Pune with high levels of vitamin D deficiency and suboptimal dietary intake of calcium, protein and calories were shorter and lighter than their aged matched South Asian and White Caucasian UK counterparts. Pune girls' had low bone mass for projected bone area in comparison to UK controls, but they had the appropriate amount of bone mineral content for their low lean body mass. The long-term consequences of these observations require further investigation.

## Acknowledgments

We thank the participating youngsters, their parents and staff at the Dhole-Patil school, Pune. We are grateful to Dr Sadanand Naik, Mr M Sayyad, Miss Neha Sanwalka, Miss Dhanashree Bhandari, Mrs. Dhole-Patil, Mrs. Shilpa Shirole, Mrs. Shamim Momin and Ms Deepa Pillay for their help with the study in Pune. We are also grateful to participants of the UK GE Lunar BMD database subjects

## REFERENCES

1. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ (Clinical research ed)*. 1996; 312:1254–1259.
2. Bailey D. The Saskatchewan pediatric bone mineral accrual study: Bone mineral acquisition during the growing years. *Int J Sports Med*. 1997; 18:191–194. [PubMed: 9187973]
3. Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. *J Bone Miner Res*. 2000; 15:2245–2250. [PubMed: 11092406]
4. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. *J Clin Invest*. 1994; 93:799–808. [PubMed: 8113412]
5. Rajeswari J, Balasubramanian K, Bhatia V, Sharma VP, Agarwal AK. Aetiology and clinical profile of osteomalacia in adolescent girls in northern India. *Natl Med J India*. 2003; 16:139–142. [PubMed: 12929856]
6. Gopalan, C.; Rama-Sastri, B.; Balasubramanian, S. Nutritive value of Indian Foods. National Institute of Nutrition Indian Council for Medical Research; Hyderabad-500 007, India: 1989.
7. Crabtree N, Oldroyd B, Truscott J, Fordham J, Kibirge M, Fewtrell M, Gordon I, Shaw N. UK paediatric DXA reference data (GE Lunar Prodigy): Effects of ethnicity, gender and pubertal status. *Bone*. 2005; 36:S42.
8. Freeman J, Cole TJ, Chinn S, Jones P, White E, Preece M. Cross sectional stature and weight reference curves for the UK, 1990. *ADC*. 1995; 73:17–24. [PubMed: 7639543]
9. Engelke K, Gluer CC. Quality and performance measures in bone densitometry: part 1: errors and diagnosis. *Osteoporos Int*. 2006; 17:1283–1292. [PubMed: 16821003]
10. Kroger H, Kontaniemi A, Vainio P, Alhava E. Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. *Bone and miner*. 1992; 17:75–85.
11. Kiebzak GM, Leamy LJ, Pierson LM, Nord RH, Zhang ZY. Measurement precision of body composition variables using the lunar DPX-L densitometer. *J Clin Densitom*. 2000; 3:35–41. [PubMed: 10745300]
12. Kalender W, Felsenberg D, Genant H, Fischer M, Dequeker J, Reeve J. European Spine Phantom - a tool for standardization and quality control in spinal bone mineral measurements by DXA and QCT. *Eur J Radiol*. 1995; 20:83–92. [PubMed: 7588873]
13. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med*. 1998; 17:407–429. [PubMed: 9496720]

14. Pettifor, JM. What is the optimal 25(OH)D level for bone in children?. In: Norman, AW.; Bouillon, R.; Thomasset, M., editors. Vitamin D endocrine system: structural, biological, genetic and clinical aspects; Proceedings of the 11th Workshop on Vitamin D 2000 Riverside (CA); University of California. p. 903-907.
15. Department of Health CMO. Dietary reference values for food energy and nutrients for the United Kingdom. HMSO; London: 1991.
16. Optimal Calcium Intake. National Institutes of Health Consensus Statement. 1994; 12:1–31.
17. Department of Health CMO. Nutrition and bone health: With particular reference to vitamin D and calcium. Report on Health and Social Subjects. The Stationary Office; London: 1998.
18. Molgaard C, Thomsen BL, Michaelsen KF. Effect of habitual dietary calcium intake on calcium supplementation in 12-14-y-old girls. *AJCN*. 2004; 80:1422–1427.
19. Frost H. Bone “mass” and the “mechanostat”: a proposal. *Anat Rec*. 1987; 219:1–9. [PubMed: 3688455]
20. Heaney R, Dowell S, Hale C, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr*. 2003; 22:142–146. [PubMed: 12672710]
21. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008; 122(5):1142–52. [PubMed: 18977996]

**TABLE 1**

Anthropometric &amp; Bone Variables for girls of three ethnic origins presented as means (SE).

	PUNE ASIANS	UK SOUTH ASIANS	UK WHITE CAUCASIAN
Number	50	34	82
Age	14.7 (0.10)	14.9 (0.11)	14.9 (0.07)
Height (cm) <sup>a,b,c</sup>	150.8 (0.8)	157.3 (1.2)	163.4 (0.7)
Weight (kg) <sup>a,b</sup>	40.1 (0.9)	53.5 (2.0)	56.4 (1.0)
Body mass index (kg/m <sup>2</sup> ) <sup>a,b</sup>	17.6(2.14)	21.6(4.13)	21.1(3.22)
Mean age at menarche(yrs) <sup>a,e</sup>	13.1(1.0)	12.3 (0.9)	12.7(1.3)
LBM adjusted for height (kg) <sup>d</sup>	29.99 (0.4)	33.71 (0.4)	34.18 (0.3)
TBBMD (g/cm <sup>2</sup> ) for age <sup>d</sup>	0.915 (0.009)	1.033 (0.011)	1.050 (0.007)
TBBA adjusted for height (cm <sup>2</sup> )	1837 (20)	1889 (20)	1838 (15)
TBBMC adjusted for TBBA (g) <sup>d</sup>	1778 (17)	1864 (18)	1864 (13)
TBBMC adjusted for LBM (g)	1853 (36)	1864 (33)	1819 (25)
LSBMD (g/cm <sup>2</sup> ) for age <sup>d</sup>	0.906 (0.016)	1.046 (0.019)	1.044 (0.012)
LSBMAD for age <sup>d</sup>	0.332 (0.005)	0.355 (0.006)	0.345 (0.004)
LSBMC adjusted for LBM (g)	35.2 (1.1)	36.9 (1.0)	36.2 (0.8)

<sup>a</sup> Pune Asian versus UK Asian p < 0.05 (from ANOVA Bonferroni post-hoc test)<sup>b</sup> Pune Asian versus UK White Caucasian p < 0.05 (from ANOVA Bonferroni post-hoc test)<sup>c</sup> UK Asian versus UK White Caucasian p < 0.05 (from ANOVA Bonferroni post-hoc test)<sup>d</sup> Pune Asian significantly different from UK Asian & White Caucasian p<0.05 (from univariate analysis of variance model).<sup>e</sup> Data not complete, n = 21 UK Caucasians, 58 UK Asians  
Values represent mean (SE)



**TABLE 2**

The biochemical parameters of Pune girls

BIOCHEMICAL PARAMETER	VALUE	NUMBER (%) PUNE GIRLS WITH ABNORMAL VALUES	REFERENCE RANGE
Total Calcium (mmol/l) <sup>a</sup>	2.09 (0.17)	37(74)	2.2 – 2.6
Ionized Calcium (mmol/l) <sup>b</sup>	0.88 (0.92-0.97)	49(98)	1.13-1.18
Inorganic Phosphate (mmol/l) <sup>a</sup>	1.2 (0.21)	*	0.7 – 1.4
Parathyroid hormone (pmol/l) <sup>b</sup>	5.7 (1.1 – 11.8)	24(48)	1.1 – 6.4
Alkaline phosphatase Activity <sup>b</sup> (i.u./l)	320.0 (230.3 – 439.8)	*	*
25-hydroxyvitamin D (nmol/l) <sup>b</sup>	23.4 (13.5 – 31.9)	**	**

<sup>a</sup>Values are mean (SD)<sup>b</sup>Values are Median (IQR)

\* Reference range of serum alkaline phosphatase activity depends on age and stage of pubertal development.

\*\* There is no reference range for adolescents, however, in adult reference optimum dietary calcium was achieved when serum 25-hydroxyvitamin D concentrations were 80–90 nmol/L [20]

**TABLE 3**

The estimated daily dietary intake of Calcium and Vitamin D of Pune girls

NUTRIENT	ESTIMATED DAILY INTAKE
Total calcium intake (mg/day)	449 (356-538) *
Calcium derived from dairy products (mg/day)	65 (31-76)
Calcium derived from non-dairy products (mg/day)	400 (296-458)
Vitamin D intake ( $\mu\text{g/day}$ )	0.1 (0.0-0.8) **
Total calories (kcal/day)	1275 (1155-1510) ***
Protein (gm/day)	45 (37-57) ****

\* Recommended intake for girls of this age in India (600 mg/day) the UK (800 mg/day) and in the USA (1200-1500 mg/day) [15-17] ..

\*\* Recommended intake in the USA is 10  $\mu\text{g/day}$  throughout childhood and adolescence [21]. There are no recommended intakes for Indian & UK adolescents.

\*\*\* Recommended intake for girls of this age in India is 2060 kcal/day [6]

\*\*\*\* Recommended intake for girls of this age in India is 65 gm/day [6]