Original Article

Vitamin D supplementation and bone mass accrual in underprivileged adolescent Indian girls

Anuradha V Khadilkar MD1, Mehmood G Sayyad MSc2, Neha J Sanwalka MSc3, Dhanshari R Bhandari MSc3, Sadanand Naik PhD4, Vaman V Khadilkar MRCP1, M Zulf Mughal FRCPCH5

1Hirabai Cowasji Jehangir Medical Research Institute, Jehangir Hospital, Pune, India
2Abeda Inamdar Senior College, Pune, India
3Interdisciplinary School of Health Science, Pune University, Pune, India
4Endocrine Biochemistry, King Edward Memorial Hospital, Pune, India
5Department of Paediatric Endocrinology, Royal Manchester Children’s Hospital, Manchester, UK

Vitamin D deficiency is common among children and adolescents in India, in spite of abundant sunshine. We conducted a pilot; double blind randomised controlled trial to investigate the effect of vitamin D supplementation on bone mineral content in underprivileged adolescent girls, in Pune, India. Fifty post-menarcheal girls aged 14 to 15 years were randomised to receive 300,000 IU (7.5 mg) of ergocalciferol or placebo orally, 4 times/year. All participants received 250 mg elemental calcium (calcium carbonate) daily. Outcome measures included change in serum 25-hydroxyvitamin D, size adjusted bone area and bone mineral content at total body and lumbar spine. Post supplementation, the median serum concentration of 25-hydroxyvitamin D was 75.2 (64.2-85.5) nmol/L in the intervention group and 28.1 (16.7-34.0) nmol/L in the placebo group. Increment in bone outcome measures was not different in the two groups. However, there was a positive effect of intervention in the size adjusted total body bone area (p<0.05), total body bone mineral content (p<0.05) and lumbar spine bone mineral content (p<0.05), and positive trend in lumbar spine bone area (p=0.07) in girls who were within 2 years of menarche. We conclude that vitamin D supplementation did not have a beneficial effect on skeletal mineralization in girls who were more than 2 years post menarche. However, there was a significant positive effect of the intervention on size adjusted total body and lumbar spine bone mineral content and a positive trend in lumbar spine bone area, in girls who were ≤ 2 years of menarche.

Key Words: vitamin D, calcium intake, PTH, menarche, India

INTRODUCTION

Adolescence is an important period for bone mass accrual, when approximately 40% of the peak bone mass, defined as the maximum amount of bone mass accrued at the end of skeletal growth and consolidation, is acquired during the two years either side of the start of menstrual periods.1,2 Provision of adequate amount of calcium (Ca) and phosphorous (P) in the diet is important in order to meet this mineral requirement for rapid skeletal mineralisation. In girls, the average rate of Ca accretion in the skeleton during adolescence (age 14 to 18 years) is estimated to be around 120 mg per day. Based on these data, the daily dietary calcium requirement for girls in this age group was estimated to be around 1000 mg per day.1, 1,2,5-dihydroxyvitamin D (1,25(OH)2D), the active metabolite of vitamin D plays and important role in facilitating gastrointestinal absorption of Ca and P, renal re-absorption of filtered Ca and promoting skeletal mineralisation. In humans, the main source of vitamin D is that produced by the action of solar ultraviolet B radiation (280-315 nm) acting on 7-dehydrocholesterol in skin. Small amounts are also derived from dietary sources such as oily fish, eggs, and fortified foods. In spite of abundant sunshine in most parts of India, vitamin D deficiency is common among children and adolescents, particularly in those from low-income groups.4,6 Dietary intake of calcium among such children is often very low.4,6 Furthermore, the calcium is often derived from non-dairy sources, which have low gastrointestinal calcium bioavailability. High intake of foods rich in phytin and oxalates further impairs gastrointestinal calcium absorption.8-10 It is possible that low intake of calcium and low body stores of vitamin D might adversely affect the bone mass accrual in adolescent Indian girls. There are few studies to date which have examined the effect of vitamin D supplementation on bone mineral density (BMD) in children and adolescents. In a study of

Corresponding Author: Dr M Zulf Mughal, Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Oxford Road, Manchester, M13 9WL, UK.
Tel: +44 161 276 6501; Fax: +44 161 276 6907
Email: zulf.mughal@cmft.nhs.uk
Manuscript received 27 April 2010. Initial review completed 27 September 2010. Revision accepted 5 October 2010.
adolescent girls with adequate calcium intake from Finland, Viljakainen et al found that lumbar spine (LS) and femoral BMD increased with vitamin D supplementation in a dose responsive manner. El Hajj Fuleihan et al in a study in adolescent girls from Beirut found that vitamin D supplementation had a positive impact on musculoskeletal parameters in pre-pubertal girls. To the best of our knowledge, there have been no studies of the effect of vitamin D supplementation on bone mass accrual in underprivileged Indian adolescents.

We conducted a pilot double blind randomised controlled trial (RCT) to investigate the effect of vitamin D₂ supplementation in underprivileged adolescent girls from Pune, India (latitude 18.34°N). All participants in the trial also received a daily oral calcium supplement, which provided them with 250 mg of elemental Ca. We hypothesised that girls in the intervention group would have a greater increase in size corrected total body and lumbar spine bone mineral content compared with those in the control group.

MATERIALS AND METHODS

Participants

This pilot RCT was carried out in post menarcheal girls aged 14 to 15 years, attending a state run school, from February 2006 to April 2007, in Pune, India (latitude 18.34°N). Ethical approval was granted by the Ethics Committee of the Hirabai Cowasji Jehangir Medical Research Institute. From 84 eligible girls, 50 were randomly recruited to take part in the pilot RCT (Figure 1). Twenty seven literate parents provided a written informed consent in Marathi. Twenty three illiterate provided an informed ‘thumbprint consent’, after the information sheet about the study was read out in Marathi. All thumbprint consents were witnessed by the school’s head teacher. One girl was Muslim and the rest were Hindus. The study subjects were derived from a low socio-economic group with the approximate average monthly per capita income of 745 Indian Rupees (approximately €10.5, US$15). A high proportion of these subjects had complaints of non-specific aches and pains (70%), skeletal deformities (Genu Varum and Valgum) (44%) and latent hypocalcaemia (Positive Gower’s sign). These findings have been described previously.

Study design

Randomisation

The present study was a 12 month, double blind, placebo controlled trial of vitamin D supplementation. After baseline data collection participants were randomized by the trial statistician (MS) into two groups so that the median serum 25OHD concentrations in the two groups were approximately equal.

Supplementation

The median dietary calcium intake of study subjects was low [438 (354-555) mg/day]. As this value fell short of 600 mg/day, the recommended daily calcium allowance

---

*Figure 1.* The CONSORT diagram for participant flow through the trial.

---

** serum ionic calcium, ALP, PTH, 25 hydroxy vitamin D, inorganic phosphorus
for Indian adolescent girls, subjects received 250 mg elemental calcium in the form of calcium carbonate (Calcium Sandoz, Novartis) daily during the trial. The tablets were supplied to participants monthly by trial staff. Each bottle contained 30 tablets and tablet count performed monthly was used to assess compliance. Subjects in the treatment group were administered 6 vitamin D2 (Ergocalciferol; Celltech, UK) tablets each containing 1.25 mg (50,000 IU) orally at 1, 4, 7 and 10 months. For the placebo group the local pharmacist prepared tablets which were identical in number, colour, size and texture to the ergocalciferol, but contained only sucrose. The subjects were observed by the study staff as they swallowed the ergocalciferol or placebo tablets. All the primary investigators of the study were totally blinded to the treatment regimen. The first dose of ergocalciferol or placebo was administered in May 2006 and the last dose in February 2007.

**Outcome measures**
All baseline assessments and measurements were made prior to randomization and at the end of the trial, approximately 12 months later.

### Table 1. Anthropometric characteristic of the trial group before and after supplementation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin D + Ca Supplemented Group</th>
<th>Placebo + Ca Supplemented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>Before (n=25) 14.6 (14.0-15.1)</td>
<td>After (n=25) 15.7 (15.2-16.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>149 (147-156)</td>
<td>152 (149-158)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>39.1 (35.4-42.7)</td>
<td>40.3 (35.5-41.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.8 (16.1-18.5)</td>
<td>16.9 (16.1-18.3)</td>
</tr>
</tbody>
</table>

Values are Median (25th percentile - 75th percentile), p value by paired t-test

### Table 2. Baseline dietary intake (per day) of adolescents before intervention

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin D + Ca Supplemented Group</th>
<th>Placebo + Ca Supplemented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (mcg)</td>
<td>4.9 (2.6-14.8)</td>
<td>5.3 (3.5-9.1)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>438 (354-555)</td>
<td>458 (339-517)</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>745 (650-880)</td>
<td>846 (673-914)</td>
</tr>
<tr>
<td>Oxalates (mg)</td>
<td>99.6 (87.6-153.2)</td>
<td>113.9 (76.4-169.1)</td>
</tr>
<tr>
<td>Phytin (mg)</td>
<td>252 (227-283)</td>
<td>255 (210-293)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>46.6 (35.2-60.4)</td>
<td>44.5 (39.1-52.3)</td>
</tr>
<tr>
<td>Total calories (kcal)</td>
<td>1362 (1212-1544)</td>
<td>1315 (1049-1473)</td>
</tr>
</tbody>
</table>

Values are median (25th percentile - 75th percentile), p value by independent t-test

### Table 3. Biochemical and hematological parameters of the trial group at baseline and at the end of the trial

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin D + Ca Supplemented Group</th>
<th>Placebo + Ca Supplemented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD (nmol/L)</td>
<td>24.5 (12.7-33.2)</td>
<td>75.2 (64.2-85.5)</td>
</tr>
<tr>
<td>Total calcium (mmol/L)</td>
<td>2.1 (2.0-2.3)</td>
<td>1.05 (1.0-1.09)</td>
</tr>
<tr>
<td>Ionised calcium (mmol/L)</td>
<td>0.94 (0.89-1.01)</td>
<td>0.91 (0.84-0.94)</td>
</tr>
<tr>
<td>Phosphorous (mmol/L)</td>
<td>1.2 (1.2-1.4)</td>
<td>1.69 (1.0-1.3)</td>
</tr>
<tr>
<td>Alkaline phosphate (U/L)</td>
<td>322 (244-490)</td>
<td>293 (206-436)</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>2.6 (0.7-9.1)</td>
<td>7.7 (2.6-11.9)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>125 (112-130)</td>
<td>120 (110-124)</td>
</tr>
</tbody>
</table>

Values are median (25th percentile - 75th percentile)
* The imbalance in serum PTH was caused by three high outlier values in the placebo group. When these were removed the median PTH values were not different in the two groups.

**Anthropometry, questionnaires and dietary food analysis**
Standing height was measured using a wall-mounted stadiometer to the accuracy of 1 mm and weight was measured using an electronic scale to the accuracy of 100 grams. A food frequency questionnaire and Gopalan et al’s tables of nutrient value of Indian foods were used to estimate daily dietary intake of calcium, protein and calories. Krause’s table of nutritive value were used to estimate the dietary intake of vitamin D.

**Biochemical variables**
Blood samples were drawn early in the morning after a fasting period of 8 hours. Subjects’ serum concentration of total calcium (tCa) was measured using a Colorimetric assay and ionised calcium (iCa) was measured using an ion selective electrode (AVL, ISE, Graz, Austria). Serum concentration of inorganic phosphate (IP) and alkaline phosphatase activity (ALP) were measured using an auto analyser (Biotech, USA). Intact serum parathyroid hormone (PTH) 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 inter-assay variation was 10%. Serum concentration of 25-hydroxy-vitamin D (25OHD; a measure of an individual’s body stores of vitamin D) was measured using radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA). The sensitivity of the assay was 3.75 nmol/L and the inter-assay variation was less than 5%.

### Bone densitometry

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 mineral apparent density (BMAD, g/cm²) and total body analyses. Bone mineral apparent density (BMAD, g/cm²) was calculated for lumbar spine (L2-L4) by the method of Carter et al.\textsuperscript{15} The precision of repeat measurements in adults DPX Pro are reported to be: 0.6% for LS BMD, 0.6% for TBBMD, 1.1% for LBM and 2.0% for fat mass (FM).\textsuperscript{16}

### Statistical analysis

The primary outcome measures were TB BMC, LS BMC and LS BMAD. Secondary exploratory outcomes were TB lean, FM and serum concentrations of biochemical parameters iCa, iP, ALP, PTH and 25OHD. Data are shown as median with the 25\textsuperscript{th} and the 75\textsuperscript{th} percentiles. The variables which were not normally distributed were transformed to normality using appropriate mathematical transformation to satisfy the underlying normality assumption. In the intervention group, linear relationship between the increase in serum 25OHD and dietary calcium intake was studied using Pearson’s correlation. In order to test the statistical significance of difference of continuous primary outcome measures (such as TB BMC, LS BMC and LS BMAD) between the two-study groups, independent sample t-test was used. Several statistical associations were adjusted for possible confounders such as baseline value of dietary calcium intake, height, weight, lean body mass and calcium compliance as necessary using ANCOVA technique. The relative percentage change in total BMC, total bone area, total fat mass, total lean mass, lumbar spine BMC and area was calculated using the formula (100×(Post supplementation value – Pre supplementation value)/Pre supplementation value). P value less than 0.05 was considered statistically significant. All analysis was performed using SPSS (version 12.0, Chicago, USA).

## RESULTS

Figure 1 shows a CONSORT diagram for participant flow though the trial. Table 1 shows baseline as well as post-supplementation comparison of anthropometric and clinical characteristics of the two study groups (study subjects supplemented with vitamin D and placebo group). There was no significant difference between baseline anthropometric characteristics of the two groups, however, as a group the girls that we studied were shorter and lighter (mean Height z score -0.6±0.9, mean weight z score -0.7±0.7, in relation to the age and gender matched Indian norms.\textsuperscript{17} The mean age at menarche and time since menarche was not different in the two groups (p=0.34 and 0.19 respectively). Mean compliance for calcium was about 91% (10.9) while that for vitamin D was 100%. No adverse events as a result of treatment were recorded.

Baseline biochemical characteristics of the study subjects are given in Table 2. There were no significant differences in the energy, protein, calcium and vitamin D intakes of the two groups. The calcium mean intake was 75% as per Indian recommendations however it was 55% of the recommended intake for girls of this age in the UK (800 mg/day) and 37% of the recommended intake for girls of this age in the US (1200 mg/day).\textsuperscript{13,18-20} The median energy intake of the whole group was 62% and protein intake was 69% of that recommended for Indian adolescents.\textsuperscript{13} There were no differences in the percentage change post supplementation for protein or calcium intake of the two groups.

Table 3 describes the biochemical and haematological characteristics of study subjects at baseline and follow up. As a group 70% of the girls had serum 25OHD concentrations below 30 nmol/L, 98% of the girls had iCa below the reference range and 48% had PTH concentration above the upper end of the reference range. The median serum 25OHD concentration was significantly raised in both groups, however the mean overall increase in the supplemented group was 68% as against the increase in the placebo group which was 19%. As shown in Figure 2, in the intervention group, the rise in serum 25OHD concentration was positively related to dietary calcium intake (r=0.8, p<0.005). The median iCa and iP concentrations were significantly raised post supplementation in the placebo group. In both groups, the median concentrations of iCa were raised and that of ALP reduced. Though the serum PTH concentrations were significantly lower in both groups post supplementation, the mean overall decrease was 27% in the vitamin D supplemented group as against 9% in the placebo group. Post supplementation, the median hemoglobin concentrations did not change in the vitamin D supplemented group, however, as shown in Table 3, there was a trend towards significance in the placebo group.

Table 4 shows comparison of the percentage change in median BA, BMC, BMAD, total body fat and lean body mass values between the two groups using ANCOVA with and without adjustment for confounders (age, height, weight, initial dietary calcium, calcium compliance). No significant effect of vitamin D supplementation was found on the percent change in LS BA, LSBA, TBBA and TB BMC. The median LS BMAD was not different in the two groups. To investigate this further we divided each study group into girls who had menarche within two years of performing the study and those who had menarche for over 2 years at the time of collection of baseline data (Table 5). There was a significant increase in the size and compliance adjusted total BMC and BA change in subjects who were within 2 years of menarche (p=0.03 and 0.04 respectively). No difference was seen in the above parameters even when the group was dichotomized into low and high baseline serum 25OHD values (<or >30 nmol/L; median value of vitamin D) (Data not shown). There was no difference in the size adjusted lean mass or fat mass. The Lumbar spine BMC (adjusted for size and compliance) was significantly higher in the group within...
2 years of menarche and the change in the Lumbar area showed a trend towards significance ($p=0.07$) with group within 2 years of menarche having a greater increase in area.

**DISCUSSION**

We found that despite an increase in serum 25OHD concentration, the increase in size adjusted total body and spinal BA and BMC of girls in the intervention group did not differ from that in the placebo group. At the end of the trial, the median serum 25OHD concentration in the intervention group was 75.2 (64.2-85.5) nmol/L. This is a level that is below the range of 80 nmol/L to 90 nmol/L, which in adults was found to be the desirable range for optimal gastrointestinal calcium absorption efficiency. Therefore, the lack of response to intervention might in part be due to an inadequate increase in serum 25OHD. Another possible reason for the lack of effect of intervention in adolescent girls that we studied might be that the median sum of calcium intake from diet plus the daily supplement was around 690 (600-805) mg/day, which is less than that recommended for adolescent girls in the UK (800 mg/day) and USA (1300 mg/day). Their daily calcium intake from diet and supplement during the trial
was also lower than 1000 mg/day, which was estimated by Vatanparast et al from the average rate of Ca accreted into the skeleton during adolescence in girls. El Hajj Fuleihan et al, found a significant increase in total hip BMC in pre-pubertal but not post-menarcheal Lebanese girls treated with vitamin D3. One possible explanation for lack of a significant effect in our trial might be our use of vitamin D2, rather than vitamin D3 as a supplement. In adults, vitamin D2 has been reported to have a lower potency relative to vitamin D3. However, others have found vitamin D2 in adults to be as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D in adults. Furthermore, Thacher et al have shown vitamin D2 and vitamin D3 are bioequivalent in their effects on calcium and vitamin D homeostasis in Nigerian children with nutritional rickets. We also examined the effect of intervention in girls who were ≤2 years of menarche and those who were ≥2 year of menarche. We observed a significant positive effect of the intervention on size adjusted total body BA, total body BMC and lumbar spine BMC and a positive trend in lumbar spine BA, in girls who were ≤2 years of menarche.

In 11 to 12 year old Finnish girls who were consuming a diet adequate in calcium, Vijakainen et al observed a positive effect of vitamin D3 supplementation of 5 and 10 µg/day on femoral BMC, and of 10µg/day on spinal BMC. In a study of 11 to 12 year old healthy girls from Denmark who received 5 or 10 microgram of vitamin D3 for a year, Molgaard et al found that there was no effect of vitamin D supplementation on whole body or spine bone mineral augmentation. In contrast to our findings and that of El Hajj Fuleihan et al, Molgaard et al found that pubertal status did not appear to modulate the effect of vitamin D status on bone. In a study to assess the effect of relatively low dosages of supplemental vitamin D on bone in Pakistani immigrant girls (mean age 12.2 years) in Denmark, Andersen et al found that though supplementation increased serum 25OHD concentrations there was no significant effect of the intervention on whole body and lumbar spine bone accretion.

Unlike El Hajj Fuleihan et al, we did not observe a positive effect of the intervention on total body lean body mass of the adolescent girls we studied. This might be due to low protein intake in our subjects; the median protein intake for the whole group was around 69 % of the recommended nutrient intake.

In the intervention group, we found the increment in serum 25OHD was greater in those with higher dietary calcium intake. This finding is in keeping with results of studies in rats and humans which suggest that calcium intake influence the serum 25OHD status. This effect is probably mediated by low calcium intake causing secondary rise in serum PTH concentrations, which in turn leads to higher serum concentrations of 1,25-dihydroxyvitamin D, which is known to degrade 25OHD to inactive 24,25-dihydroxyvitamin D, thereby depleting body stores of vitamin D.

Our trial has a number of limitations. We did not measure BMC or BMD of the hip a site where positive effects of intervention have been reported. There was a significant difference in baseline median serum PTH concentrations of girls in the intervention groups and placebo groups. However, this difference disappeared when the analysis was re-run leaving out three subjects with very high serum PTH values. These three individuals had very low serum 25OHD concentration (mean value 17.3 nmol/L) which is known to result in secondary hyperparathyroidism. Results did not change when these subjects were excluded from analysis. All subjects with high intact PTH levels from the placebo group underwent treatment with vitamin D after the study.

In summary, we found that underprivileged post-menarcheal Indian girls randomised to treatment with 300,000 IU of ergocalciferol, administered orally 4 times in a year, did not show a greater increase in size corrected total body and spinal bone mineral content, compared with those in the placebo group. However, a positive effect of the intervention on these outcomes was observed in girls who were ≤2 years of menarche, suggesting that pre-pubertal period might be an important period for improving skeletal mineralisation through vitamin D supplementation. An adequately powered clinical trial of vitamin D supplementation in prepubertal children consuming a diet adequate in calcium is required to determine if it results in a greater increase in whole body and regional bone mineral content.

ACKNOWLEDGEMENTS

We thank the participating adolescents, their parents and staff at the Dhole Patil School, Pune. We are grateful to Dr. Dive, Director HCJMRI, Mrs. Shamim Momin and Ms Deepa Pillay for their help with the trial. We thank Dr. Kate Ward for her comments on the manuscript.

AUTHOR DISCLOSURES

None declared.

REFERENCES


Original Article

Vitamin D supplementation and bone mass accrual in underprivileged adolescent Indian girls

Anuradha V Khadilkar MD¹, Mehmood G Sayyad MSc², Neha J Sanwalka MSc³, Dhanshari R Bhandari MSc³, Sadanand Naik PhD⁴, Vaman V Khadilkar MRCP¹, M Zulf Mughal FRCPCH⁵

¹Hirabai Cowasji Jehangir Medical Research Institute, Jehangir Hospital, Pune, India
²Abeda Inamdar Senior College, Pune, India
³Interdisciplinary School of Health Science, Pune University, Pune, India
⁴Endocrine Biochemistry, King Edward Memorial Hospital, Pune, India
⁵Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, UK

印度貧困青少女補充維生素 D 對骨質增長之效果

儘管有充足的日曬，印度的兒童與青少年，維生素 D 缺乏的情況仍然相當常見。以一項雙盲隨機對照試驗，探討印度普那地區貧困青少女攝取維生素 D 補充劑對於骨礦物質含量之效果。受試者為 50 位已歷初經後，且年齡為 14-15 歲的少女，隨機分派為介入組或安慰劑組，並分別在一年內各補充四次，每次 7.5 mg 之維生素 D₂ 或安慰劑，同時給予所有受試者每天 250 mg 之元素鈣 (碳酸鈣)。結果測量包括，血清 25-OHД、全身與腰椎之骨面積與骨礦質含量的變化。補充試驗後發現，介入組的血清 25-OHД 濃度之中位數為 75.2 nmol/L，而在安慰劑組為 28.1 nmol/L。但是骨質增長部分，兩組之間沒有顯著差異。然而在初經來潮兩年內少女之全身骨質面積、骨礦質含量及腰椎骨礦質含量呈現介入之正向效應 (p<0.05)，腰椎骨質面積亦呈現介入的正向趨勢 (p=0.07)。綜合以上，對初經來潮兩年後的少女，維生素 D 的補充，對於骨骼礦化沒有增益效果。然而對初經後兩年內的少女，其全身及腰椎骨礦質含量則有介入之顯著正向效應，並且在腰椎骨質面積顯示介入的正向趨勢。

關鍵字：維生素 D、鈣攝取、甲狀旁腺激素、初經、印度