Hypovitaminosis D among healthy adolescent girls attending an inner city school

G Das, S Crocombe, M McGrath, J L Berry, M Z Mughal

To determine whether vitamin D status is related to hypovitaminosis D among healthy adolescent schoolgirls attending an inner city multiethnic girls’ school.

Methods: Fifty one (28%) of 182 girls (14 white, 37 non-white; median age 15.3 years, range 14.7–16.6) took part in the study. Biochemical parameters, dietary vitamin D intake, muscle function parameters, duration of daily sunlight exposure (SE), and percentage of body surface area exposed (%BSA) were measured.

Results: Thirty seven (73%) girls were vitamin D deficient (25-hydroxyvitamin D (25OHD) <30 nmol/l) and 9 (17%) were severely deficient (25OHD <12.5 nmol/l). The median (range) 25OHD concentration of non-white girls (37.3 nmol/l (18.3–73.3)) was higher than that of non-white girls (14.8 nmol/l (5.8–42.8)). The median (range) concentration of parathyroid hormone in white girls (2.8 pmol/l (1.0–3.7)) was lower than that of non-white girls (3.4 pmol/l (1.7–34.2)). Serum Ca, inorganic phosphate, alkaline phosphatase, and 1,25-dihydroxyvitamin D were not different in white and non-white girls. For the whole group, 25OHD concentration was related to the estimated SE and %BSA, but not to estimated intake of vitamin D. In white girls, the estimated SE and %BSA were significantly higher than that of non-white girls. The median times taken to complete the Gower’s manoeuvre and grip strength were not different in the two groups; these variables were not related to serum 25OHD.

Conclusions: Hypovitaminosis D is common among healthy adolescent girls; non-white girls are more severely deficient. Reduced sunshine exposure rather than diet explains the difference in vitamin D status of white and non-white girls.

Methods

Fifty one (28%) of 182 girls, 14 white Caucasian and 37 non-white (20 were of Pakistani origin, 11 of Bangladeshi origin, 5 of Middle Eastern origin, and 1 was Black British), attending year 10 of an inner city multiethnic girls’ school took part in the study between 21 and 23 May 2003. The study was approved by the Central Manchester local research ethics committee and written consent was obtained from both parents and subjects. Standing height (m) was measured for each participant using a Microtoise (Chasmors, London), and weight (kg) was measured using Seca 761 mechanical weighing scales. BMI was calculated using the formula kg/m².

The daily intake of vitamin D and calcium were estimated using a food frequency questionnaire and the Comp-Eat v.5 for Windows Nutritional Software (CompEat Nutrition Systems, Closterworth, Grantham, UK), which incorporates the 6th edition of McCance and Widdowson’s tables of food values. The subjects also completed a questionnaire that was used to determine the daily sunlight exposure (SE). The percentage of body surface area exposed (%BSA) to sunlight, when wearing their most commonly worn daytime clothes, was estimated using the “rule of nines” used in clinical practice to estimate the burnt area of skin. The Gower’s manoeuvre was assessed by two independent observers, who used electronic stop watches to measure time taken in seconds to stand erect from a supine lying position. Grip strength (kg) of the non-dominant hand was determined using a hand held dynamometer (JAMAR Hydraulic Hand Dynamometer, Preston, Jackson, MI, USA); in each subject the 6th edition of McCance and Widdowson’s tables of food values.

Abbreviations: 1, 25(OH)2D; 1, 25-dihydroxyvitamin D; 25OHD; 25-hydroxyvitamin D; ALP, alkaline phosphatase; P, inorganic phosphate; PTH, parathyroid hormone.
three consecutive measurements were taken and the mean value was used for analysis. The in-house CV of this method is less than 10%.

Serum concentrations of calcium adjusted for albumin (Ca) and inorganic phosphate (P), and alkaline phosphatase activity (ALP) were measured using the Hitachi 917 auto-analyser (Hitachi, Tokyo, Japan). Serum intact parathyroid hormone (PTH) was measured using an immunoradiometric assay (Nichols Institute Diagnostics, San Juan, Capistrano, USA) (adult reference range 1.1–6.4 pmol/l, sensitivity 0.1 pmol/l, intra- and inter-assay coefficients of variation 3% and 6% respectively). Vitamin D metabolites were measured by in-house assays as described in detail previously. 10–12 Briefly, samples were extracted using acetonitrile and applied to C18 Silica Sep-paks. Separation of metabolites was by straight phase HPLC (Waters Associates, Milford, MA) using a Hewlett-Packard Zorbax-Sil Column (Hichrom, Reading, UK) eluted with hexane:propan-2-ol (98:2) and quantified by UV absorbance at 265 nm and corrected for recovery (sensitivity 5 nmol/l, intra- and inter-assay coefficients of variation 3.0% and 4.2% respectively). 11 Following separation by HPLC, 1,25(OH)2D was quantified by radioimmunoassay as described in detail elsewhere 11 (adult reference range 48–120 pmol/l, sensitivity 3 pmol/assay tube, intra- and inter-assay coefficients of variation 7.8% and 10.5% respectively).

The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) for Microsoft Windows was used for statistical analysis. The data are presented as median (range) and the differences in the two groups were compared using the Mann-Whitney U test. *Normal range in parentheses.
†Three non-white girls had PTH levels outside the normal range (see table 2). Reference range of serum alkaline phosphatase activity depends on age and stage of pubertal development.

### RESULTS

The age, anthropometric parameters, dietary vitamin D intake, duration of daily sunlight exposure, the percentage of body surface area exposed by the most commonly worn clothes during daytime, and biochemical parameters of white and non-white girls are shown in table 1. In all the subjects studied, serum concentration of 25(OH)D2 was below the limit of assay sensitivity. The 25OHD values stated therefore represent serum 25(OH)D3 concentrations. Thirty seven (73%) girls had serum 25OHD concentrations <30 nmol/l, a level that is conventionally regarded as the lower limit of vitamin D adequacy, and 9 (17%) had serum 25OHD concentrations <12.5 nmol/l, a level that is often associated with rickets and osteomalacia. 21 24 The median 25OHD concentration of white girls was significantly higher (p < 0.001) than that of non-white girls. For the whole group, serum 25OHD concentration was related to the estimated SE (r = 0.38; p = 0.007) and %BSA (r = 0.41; p = 0.003), but not to estimated intake of vitamin D (r = −0.01; p = 0.94). In white girls the estimated SE and %BSA were significantly higher than that of non-white girls (p = 0.003 and p = 0.001 respectively). The median concentration of PTH in white girls was lower (p = 0.02) than that of non-white girls. In spite of their low median serum 25OHD concentration and significantly raised median PTH concentration, median serum Ca, P, ALP, and 1,25(OH)2D concentrations of non-white girls were not different from those of white girls. Three non-white girls had serum PTH concentrations above the upper limit of the assay; their serum Ca, P, ALP, 25OHD, 1,25(OH)2D, and PTH concentrations are shown in table 2. The median time taken to complete the Gower’s manoeuvre or the grip strength was not different between white and non-white girls; for the whole group, these muscle function parameters were not associated with serum 25OHD, 1,25(OH)2D, or PTH concentrations. One non-white girl (serum PTH concentration 34.2 pmol/l; table 2) complained of non-specific limb pains.

### DISCUSSION

We found that hypovitaminosis D was common among healthy adolescent girls, with non-white girls being more severely deficient. This finding is in keeping with reports from European countries, 20–24 the USA, 24 and a sun-rich country like Lebanon. 24 Furthermore, we found that reduced exposure of skin to sunlight rather than dietary vitamin D intake explained the difference in vitamin D status of non-white and white girls. This is in keeping with the fact that the main source of vitamin D is that produced by the action of solar ultraviolet B radiation acting on 7-dehydrocholesterol in skin; only small amounts are obtained from dietary sources. Avoidance of exposure to sunshine for religious and cultural beliefs that encourage wearing of concealing clothing and

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**Table 1** Age, height, weight, dietary vitamin D intake, duration of daily sunlight exposure, the percentage of body surface area exposed by the most commonly worn clothes during daytime, and biochemical parameters of white and non-white girls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White girls (n = 14)</th>
<th>Non-white girls (n = 37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.2 (14.8–16.6)</td>
<td>15.3 (14.7–16.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.0 (45–76)</td>
<td>56.5 (42–78.5)</td>
<td>0.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.5 (150–170)</td>
<td>158.0 (148.7–168.7)</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>20.6 (17.8–30.8)</td>
<td>25.0 (17.0–31.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Dietary vitamin D intake (µg/day)</td>
<td>1.2 (0.3–5.6)</td>
<td>1.4 (0.4–6.0)</td>
<td>0.6</td>
</tr>
<tr>
<td>Daily sun exposure (min)</td>
<td>60 (34–60)</td>
<td>34 (15–60)</td>
<td>0.003</td>
</tr>
<tr>
<td>% Body surface area exposed</td>
<td>19 (9–33)</td>
<td>9 (7–43)</td>
<td>0.001</td>
</tr>
<tr>
<td>Time taken to perform the Gower’s test (sec)</td>
<td>2.6 (2.3–3.5)</td>
<td>2.7 (2.0–4.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>16.4 (9–21)</td>
<td>16.7 (9–30)</td>
<td>0.6</td>
</tr>
<tr>
<td>Ca (mmol/l)†</td>
<td>2.2 (2.2–2.6 mmol/l)</td>
<td>2.4 (2.3–2.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>P (mmol/l)†</td>
<td>0.7 (1.4 mmol/l)</td>
<td>1.1 (0.8–1.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>PTH (pmol/l)†</td>
<td>1.1 (1.0–1.4)</td>
<td>1.3 (1.0–1.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Alkaline phosphatase activity (IU/l)</td>
<td>232 (156–773)</td>
<td>226 (124–597)</td>
<td>0.6</td>
</tr>
<tr>
<td>25(OH)D3(mmol/l)</td>
<td>37.3 (18.3–73.3)</td>
<td>14.8 (5.8–42.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1,25(OH)2D (pmol/l)</td>
<td>87.6 (50.4–115.2)</td>
<td>93.6 (60–139.2)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are presented as median (range) and the differences in the two groups were compared using the Mann-Whitney U test.

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Table 2  Serum calcium (Ca), inorganic phosphate (P), alkaline phosphatase (ALP), 25-hydroxyvitamin D (25OHD), and 1,25-dihydroxyvitamin D (1,25(OH)2D) in three non-white girls with raised serum parathyroid hormone (PTH)

<table>
<thead>
<tr>
<th>Non-white girls</th>
<th>PTH* (1.1–6.4 pmol/l)</th>
<th>Corrected Ca+ (2.2–2.6 mmol/l)</th>
<th>P* (0.7–1.4 mmol/l)</th>
<th>ALP* (IU)</th>
<th>25OHD (nmol/l)</th>
<th>1,25(OH)2D* (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.6</td>
<td>2.4</td>
<td>1.2</td>
<td>295</td>
<td>17.3</td>
<td>136.8</td>
</tr>
<tr>
<td>2</td>
<td>8.3</td>
<td>2.4</td>
<td>1.1</td>
<td>193</td>
<td>10.0</td>
<td>72.0</td>
</tr>
<tr>
<td>3</td>
<td>34.2</td>
<td>2.3</td>
<td>1.8</td>
<td>597</td>
<td>13.8</td>
<td>117.6</td>
</tr>
</tbody>
</table>

*Reference range in parentheses.
†Reference range of serum alkaline phosphatase activity depends on age and stage of pubertal development.
‡Adult reference range: 48–120 pmol/l.

While very low serum concentrations of 25OHD in the face of secondary hyperparathyroidism are associated with rickets and osteomalacia, there is poor understanding between the relationship of serum 25OHD concentration and other health outcomes, such as optimal skeletal mineralisation in a growing child. Over 35% of the peak bone mass of a mature adult is accrued during the four years surrounding the peak pubertal growth spurt, and it is widely accepted that subjects who attain a lower peak bone mass at maturity have a higher risk of sustaining osteoporotic fractures in later life. In 14–16 year old Finnish girls, Outila et al found lower (p = 0.04) mean radial bone mineral density (BMD) in those with serum 25OHD concentration <40 nmol/l, compared to those with serum 25OHD concentration >40 nmol/l.13 Lehtonen-Vermoa et al observed a positive relationship between serum 25OHD concentrations and lumbar spine and femoral neck BMD among peri-pubertal Finnish girls.15 Further studies are required to determine if subclinical vitamin D deficiency, by limiting gastrointestinal calcium absorption and causing secondary hyperparathyroidism and associated skeletal demineralisation, results in reduced bone mass accrual around puberty, and whether vitamin D supplementation can help to prevent this.

This study has a number of limitations. The estimated duration of subjects’ sunshine exposure and percentage of body surface area exposed apply only to early summer. The results of this study may not apply to adolescents from widely diverse ethnic communities within the UK. We did not enquire about the use of sunscreen creams, which are known to reduce cutaneous vitamin D synthesis.12

In conclusion, we found that subclinical hypovitaminosis D was common among healthy adolescent girls; Non-white girls were more severely deficient. Reduced sunshine exposure rather than diet explained the difference in vitamin D status of white and non-white girls. Vitamin D deficiency during childhood and adolescence might impair the acquisition of peak bone mass at the end of skeletal growth and maturation, thereby increasing the risk of osteoporotic fracture in later life.

ACKNOWLEDGEMENTS
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Competing interests: none declared

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What is already known on this topic

- There has been a resurgence of symptomatic vitamin D deficiency among adolescents of South Asian and Middle Eastern origin living in the UK
- Adolescent vitamin D deficiency is commonly associated with hypocalcaemic tetany and non-specific symptoms, e.g. limb pains, muscle weakness

What this study adds

- Hypovitaminosis D was found in over 70% of adolescent girls attending an inner city school
- Biochemical vitamin D was not associated with symptoms

REFERENCES