

## ORIGINAL ARTICLE

## Hypovitaminosis D among healthy adolescent girls attending an inner city school

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**Aims:** To determine the prevalence of 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 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.

There has been a resurgence of vitamin D deficiency rickets among toddlers in the UK,<sup>1-4</sup> and recently there have been reports of adolescents presenting with symptoms of vitamin D deficiency; carpedal spasms, hypocalcaemic seizures, limb pains, muscle weakness, difficulty in walking/climbing stairs, and lower limb deformities.<sup>4,5</sup> These subjects had low serum concentrations of 25-hydroxycholecalciferol (25OHD; a measure of an individual's vitamin D status) and raised concentrations of serum parathyroid hormone (PTH); some also had radiological features of rickets.

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: oily fish, eggs, and fortified foods, such as margarine and breakfast cereals. Serum 25OHD concentration is considered to be an index of an individual's vitamin D status; it reflects both the vitamin D derived from cutaneous synthesis and that obtained from diet.

The Department of Health guidelines in the UK recommend that all pregnant women, lactating mothers, and children up to the age of 3 years who are at risk of vitamin D deficiency should be encouraged to take supplements.<sup>6</sup> However, there are no clear recommendations for vitamin D supplementation in adolescents. The aims of this cross-sectional study were:

- To assess the vitamin D status of healthy adolescent schoolgirls attending an inner city school
- To determine whether vitamin D status was related to sunshine exposure or dietary intake in these subjects
- To determine whether vitamin D status is related to muscle function, as assessed by the grip strength and time taken by subjects to complete the Gower's manoeuvre.

## 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/m<sup>2</sup>.

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,<sup>7</sup> was estimated using the "rule of nines" used in clinical practice to estimate the burnt area of skin.<sup>8</sup> The Gower's manoeuvre<sup>9</sup> 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

**Abbreviations:** 1, 25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D; ALP, alkaline phosphatase; P, inorganic phosphate; PTH, parathyroid hormone

**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

	White girls (n = 14)	Non-white girls (n = 37)	p value
Age (years)	15.2 (14.8–16.6)	15.3 (14.7–16.3)	0.4
Weight (kg)	53.0 (45–76)	56.5 (42–78.5)	0.2
Height (cm)	159.5 (150–170)	158.0 (148.7–168.7)	0.3
Body mass index (kg/m <sup>2</sup> )	20.6 (17.8–30.8)	25.0 (17.0–31.8)	0.09
Dietary vitamin D intake (µg/day)	1.2 (0.3–5.6)	1.4 (0.4–6.0)	0.6
Daily sun exposure (min)	60 (34–60)	34 (15–60)	0.003
% Body surface area exposed	19 (9–33)	9 (7–43)	0.001
Time taken to perform the Gower's test (sec)	2.6 (2.2–3.5)	2.7 (2.0–4.4)	0.6
Grip strength (kg)	16.4 (9–21)	16.7 (5.3–30.6)	0.6
Ca (mmol/l) (2.2–2.6 mmol/l)*	2.4 (2.3–2.6)	2.4 (2.2–2.7)	0.2
P (mmol/l) (0.7–1.4 mmol/l)*	1.1 (1.0–1.4)	1.1 (0.8–1.8)	0.5
PTH (pmol/l)*† (1.1–6.4 pmol/l)	2.8 (1.0–3.7)	3.4 (1.7–34.2)	0.02
Alkaline phosphatase activity (IU)‡	232 (156–773)	226 (124–597)	0.5
25(OH)D (nmol/l)	37.3 (18.3–73.3)	14.8 (5.8–42.8)	<0.001
1,25(OH) <sub>2</sub> D (pmol/l)§	87.6 (50.4–115.2)	93.6 (60–139.2)	0.2

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.

§Adult reference range: 48–120 pmol/l.

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.<sup>10–12</sup> 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:methanol (92:4:4). Serum 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> were measured separately by application to a second Zorbax-Sil Column 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).<sup>11</sup> Following separation by HPLC, 1,25(OH)<sub>2</sub>D was quantified by radioimmunoassay as described in detail elsewhere<sup>11</sup> (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).<sup>12</sup>

The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) for Microsoft Windows was used for statistical analysis. The data are presented as median and range; the two tailed Mann-Whitney test was used to compare the differences between variables in the two groups. Pearson's correlation was used to determine association between the variables; p values less than 0.05 were considered statistically significant.

## RESULTS

The age, anthropometric parameters, dietary vitamin D intake, muscle function parameters, duration of daily sunlight exposure, the percentage of body surface area exposed during daytime, and biochemical parameters of white and non-white girls are shown in table 1. In all the subjects studied, serum concentration of 25OHD<sub>2</sub> was below the limit of assay sensitivity. The 25OHD values stated therefore

represent serum 25OHD<sub>3</sub> 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,<sup>13</sup> and 9 (17%) had serum 25OHD concentrations <12.5 nmol/l, a level that is often associated with rickets and osteomalacia.<sup>2–14</sup> 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)<sub>2</sub>D 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)<sub>2</sub>D, 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)<sub>2</sub>D, 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,<sup>15–17</sup> the USA,<sup>18</sup> and a sun-rich country like Lebanon.<sup>19</sup> 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

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

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

\*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.

restriction of outdoor activities has previously been reported as a risk factor for vitamin D deficiency in Saudi Arabian adolescents.<sup>20</sup> Increased skin pigmentation is a further factor that might explain the difference in vitamin D status of white and non-white girls in our study.<sup>21</sup>

The most important function of vitamin D is to maintain serum calcium concentrations within the normal range by stimulating its absorption from the diet. In the early stages of vitamin D deficiency, plasma calcium concentration is low, with a normal plasma phosphate concentration. Hypocalcaemia leads to secondary hyperparathyroidism, which in turn results in an increase in plasma 1,25(OH)<sub>2</sub>D concentration, normalisation of plasma calcium concentration, and a decrease in serum phosphate concentration. At this stage, plasma 25OHD concentration is low and the concentration of 1,25(OH)<sub>2</sub>D is normal or high. This biochemical state is maintained at the expense of the resorptive action of PTH on bone. Long standing vitamin D deficiency eventually leads to recurrence of hypocalcaemia. Unlike the nine South Asian adolescents who presented to our unit with symptomatic vitamin D deficiency,<sup>3</sup> as a group, the subjects in this study did not have disturbances of their serum Ca, P, and ALP concentrations or evidence of muscle weakness, as determined by grip strength and the Gower's manoeuvre. While the median PTH concentration of non-white girls was higher than that of white girls, it was within the reference range for the assay; the failure of serum PTH to rise above the upper limit of the normal range in the face of low serum 25OHD concentration has previously been reported in asymptomatic Finnish adolescent girls.<sup>15</sup> In contrast, the median serum 25OHD concentration of the nine South Asian adolescents with symptomatic vitamin D deficiency (6.8 nmol/l, range 0–25) was significantly lower ( $p = 0.004$ ), and their median PTH (26.5 pmol/l, range 15.5–124.3) significantly higher ( $p < 0.001$ ) compared to median concentrations of these variables in 37 girls who were vitamin D deficient (25OHD  $< 30$  nmol/l) in the current study. Taken together, these findings suggest that there is a long latency period before symptoms and signs of vitamin D deficiency become apparent and that they are more likely to occur when secondary hyperparathyroidism has developed on depletion of the 25OHD substrate, which in turn leads to a fall in serum concentration of 1,25(OH)<sub>2</sub>D.

While serum 25OHD is an indicator of an individual's vitamin D status, there is considerable debate about what level constitutes "deficiency" or "sufficiency". In one study in the USA, adults with serum 25OHD concentrations  $< 20$  nmol/l were considered to have severe hypovitaminosis D, those with serum 25OHD concentrations 20–37.5 nmol/l to have moderate hypovitaminosis D, and those with 25OHD concentrations  $> 37.5$  nmol/l to have adequate vitamin D stores.<sup>22</sup> With regard to children, serum 25OHD concentrations of 27.5 nmol/l<sup>23</sup> or 30 nmol/l<sup>13</sup> are considered to be deficient. Regardless of the level of 25OHD that is used to define vitamin D deficiency, hypovitaminosis D was common among adolescent girls in this study.

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,<sup>24</sup> 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  $\leq 40$  nmol/l, compared to those with serum 25OHD concentration  $> 40$  nmol/l.<sup>15</sup> Lehtonen-Veromaa *et al* observed a positive relationship between serum 25OHD concentrations and lumbar spine and femoral neck BMD among peri-pubertal Finnish girls.<sup>25</sup> 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.<sup>26</sup>

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.

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Competing interests: none declared

### 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

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