Factors influencing the vitamin D status of 10-year-old urban South African children

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Abstract

Objective: Assessment of vitamin D status in a cohort of healthy 10-year-old urban children and the factors that influence vitamin D status in these children. Design: A cross-sectional study. Blood samples were collected across four seasons of the year for the biochemical determination of serum 25-hydroxyvitamin D [25(OH)D]. Anthropometric measurements (height and weight), BMI and total fat and lean mass (determined by the dual energy X-ray absorptiometry) were measured. 25(OH)D concentrations were assessed by chemiluminescent assay. Setting: Study of children in the Greater Johannesburg area of South Africa who form the Bone Health sub-cohort of the longitudinal Birth to Twenty cohort. Subjects: Three hundred and eighty-five children who form the Bone Health sub-cohort of the longitudinal Birth to Twenty cohort.

Results: White children had significantly higher 25(OH)D than their black peers (120·0 (sp 36·6) nmol/l v. 93·3 (sp 34·0) nmol/l, respectively). Seasonal variations in 25(OH)D levels were found only in white children, with 25(OH)D levels being significantly higher in white than in black children during the autumn and summer months. In multiple regression analysis, season, ethnicity, sex and total fat mass were the factors found to have an influence on 25(OH)D. Vitamin D deficiency (7%) and insufficiency (19%) were uncommon among the 10-year-old children.

Conclusions: Vitamin D supplementation or fortification is not warranted in healthy children living in Johannesburg. However, further studies need to confirm this in other regions of the country, especially in those living further south and with less sunshine during the winter months.

Keywords
25(OH)D
Ethnicity
Season
Gender
Body composition
South Africa
Children

The classical role of vitamin D in man is to increase the absorption of calcium and phosphate from the gastrointestinal tract for the mineralisation of the skeleton. A deficiency of vitamin D leads to hypocalcaemia and bone disease (rickets or osteomalacia). Vitamin D has two main forms: vitamin D₃ or cholecalciferol, which is formed in the skin after exposure to ultraviolet (UV) light; and ergocalciferol or vitamin D2, which is obtained by irradiation of ergosterol in plants and foods⁽¹⁾. Serum 25-hydroxyvitamin D [25(OH)D] concentrations serve as a marker of vitamin D status; however, the optimal levels for health remain a subject of much debate⁽²⁾. This is attributed to the difficulty in reaching a consensus in defining optimal 25(OH)D concentrations not only for bone health at different ages but also for the other non-classical actions of vitamin D on cell differentiation, proliferation and function (3,4). Some of the uncertainty has also been caused by a lack of standardisation of and variation in 25(OH)D assays⁽⁵⁾.

There are a variety of factors that are reported to influence vitamin D status in children and adults. These include

duration of sun exposure, season, cloud cover, latitude, pollution, sunscreen use, skin coverage by clothing, ethnicity and body composition. The paucity of data on the vitamin D status of healthy children in Johannesburg has led us to assess the vitamin D status in healthy 10-year-old children and to determine the influence of body composition, seasonal change, sex and ethnicity on vitamin D status.

Our hypothesis was that children living in Johannesburg would generally be vitamin D-sufficient as Johannesburg has greater than eight sunshine hours daily throughout the year⁽⁶⁾; however, black children in general would have lower 25(OH)D concentrations than white children due to their higher skin pigmentation.

Methods

Subjects

This was a cross-sectional study of 475 children aged 10 years, who formed the Bone Health sub-cohort of the

Birth to Twenty cohort. The Birth to Twenty cohort is a longitudinal study of child health and development, which has followed the development of 3273 children in the Greater Johannesburg area of South Africa since their birth in 1990⁽⁷⁾. The Bone Health cohort, which is a representative of the larger Birth to Twenty cohort, consists of 475 black and white children who since the age of 9 years have been intensively studied to investigate factors influencing bone mass accrual during adolescence.

The selection of the participants and cross-checks for key demographic variables between the Birth to Twenty and Bone Health cohorts have been reported elsewhere⁽⁸⁾. Participants with chronic illness (juvenile ideopathic arthritis, asthma or epilepsy), on medications which affect growth or bone mass development, or on mineral or vitamin D supplements were excluded. Guardians gave written informed consent for their children to be studied, and verbal assent was obtained from the children. The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg (Approval no. M980810).

All children lived in Johannesburg at a latitude of approximately 26°S and altitude of 1750 m. Seasons of the year for the statistical analysis of 25(OH)D concentrations were categorized as follows: autumn (March-May), winter (June-August), spring (September-November) and summer (December-February). The average daily sunshine hours and maximum temperatures in Johannesburg during the seasons are as follows: autumn (8.2 h, 22°C), winter (9.1 h, 18°C), spring (8.8 h, 24°C) and summer (8.2 h, 26°C)⁽⁶⁾. With regard to the sampling procedure of study participants across the seasons, eighty-two participants were sampled in autumn (seventy-two blacks and ten whites), 105 in winter (seventy-six blacks and twenty-nine whites), ninety-four in spring (ninety-one blacks and three whites) and 104 in summer (fifty-six blacks and forty-eight whites), totalling to 385 subjects.

There were major differences in socio-economic status between the black and white children. Black South African children are exposed to a multitude of factors known to impact negatively on their health in general such as poor nutrition, low dietary calcium intake, less physical activity as well as compromised growth and delayed onset of puberty compared with their white peers (9–14).

Anthropometric measurements and body composition

Height was measured in millimetres using a wall-mounted stadiometer (Holtain, Crymych, UK) and weight in kilograms using a digital electronic instrument (Dismed, Quebec, Montreal, Canada). Both instruments were regularly calibrated and the participants wore minimal clothing when being weighed. BMI was calculated as the participant's weight in kilograms divided by the square of their height in metres (kg/m²). Total and percentage of fat mass and lean mass were measured by dual-energy X-ray absorptiometry,

using a Hologic QDR 4500 instrument (Hologic Inc., Bedford, MA, USA). The data were analysed with the software supplied by the manufacturer (version 11·2).

Biochemical analysis

Blood samples (20 ml) were drawn by venepuncture from fasting participants into plain tubes by registered nurses. The blood samples were allowed to clot for a minimum of 20 min, the serum was aliquoted and stored in Eppendorf tubes at -70°C until analysed. 25(OH)D was measured by a chemiluminescent assay using DiaSorin Liaison kits (DiaSorin, Stillwater, MN, USA). All the samples were run in duplicate. Our laboratory is currently participating in the International Vitamin D External Quality Assessment Scheme (DEQAS) and was given the certificate of efficiency, as the laboratory has achieved the performance target set by the DEQAS advisory panel, i.e. 80% or more of the results fell within ±30% of the all laboratory trimmed mean. The inter-assay CV for low and higher 25(OH)D controls was 10% and 9%, respectively, whereas the intra-assay CV was 8% and 6% for low and higher 25(OH)D controls, respectively. Blood samples were collected from only 385 of the 475 participants in the cohort as the others refused blood sampling, were not available, or blood samples could not be obtained.

The following categories were used to define vitamin D status: vitamin D deficiency (<50 nmol/l), insufficiency (50-74 nmol/l) and sufficiency ($\geq 75 \text{ nmol/l}$).

Statistical analysis

The results are expressed as mean and $_{\rm SD}$, unless otherwise indicated. The data were analysed using the Statistica software package version 6 (StatSoft, Tulsa, OK, USA). Unpaired t tests were used to compare the means of different groups. All tests were two-tailed and a P value <0.05 was considered statistically significant.

Results

Blood samples were available from 385 (140 black female, 47 white female, 155 black male and 43 white male participants) of the 475 children. Only those participants with 25(OH)D values were included in the study (Table 1).

There were no significant sex or ethnic differences with respect to age between the study groups. Black girls were heavier and taller than black boys (P = 0.01). There were no significant differences in weight, height or BMI between white boys and white girls. White boys and girls were taller than their black peers (P = 0.0001). There were no significant differences in BMI between any of the groups.

Girls had a higher percentage of fat and a lower percentage of lean tissue and total lean tissue than their male peers. There were no significant differences between black and white girls with respect to total fat tissue, percentage of fat or percentage of lean tissue; however, white girls had 336 MA Poopedi *et al.*

Table 1 Gender and ethnic differences in age, anthropometry, body composition and 25(OH)D of 10-year-old children

	Female				Male				
Variables	Black (n 140)		White (n 47)		Black (n 155)		White (n 43)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P value
Age (years)	10.5	0.3	10.6	0.3	10.5	0.3	10.6	0.3	P ₁ = NS P ₂ = NS
Anthropometry Weight (kg)	34.8*	8.5	35.9	7.6	32.6*	6.4	35.8	6.8	$P_1 = 0.01$ and 0.97 $P_2 = NS$
Height (mm)	139·2*†	6.4	143-6†	7.3	137·3*†	6·1	143-2†	7.1	$P_1 = 0.01$ and NS $P_2 = 0.0001$ and 0.0001
BMI (kg/m²)	17.8	3.4	17-2	2.3	17·2	2.6	17.4	2.5	$P_1 = NS$ $P_2 = NS$
Body composition Total fat tissue (g)	10 069*	5166	9893	4049	7307*	3771	8112	3462	$P_1 = 0.0001$ and NS $P_2 = NS$
Fat (%)	28*	7.0	27*	5.5	22*	6.3	22*	5.2	$P_1 = 0.0001$ and 0.0004 $P_2 = NS$
Total lean tissue (g)	23892*†	4009	25 164*t	4020	24 152*t	3151	26 825*t	3849	$P_1 = 0.0001$ and 0.03
Lean tissue (%)	70*	6.8	70*	5.3	75*	6.0	75*	5∙0	$P_2 = 0.04$ and 0.0001 $P_1 = 0.0001$ and 0.0003 $P_2 = NS$
Blood biochemistry 25(OH)D (nmol/l; total)	86*†	31·1	112* †	34.8	100*†	34·4	129* †	37∙1	$P_1 = 0.0004$ and 0.02 $P_2 = 0.0001$ and 0.0001

25(OH)D, serum 25-hydroxyvitamin D; P_1 , gender difference (black (female v. male) and white (female v. male)); P_2 , ethnic difference (female (black v. white) and male (black v. white)).

A P value <0.05 is considered statistically significant.

Mean values were significantly different between genders: *P < 0.05.

Mean values were significantly different between ethnicities: $\pm P < 0.05$.

greater total lean tissue than black girls (P = 0.04). Similarly, there were no significant differences between black and white boys with respect to total fat tissue, percentage of fat or percentage of lean tissue, but white boys had greater total lean tissue than black boys (P = 0.0001).

Boys (black (100 (sp $34\cdot4$) nmol/l) and white (129 (sp $37\cdot1$) nmol/l) had significantly higher 25(OH)D than girls (black (86 (sp $31\cdot1$) nmol/l) and white (112 (sp $34\cdot8$) nmol/l) in each ethnic group ($P=0\cdot0004$ and $0\cdot02$ for black and white children, respectively).

Seasonal variations in 25(OH)D were found in white children, with values being highest in summer and autumn. No seasonal variations were noted in black children. 25(OH)D values were significantly higher in white than black children during the autumn (whites (119 (sp $34\cdot1$) nmol/l) v. blacks (89 (sp $32\cdot3$) nmol/l), $P=0\cdot01$) and summer months (whites (137 (sp $34\cdot9$) nmol/l) v. blacks (113 (sp $30\cdot9$) nmol/l), $P=0\cdot0001$; Fig. 1).

The percentages of black and white participants with vitamin D sufficiency were 70% and 87%, respectively, whereas 22% and 12%, respectively, were vitamin D-insufficient, and 8% black and 1% white children were vitamin D-deficient. For the overall study population, 7% had vitamin D deficiency, 19% had vitamin D insufficiency and 74% had vitamin D sufficiency.

In both black and white children, 25(OH)D concentrations correlated negatively with the percentage of fat tissue (r = -0.14, P = 0.02; and r = -0.3, P = 0.01,

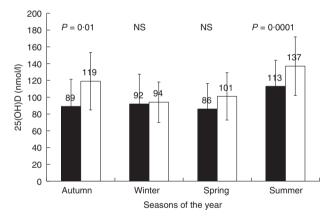


Fig. 1 Serum 25-hydroxyvitamin D [25(OH)D] concentrations (nmol/l) by season in black (■) and white (□) 10-year-old children. Values are mean and sp: black (autumn) = 89 (sp $32\cdot3$), white (autumn) = 119 (sp $34\cdot1$); black (winter) = 92 (sp $35\cdot3$), black (winter) = 94 (sp $24\cdot2$); black (spring) = 86 (sp $30\cdot4$), white (spring) = 101 (sp $28\cdot2$), black (summer) = 113 (sp $30\cdot9$), white (summer) = 137 (sp $34\cdot9$)

respectively) and positively with the percentage of lean tissue (r=0.14, P=0.02 (black); and r=0.27, P=0.01 (white)). After adjusting fat mass and lean mass for height a significant correlation was found between fat mass and 25(OH)D (r=-0.13, P=0.01).

The relationship between 25(OH)D and parathyroid hormone (PTH) was not significant in either ethnic or gender groups (data not shown).

Discussion

Vitamin D sufficiency was present in 74% of our study population. As we had hypothesised, vitamin D insufficiency is not a major public health issue among 10-yearold South African children living in Johannesburg. These findings differ from other studies reported from North Africa⁽¹⁶⁾, USA^(17,18), Europe⁽¹⁹⁾, the Middle East^(20,21), Asia^(22,23), New Zealand⁽²⁴⁾ and elsewhere⁽²⁵⁾. South Africa, and in particular Johannesburg, has abundant sunshine throughout the year (6); thus, despite few foods being fortified with vitamin D, vitamin D sufficiency is maintained in healthy ambulatory children. It should be noted, however, that the majority of children included in the study lived in small separate houses with yards; thus the findings might be very different if children living in high-rise apartment blocks in the inner city of Johannesburg had been studied. Factors such as latitude, the lack of excessive clothing coverage(21,25-27) and not living in high-rise urban inner city ghettos may have played significant roles in reducing hypovitaminosis D in the present study.

As in other studies, ethnic differences in 25(OH)D levels were found in the present study. White children had significantly higher 25(OH)D than their black peers (Table 1). The dark skin colour of black participants is known to restrict the solar UVB photons that penetrate the skin, thereby reducing the cutaneous production of vitamin D₃⁽²⁸⁾. Numerous studies comparing black and white young and old participants in different countries have reported that blacks have lower 25(OH)D levels than whites^(3,18,29). However, studies^(28,30) have shown that darker-skinned participants have the capacity to produce vitamin D of similar amount to light-skinned participants when exposed to prolonged UVB light. In our study, the lower 25(OH)D in black children during summer probably reflects this difference in the capacity to produce vitamin D with similar exposures.

Dietary calcium intake in this cohort of children at the age of 9 years was significantly lower in blacks of both sexes (347 mg/d), compared with white female (719 mg/d) and male participants (778 mg/d)⁽³¹⁾. Low calcium intake in black children could result in increased catabolism of 25(OH)D and thus accentuate the lower 25(OH)D. We did not find a relationship between 25(OH)D and PTH in either ethnic or sex groups (data not shown), supporting the contention that the majority of children were vitamin D-replete.

One of the factors that influence vitamin D status in both young and adult participants is season. Seasonal changes in 25(OH)D levels have been reported in black and white participants and other ethnic groups $^{(24,32)}$. In the present study, the effect of season on serum 25(OH)D levels was only seen in the white children. The biggest difference in 25(OH)D concentrations between black and white children was noticed during autumn and summer (P=0.01 and 0.0001, respectively), while there was no significant ethnic difference in 25(OH)D in winter and

spring (Fig. 1). The reason for the lack of seasonal variation in black children is not clear, but it is interesting to speculate that black children do not spend time lying in the sun at swimming pools during the summer months because of the differences in socio-economic status and the lack of facilities in the mainly black communities. This finding of a lack of seasonal variation in black children is different to that reported in some studies of children (24,33), but similar to others⁽²⁹⁾. We have previously shown that in vitro vitamin D synthesis is relatively constant throughout the year. Thus, if skin exposure remains constant throughout the year, one would not expect major seasonal fluctuations in vitamin D status. In a multiple regression analysis, season $(\beta = 0.28, P = 0.0001)$, ethnicity $(\beta = -0.29, P = 0.0001)$, sex ($\beta = -0.16$, P = 0.003) and total fat mass ($\beta = -0.15$, P = 0.01) were the only factors found to have an influence on 25(OH)D. Higher 25(OH)D levels were found in male rather than female participants in the present study, a finding that is similar to other studies (24,32). The gender difference in our present study may reflect the fact that male participants may spend more time outdoors (but this was not measured). Studies of physical activity in the same group of children have previously revealed that male participants have higher physical activity than their female peers in both ethnic groups (34,35). Some researchers have suggested that gender differences in vitamin D status may be linked to androgenrelated differences in vitamin D-binding protein levels, to differences in precursor production in the skin, to differences in 25-hydroxylation by the liver (36), or to gender differences in body fat. The girls in our study had significantly greater fat mass than the boys, thus possibly providing another reason for the sex differences in 25(OH)D concentrations in our cohort. Vitamin D is a fat-soluble vitamin, and the inverse relationship between 25(OH)D and fat mass has been reported previously by researchers (37,38). This association has been attributed to the sequestration into adipocytes of vitamin D generated in the skin or orally ingested, before it can be transported to the liver and converted to 25(OH)D^(37,38). However, it still remains unclear whether adiposity (or a percentage of body fat) should be taken into consideration while assessing vitamin D requirements in the general population. We found a similar inverse relationship between fat mass and 25(OH)D in our black and white children.

In conclusion, despite seasonal variations in 25(OH)D levels, vitamin D deficiency (25(OH)D < 50 nmol/l) and vitamin D insufficiency (25(OH)D = 50–74 nmol/l) were uncommon findings in our black and white 10-year-old children. We therefore believe that vitamin D supplementation or fortification should not be considered in healthy South African children living in Johannesburg. Whether similar findings hold true in other regions of the country need to be confirmed; however, of particular interest would be studies in Cape Town, where an earlier study has shown only limited vitamin D synthesis *in vitro* from April through to September⁽³⁹⁾.

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