Is vitamin D status associated with non-communicable disease risk in children? A cohort study

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Background: Studies in children and adults have reported variations in 25-hydroxyvitamin D (25(OH)D), body mass index (BMI) and blood pressure (BP) over time. Furthermore, there has been a reported association of 25(OH)D with BMI, BP and lipid levels in some cross-sectional and longitudinal studies.

Methods: This is a longitudinal study of a group of adolescents with measurements of 25(OH)D, BP, anthropometry and lipids at the ages of 11, 12 13, 15 and 18–20 years. For age-related changes, year 12 participants ($n = 261$) were matched with year 18–20 participants ($n = 368$), resulting in 200 paired participants. Longitudinal analyses using the Generalized Estimating Equations (GEE) comprised the following groups of participants, Year 11 ($n = 288$), Year 12 ($n = 253$), Year 13 ($n = 292$), Year 15 ($n = 238$) and Year 18–20 ($n = 368$). The relationship of 25(OH)D with BMI, BP and lipid levels over a period of 10 years was assessed.

Results: There were significant increases in mean BMI and BP, and decreases in 25(OH)D levels with age (all p-values < 0.0001). In females, systolic BP was significantly higher in older participants (18–20) years than younger participants (12 years), but 25(OH)D was significantly higher in younger than older participants. In males, there was significant increase in BP in participants between age 12 years and 18–20 years. 25(OH)D, total cholesterol (TC) and low-density lipoprotein (LDL-C) were significantly lower in 18–20-year-old participants compared with 12-year-old participants. Longitudinally, 25(OH)D was inversely associated with LDL-C.

Conclusion: There is evidence of changes in 25(OH)D, BMI and BP in adolescents over a period of 10 years. After adjusting for covariates, BMI and LDL-C were significantly negatively associated with 25(OH)D, which suggests that vitamin D status might be associated positively with favourable lipid profiles in children and adolescents.

Keywords: vitamin D, lipids, BMI, lifestyle, blood pressure, adolescence

Introduction

The classical function of vitamin D is to facilitate the absorption of calcium and phosphorus from the gastrointestinal tract (GIT) for skeletal mineralisation.[1] However, recent evidence suggests that vitamin D status may be associated with a variety of non-communicable disease (NCD) risks (such as hypertension, obesity, metabolic syndrome and diabetes mellitus to mention but a few).[2-6] The mechanisms by which hypovitaminosis D might be related to these diseases remain unclear, although vitamin D may influence immunomodulation, insulin release and cardiovascular function.[7,8] Vitamin D status is dependent on both dietary intake and cutaneous synthesis through exposure to ultraviolet β radiation, the latter being affected by factors such as latitude, season, skin pigmentation, lifestyle and cultural practices.[9-12] Studies in children and adults have found obesity and sedentary lifestyles to be associated with reduced vitamin D status.[10,13] An abnormal lipid profile and high BP have also been associated with vitamin D deficiency.[14,15] However, in some studies,[16,17] blood pressure (BP) levels remained unchanged with the improvement in serum 25-hydroxyvitamin D (25(OH)D) levels. Given the equivocal findings relating vitamin D status with lipid profiles and BP in children, our aim was to study the influence of vitamin D status on BMI, serum lipids and blood pressure in a longitudinal cohort of adolescents living in the Johannesburg Metropolitan area (latitude 26°S, altitude 1600 m), South Africa. We hypothesised that an increase in vitamin D status would be associated with a reduction in non-communicable diseases risk factors.

Methods

Study participants

Children from the Bone Health sub-cohort of the Birth to Twenty Plus longitudinal cohort in Johannesburg were studied. The Birth to Twenty cohort consists of participants who have been followed since their birth in 1990. A total of 3 273 black and white singleton deliveries were enrolled within the Greater Johannesburg Metropolitan area over a seven-week period. Children in the Bone Health cohort have, since the age of nine years, been investigated annually for factors affecting bone growth and bone mass accrual. Children with chronic illnesses, or on medication or drugs and mineral supplements known to affect bone metabolism, were excluded from participating in the study.

Anthropometric variables (weight, height and BMI), BP (systolic blood pressure [SBP] and diastolic blood pressure [DBP]) and 25(OH)D levels were measured in children aged 11, 12 13, 15 and 18–20 years. Lipid levels, total cholesterol (TC), triglycerides (Trig), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured only in years 12 and 18–20. For the longitudinal assessment of age-

related changes in serum lipids, only participants who had measurements at both years 12 and 18–20 were included, resulting in the analysis of 200 participants. The data for longitudinal analysis of anthropometry, BP and 25(OH)D using the generalised estimating equation (GEE) comprised the following groups of participants: Year 11 ($n = 288$), Year 12 ($n = 253$), Year 13 ($n = 292$), Year 15 ($n = 238$) and Year 18–20 ($n = 368$). Eight participants, who had measurements taken only once during the study, were excluded from the GEE data analysis. For participants aged 11–15 years, the BMI for age cut-offs for underweight or healthy weight were defined as < 85th percentile and ≥ 85th percentile for overweight or obese participants.[18] For participants aged 18–20 years, underweight or normal weight was defined as a BMI of < 25 kg/m^2 and overweight or obese as a BMI of ≥ 25 kg/m^2.[19] Age- and height-adjusted reference values were used to define normal BP in children aged 11–15 years,[20] while in participants aged 18–20 years normal BP was defined as SBP ≤ 140 mm Hg and DBP ≤ 90 mmHg. Cut-offs for normal lipid profiles were TC ≤ 5 mmol/l, Trig ≤ 1.7 mmol/l, LDL-C ≤ 3.0 mmol/l, HDL-C ≥ 1.0 mmol/l in males and ≥ 1.2 mmol/l in females.[21] Vitamin D status was defined deficient when 25(OH)D was < 50 nmol/l and sufficient as ≥ 50 nmol/l.[22]

The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg (Approval no. M980810). Consent was obtained from the adolescents' guardians and assent from the participants.

Anthropometric and blood pressure measurements
Height was measured in millimetres using a wall-mounted stadiometer (Holtain, Crymych, UK) and weight in kilograms using a digital electronic instrument (Dismed, Montreal, Canada). Height was measured in bare feet to the nearest 0.1 cm using a rigid stadiometer and weight was measured with participants wearing light clothes without shoes to the nearest 0.1 kg using a calibrated scale balance. BMI was calculated by dividing weight in kilograms by height in metres squared (kg/m^2).

SBP and DBP (mmHg) were measured at the right upper arm using an automated oscillometric device (Omron M6 HEM-7001-E, Omron Corporation, Kyoto, Japan). Prior to BP readings, each participant rested quietly for five minutes. After each reading the cuff was automatically deflated to 0 mmHg. BP was measured three times with the participants seated. The average of the three readings was used for systolic and diastolic blood pressure.

Biochemical measurements and calculations
Blood samples (20 ml) were obtained after an overnight fast from each participant. The blood samples for 25(OH)D and lipid concentrations were collected into plain red-top tubes. The blood was allowed to clot at room temperature for a minimum of 20 minutes, before being centrifuged, and the serum separated and stored at –80°C. Serum 25(OH)D was measured by a chemiluminescence assay using the Diasorin Liaison instrument (Diasorin Liaison, Stillwater, MN, USA) (intra-assay and inter-assay variations were < 5% and < 8% respectively). Our laboratory participates in an international quality assurance programme (DEQAS—International Vitamin D External Quality Assessment Scheme, London, UK). Since our participation in DEQAS external quality control, our laboratory had received the certificates of efficiency on yearly basis (i.e. 80% or more results fell within 30% of the all-laboratory

trimmed mean). Lipids were measured by a Randox Daytona Instrument (Randox Laboratories Ltd, Antrim, UK) (intra- and inter-assay variation < 5%).

Statistical analyses
The data were analysed using STATA software package version 11 (StataCorp, College Station, TX, USA, July 2009). All continuous variables were presented as means and standard deviations (mean ± SD). Because of missing data at each longitudinal time point, the association of 25(OH)D levels with risk factors was determined using GEE, which accommodates missing values and allows repeated measures. The bivariate GEE models assessed the relationship of 25(OH)D with BMI, BP and lipids profiles; the covariates (age, gender, ethnicity and season) were controlled for in a multivariate GEE model.

Results
The anthropometric and biochemical data are given in Tables 1 and 2. There was a significant increase in BMI and BP and a significant decrease in 25(OH)D with age during the 10 years of study (years 11, 12, 13, 15 and 18–20). Serum 25(OH)D, TC and LDL-C were higher in males at 12 years of age than at 18–20 years of age. In female participants only 25(OH)D was higher when they were younger. Over the period from 12 years of age to 18–20 years, 25(OH)D was found to be significantly negatively associated with LDL-C, when adjusted for age, BMI, gender, ethnicity and season of the year.

Over a period of 10 years, there was a relative significant increase in mean BMI, SBP and DBP (all p-values < 0.0001) and a significant decrease in 25(OH)D (p-value < 0.0001) with age in study participants. The overall prevalence of abnormal BMI and vitamin D deficiency was 273 (20%) and 350 (23%) respectively. There was also an overall low prevalence of abnormal SBP: $n = 32$ (3%) as compared with abnormal DBP: $n = 173$ (14%).

Table 2 lists comparisons of the same participants at age 12 years and age 18–20 years. In female participants, SBP was significantly higher at participant age 18–20 years, while 25(OH)D levels were significantly lower. There were no significant changes in lipid levels in the females in the two age groups. In males the pattern was similar to that of females over time with increases in BP. 25(OH)D levels were significantly lower in the group of males aged 18–20 years than at 12 years of age. TC and LDL-C were significantly lower in males at 18–20 years of age compared with when the participants were 12 years, with the triglycerides remaining constant over the period. There was a slight decrease in abnormal DBP in female participants from 15 (17%) (younger) to 9 (10%) (older), which was in contrast to the males, in whom abnormal DBP increased from 6 (5%) (younger) to 31 (28%) (older). Younger participants had similar percentages of abnormal TC (females 14 [16%] and males 19 [17%]); furthermore, younger males had abnormally increased LDL-C (21 [19%]) compared with that in older males (9 [8%]). The prevalence of abnormal HDL-C in females ranged from 15(17%) (younger) to 20 (23%) (older) and in males from 25(22%) (younger) to 35 (31%) (older). There was 43% increase in vitamin D deficiency in females as compared with a 1% increase of vitamin D deficiency on males.

In GEE bivariate regression analysis, 25(OH)D was significantly negatively associated with BMI, SBP and DBP, and positively associated with triglyceride levels and better BP status. In the

Table 1: Cross-sectional assessment of BMI, blood pressure and vitamin D status at different time points

Variables	BMI (kg/m²) Total n (mean ± SD)	Abnormal BMI (kg/m²)* F	M	Mean (%)	SBP (mmHg) Total n (mean ± SD)	Abnormal SBP (mmHg)# F	M	Mean (%)	DBP (mmHg) Total n (mean ± SD)	Abnormal DBP (mmHg) F	M	Mean (%)	25(OH)D (nmol/l) n (mean ± SD)	Vitamin D deficiency (25(OH) < 50 nmol/l) F	M	Mean (%)
Year 11	256 (18 ± 2.9)	n = 117 31 (26%)	n = 139 12 (9%)	17	283 (100 ± 10.1)	n = 133 3 (2%)	n = 150 1 (1%)	1.4	283 (65 ± 8.2)	n = 133 13 (10%)	n = 150 6 (4%)	7	288 (73 ± 30.8)	n = 135 14 (30%)	n = 153 34 (22%)	26
Year 12	253 (19 ± 3.1)	n = 119 29 (24%)	n = 134 16 (12%)	18	211 (105 ± 10.5)	n = 95 2 (2%)	n = 116 2 (2%)	2	211 (68 ± 7.9)	n = 95 22 (23%)	n = 116 7 (6%)	14	261 (81 ± 25.7)	n = 124 16 (13%)	n = 137 7 (5%)	9
Year 13	291 (20 ± 3.7)	n = 138 36 (26%)	n = 153 22 (14%)	20	226 (108 ± 10.5)	n = 102 6 (6%)	n = 124 3 (2%)	4	226 (71 ± 8.6)	n = 102 32 (31%)	n = 124 13(10%)	20	292 (70 ± 23.3)	n = 139 26 (19%)	n = 153 22 (14%)	16
Years 15	236 (21 ± 3.7)	n = 117 29 (25%)	n = 119 13 (11%)	18	214 (109 ± 11.8)	n = 104 3 (3%)	n = 110 1 (1%)	2	214 (69 ± 7.5)	n = 104 4 (4%)	n = 110 2 (2%)	3	238 (62 ± 18.5)	n = 139 26 (19%)	n = 153 22 (14%)	20
Year 18–20	339 (23 ± 4.3)	n = 159 57 (36%)	n = 180 28 (16%)	25	327 (119 ± 11.6)	n = 155 3 (2%)	n = 172 42 (24%)	3	327 (80 ± 17.5)	n = 155 32 (21%)	n = 172 42 (24%)	23	368 (53 ± 17.6)	n = 173 94 (54%)	n = 194 62 (32%)	42
p-value and overall prevalence	p-value• 0.0001	Prevalence = 273 (20%)			P-value • 0.0001	Prevalence = 32 (3%)			p-value • 0.0001	Prevalence = 173 (14%)			p-value • 0.0001	Prevalence = 350 (23%)		

*Abnormal BMI for Year 11 to Year 15, ≥ 85th percentiles for age and sex, and abnormal BMI for Year 18–20, ≥ 25 kg/m².
#Hypertension status for Year 11 to Year 15, ≥ 95th percentile for age, sex, and height and for Year 18–20, SBP ≥ 140 mm Hg and DBP ≥ 90 mmHg.
• Significant variations in mean BMI, SBP, DBP and 25(OH)D over a period of 10 years were calculated using ANOVA test.

Table 2: Change in blood pressure, anthropometry, body composition and biochemical variables in adolescents between the ages of 12 and 18–20 years stratified by sex

Variables	Female Year 12 (N = 88)	Abnormal n (%)	Years 18–20 (N = 88)	Abnormal n (%)	p-value*	Male Year 12 (N = 112)	Abnormal n (%)	Years 18–20 (N = 112)	Abnormal n (%)	p-value*
Systolic BP	106 ± 9.5	1 (1)	112 ± 8.4	0 (0)	**0.0001**	105 ± 10.3	2 (2)	121 ± 10.6	6 (5)	**0.0001**
Diastolic BP	70 ± 8.0	15 (17)	70 ± 7.9	9 (10)	0.64	67 ± 7.5	6 (5)	72 ± 7.9	31 (28)	**0.003**
Height (cm)	153 ± 8.5		161 ± 6.8		**0.0001**	149 ± 8.3		172 ± 7.9		**0.0001**
Weight (kg)	46 ± 10.8		63 ± 14.3		**0.0001**	41 ± 8.0		63 ± 10.2		**0.0001**
BMI (kg/m²)	19 ± 3.6	21 (24)	24 ± 5.0	36 (41)	**0.0001**	18.0 ± 2.5	14 (13)	21 ± 2.8	11 (10)	**0.0001**
25(OH)D (nmol/l)	72 ± 22.8	13 (15)**	47 ± 19.2	51 (58)**	**0.0001**	84 ± 26.4	6 (5)**	58 ± 15.6	7 (5)**	**0.0001**
Total cholesterol (mmol/l)	4.21 ± 1.0	14 (16)	4.14 ± 1.1	10 (11)	0.63	4.28 ± 0.8	19 (17)	3.86 ± 0.7	7 (6)	**0.0003**
Triglycerides (mmol/l)	0.83 ± 0.4	6 (7)	0.74 ± 0.4	3 (3)	0.08	0.77 ± 0.3	8 (2)	0.76 ± 0.3	1 (1)	0.78
HDL-C (mmol/l)	1.22 ± 0.3	15 (17)	1.30 ± 0.3	20 (23)	0.34	1.20 ± 0.3	25 (22)	1.14 ± 0.3	35 (31)	0.14
LDL-C (mmol/l)	2.30 ± 0.80	12 (14)	2.11 ± 0.82	12 (14)	0.11	2.40 ± 0.7	21 (19)	2.01 ± 0.6	9 (8)	**0.0001**

*p-values were calculated using paired Student's t-test and significant p-values are shown in bold.

unadjusted multivariate model, 25(OH)D was only inversely associated with DBP. After adjustment for possible confounding variables (age, gender, ethnicity and season) in Model 2[b], 25(OH)D was significantly inversely associated with BMI and LDL-C.

Discussion

Unlike other studies reporting higher percentages of hypovitaminosis D,[23] the present Bone Health cohort had vitamin D deficiency (25(OH)D < 50 nmol/l) in 17% of the participants (age 11–15 years), and 42% of participants (age 18–20 years).[24,25] There was a significant increase in mean BMI and BP, and a decrease in 25(OH)D over a period of 6–8 years, which was to be expected in children going through adolescence.[26] Lipids levels measured in 12- and 18–20-year-old participants were significantly higher in the male cohort at 12 years of age than at 18–20 years of age, changes not seen in the female cohort. Over a period of 6–8 years, 25(OH)D was found to be significantly negatively associated with LDL-C (Model 2[b]), which may suggest that increased 25(OH)D levels are associated with favourable lipid profiles.[27,28]

25(OH)D is by nature hydrophobic, hence is easily sequestrated into the body fat cells, which may result in vitamin D deficiency.[29] Despite vitamin D deficiency been linked to altered bone mineral metabolism,[30] vitamin D deficiency has been found to be inversely associated with BMI and body fat (total and percentage fat mass, visceral adipose tissue).[31,32] In the present study (Table 3), 25(OH)D was found to be inversely associated with BMI, before ($\beta = -1.59$, $p = 0.0001$) and after ($\beta = -0.74$, p-value = 0.0001) adjustment for other covariates (age, gender, ethnicity and season). This may reflect an independent association of BMI with 25(OH)D and the sequestration of 25(OH)D in the body fat adipocytes.[10,29] A study by Blum et al.[33] showed a strong relationship between fat tissue vitamin D$_3$ content and serum vitamin D$_3$, but the researchers failed to show a correlation between bodyweight and vitamin D$_3$, possibly because of small sample size ($n = 17$). But others have suggested that total body volume is an important mechanism in the explanation of the relationship.[34] This is because, according to Drincic et al.,[34] dilution of ingested or cutaneously synthesised vitamin D in the large fat mass of obese patients may fully explain their typically low vitamin D status. Another possible explanation for low vitamin D in obesity has been described in animal studies, in which obesity was associated with a down-regulation of CYP2R1, the major hepatic hydroxylase responsible for the conversion of vitamin D to 25(OH)D.[35]

Vitamin D deficiency has been reported to be associated with diseases that are not bone related and, hypertension is one of them.[14,36] The importance of vitamin D in hypertension has also been investigated in animal studies, where vitamin D was shown to reduce blood pressure through inhibition of the renin-angiotensin system.[37] Researchers have found low 25(OH)D to be associated with increased blood pressure in cross-sectional and longitudinal studies around the globe.[14,26,36] But no association was found in randomised clinical trials and case–control studies.[38,16] In the present study, an inverse relationship between 25(OH)D and BP was found in bivariate linear regression analysis but the relationship was not statistically significant in the adjusted model. Hence, researchers have suggested that understanding the relationship between 25(OH)D and cardiometabolic risk factors is a challenge due to confounding.[39] More insight into this relationship could be provided by randomised clinical trials, which will directly investigate the relationship of 25(OH)D with cardiometabolic risk factors.

Cross-sectional and a few prospective studies have reported that the association of 25(OH)D and abnormal lipid levels was related to obesity in children and adults, which may lead to cardiovascular complications.[40-42] The present study found 25(OH)D to be significantly positively related to triglyceride levels in bivariate analysis and negatively associated with LDL-C in multivariate analysis (Model 2[b]). However, these findings have not been consistently found in children and adolescents.[40,43]

The strength of the study was due to its longitudinal data analysis. However, there are several limitations associated with the study: loss to follow up over the 6–8 years of the study resulted in a reduction in participants with measurements at both 12 and 16–18 years and no information was collected on time spent outdoors or of physical activity and sunscreen use. The study used BMI as a measure of fat mass, which is unable to distinguish lean mass from fat mass. The present study was restricted to the Greater Johannesburg metropolitan area; hence the findings cannot reflect the general population in South Africa.

Table 3: GEE bivariate and multivariate regression analysis of 25(OH)D with blood pressure, BMI (over a 10-year period) and lipids (at 12 and 18–20 years)

Cardio-metabolic risk factors versus 25(OH)D	BMI β(p-value)	SBP (mmHg) β(p-value)	DBP (mmHg) β(p-value)	BP status 0 = abnormal BP 1 = normal BP	TC (mmol/l) β(p-value)	Trig (mmol/l) β(p-value)	HDL-C (mmol/l) β(p-value)	LDL-C (mmol/l) β(p-value)	Lipid status 0 = abnormal lipid profile 1 = normal BP
Bivariate Analysis	−1.59 (0.0001)	−0.32 (**0.0001**)	−0.31 (**0.0001**)	6.85 (**0.001**)	1.11 (0.26)	5.13 (**0.04**)	−5.11 (0.06)	−1.18 (0.34)	2.51 (0.25)
Model 1[a] (multivariate —unadjusted)	_	−0.08 (0.56)	−0.17 (**0.01**)	−3.35 (0.35)	1.84 (0.11)	5.02 (0.09)	−4.51 (0.15)	−2.49 (0.08)	3.61 (0.15)
Model 2[b] (multivariate —adjusted)	−0.74 (0.0001)	−0.03 (0.85)	0.09 (0.92)	−1.11 (0.75)	0.24 (0.82)	0.05 (0.98)	−5.8 (0.05)	−3.7 (**0.01**)	3.04 (0.20)

Model 1[a]—multivariate analysis.
Model 2[b]—multivariate analysis adjusted for age, gender, ethnicity and season of the year.

Conclusion

Although we were able to show relationships using bivariate analyses between vitamin D status and BMI, BP and lipid levels over a 6–10-year period during adolescence, the relationships were not statistically significant following adjustment for covariates, except for the relationship of 25(OH)D with BMI and LDL-C, which persisted after adjustment. One possible reason for lack of relationships with 25(OH)D might relate to the relatively good vitamin D status of most of the participants.

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