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ORIGINAL ARTICLE

Serum 25-Hydroxyvitamin D associated with indicators of body fat and insulin resistance in prepubertal chilean children

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BACKGROUND: Consistent data on the relation between vitamin D, body fat and insulin resistance (IR) in children are lacking.
OBJECTIVES: (1) To evaluate the association between serum 25-Hydroxyvitamin D [25(OH)D] and key indicators of: adiposity (total and central), IR, and (2) to estimate serum 25(OH)D cut-offs that best reflect IR and total and central adiposity in children.
SUBJECTS/METHODS: Prepubertal children ($n = 435$, ~53% girls; ~age 7 years) from the Growth and Obesity Chilean Cohort Study were evaluated for potential associations between serum 25(OH)D and indicators of: (1) total adiposity (body mass index by age (BAZ), body fat (including three-component model)), central adiposity (waist circumference and trunk fatness); (2) IR (homeostasis model assessment of IR) and insulin sensitive (quantitative insulin sensitivity check index) using standardized multiple regression models with standardized coefficients and receiver operating characteristic curves.
RESULTS: Overall, mean serum 25(OH)D was 32.1 ± 9.2 ng ml^{-1}, while 19.4% of children were obese (BAZ $\geqslant 2$ s.d.). Serum 25(OH)D was inversely associated with indicators of total and central adiposity and with IR indicators. Effect sizes were moderate in girls (~0.3 for adiposity and IR indicators), while, weaker values were found in boys. Serum 25(OH)D estimated cut-offs that best predicted total, central adiposity and IR were ~30 ng ml^{-1}. Children with suboptimal serum 25(OH)D (< 30 ng ml^{-1}) had a higher risk (two to three times) of being obese (high BAZ, body fat percent and/or central adiposity); and three to four times greater risk for IR.
CONCLUSIONS: Serum 25(OH)D was inversely associated with adiposity (total and central) and IR indicators in prepubertal Chilean children. The conventional cut-off of vitamin D sufficiency ($\geqslant 30$ ng ml^{-1}) was adequate to assess obesity and IR risk in this age group.

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INTRODUCTION

Vitamin D (VD) stimulates intestinal calcium absorption and is important to maintain adequate phosphate levels for bone mineralization, bone growth and remodeling.[1] However in the past decade, VD receptor and VD-metabolizing enzymes have been described in other tissues (for example, adipose, muscle and pancreas),[2–4] suggesting regulatory roles beyond bone health.[5,6]

In adults, suboptimal VD has been related with higher mortality from cardiovascular and metabolic disorders among several other diseases;[7] the association of VD deficiency with increased adiposity and insulin resistance (IR) has been suggested as the common underlying factor for these conditions.[8–10] In children, the evidence for an extraskeletal role for VD is scarce, particularly in developing countries. However, an inverse relationship between VD and adiposity has been shown in some studies;[11–20] while others, show no association.[21–23] These inconsistencies may be partly due to the use of indirect indicators of adiposity (for example, body mass index (BMI), skinfolds).[24] Observational studies in children suggest an inverse association between VD and IR; however, this association varies with age.[14–16,21–23,25–32] Serum 25-Hydroxyvitamin D [25(OH)D] is accepted as a biomarker of VD status since it is the dominant and more stable form of VD. It reflects the combined effect of intake, skin synthesis, storage, blood transport protein and catabolism.[33] Serum 25(OH)D cut-offs serve to define VD status; however, since these cut-offs are based on information related to calcium metabolism and risk of rickets,[34] it has been questioned if they are also potentially valid to assess extraskeletal VD status.[35,36]

Thus, the aims of the present study were: (1) to evaluate the association between serum 25(OH)D and multiple indicators of total and central adiposity and IR; and (2) to define the range of serum 25(OH)D that are more strongly associated to whole body and central adiposity and IR in a sample of >400 prepubertal Chilean children participating in a longitudinal follow-up study that includes periodic monitoring of body adiposity and associated metabolic markers.

METHODS

Subjects

The study sample was composed of 435 prepubertal children of both sexes (mean age was ~6 years for girls and 8 years for boys) participants of the Growth and Obesity Chilean Cohort Study (GOCS) conducted in Santiago, Chile (latitude 33° 27` S). Growth and Obesity Chilean Cohort Study is a study of low-middle income Chilean children born in 2002–2003 ($n = 1196$, ~50% girls) at 37 and 42 weeks gestational age with birth weight $\geqslant 2500$ g. Growth and Obesity Chilean Cohort Study assessments include repeated anthropometric, growth and maturation assessments; as well as biological specimen collection at defined time points. Details on recruitment procedures and study design have been previously published.[37]

Study design

For the present study we identified all Children recruited to the primary study with a negative family history of genetic metabolic disease

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(for example, type 1 diabetes), not receiving VD supplements in past 6 months and had a serum sample drawn during last visit before initiation of puberty ($n = 1050$). For the present study, we included a random sample of 435 children (~53% girls). Sample size was estimated to assess magnitude of associations previously reported assuming 80% power, a two-tail significance $P < 0.05$ considering a small effect size.[38] Children included in the study did not differ in age (7.1 ± 0.04; 7.05 ± 0.06), *z*-score BMI for age (BAZ) (0.91 ± 0.04; 0.91 ± 0.05), *z*-score height to age (0.21 ± 0.03; 0.21 ± 0.04) or waist circumference (WC) (60.5 ± 0.4; 60.4 ± 0.3) relative to children not included (all *P*-value > 0.05). The Institutional Review Board of the Institute of Nutrition and Food Technology of the University of Chile approved the study. Informed consent was obtained from all parents or guardians of the children.

Vitamin D

A single nurse obtained all blood samples early in the morning (before 1000 h). Concentrations of serum 25(OH)D were measured in serum using the Liaison 25(OH)D total (D2 & D3, DiaSorin Inc, Stillwater, MN, USA), which had an inter-assay and intra-assay coefficient variation of 14.2% and 6.1%, respectively.

Adiposity

Anthropometric measures. At the time of the VD sample collection, a registered dietician measured the weight, height, WC and skinfold thickness of children using standardized procedures. Weight was measured using a portable electronic scale (Seca 770; Seca, Hamburg, Germany) with 0.1 kg precision. Height was measured with a portable stadiometer (Harpenden 603; Holtain Ltd, Crosswell, UK) to the nearest 0.1 cm. WC (minimum circumference between the iliac crest and the rib cage) was measured with a metal inextensible tape (model W606PM; Lufkin, Baltimore, MD, USA) to the closest 0.1 cm. In addition, the indicator WC divide by height (WC/H) was calculated. Skinfold thickness was measured in triplicate with a Lange caliper to the nearest 0.5 mm; the mean value was used in the analysis. For all measurements the intraobserver technical measurement and the mean average bias of the observer were within the limits suggested by the World Health Organization.[39]

Bioelectrical impedance measurements (BIA). BIA was measured using a Tanita BC-418 MA, eight-electrodes, hand-to-foot system (Tanita Corporation, Tokyo, Japan). BIA measurements were obtained in the morning, with limited physical exertion and with empty bladders and at measurement frequency of 50 kHz. Height, sex and age were entered manually, while weight was recorded automatically (accuracy within 0.1 kg).

BOD POD and deuterium dilution measurements. in a convenience subsample of 283 children (36% girls) body volume was measured by air displacement plethysmography (BOP POD, Life Measurement Instruments, Concord, CA, USA) and total body water (TBW) was determined by D2O dilution (98.9 atom % excess; Europa Scientific, Crewe, UK). This allowed us to estimate body fat (BF) using a reference method for body composition, the three-component model (3C model). Children in this subsample were slightly older than those of the total sample (8.4 ± 0.06 vs 7.1 ± 0.04, respectively; $P < 0.05$), but had similar BAZ (1.09 ± 0.09 vs 0.91 ± 0.04, $P > 0.05$) and WC (60.5 ± 0.4 vs 61.1 ± 0.4, $P > 0.05$).

Indicators of total adiposity. total adiposity was defined as: (1) BMI: weight (kg)/height $(m)^2$, expressed as BMI for age *z*-score (BAZ), World Health Organization 2007 growth reference.[40] (2) Skinfolds: BF percentage (FM (%)) = (1.825*BAZ) + (0.7825*Triceps Skinfold) + (0.3072*Biceps Skinfold) + 15.558.3), BIA: BF estimated based on the equipment's equation and (3) the three-component model (3C model): FM (kg) = ((2.220 × BV) – (0.764 × TBW)) – (1.465 × BW),[41] where BV is body volume in litters, TBW in litters and BW, body weight in kilograms. Obesity was defined as BAZ ⩾ 2 s.d. and overweight as BAZ ⩾ 1 ⩽ 2 s.d. High BF % was defined as ⩾ 75th percentile of the sample distribution for skinfolds, BIA, and the 3C-Model.

Indicators of central adiposity. High central adiposity was defined as: (1) WC NHANES III > 75th percentile of Hispanic population specific by age and sex (girls at 6 years: 60.4 cm; and boys at 8 years: 66.2 cm);[42] (2) WC/H as ⩾ 0.5 cm; and (3) truncal adiposity (abdominal, suprailiac, and subscapular skinfold thicknesses)[43] ⩾ 75th percentile of the sample distribution.

Insulin resistance (IR)

Serum glucose concentrations. were measured with an enzymatic colorimetric technique (HUMAN; Gesellschaftfür Biochemica und Diagnostica, Weisbaden, Germany); and serum insulin concentrations were assessed with a radioimmunoassay kit (Linco Research Inc, St Charles, MO). The analyses were conducted at the Nutritional Laboratory of Catholic University of Chile. This laboratory conducts daily assessments of the accuracy of the measurements by using UNITY quality control software (Bio-Rad Laboratories Inc, Hercules, CA, USA).

Insulin resistance indicators. We calculated the homeostasis model assessment of IR (HOMA-IR) as fasting glucose (mg dl^{-1}) x fasting insulin (mU ml^{-1})/405 and quantitative insulin sensitivity check index as 1/(log fasting insulin (mU ml^{-1})+ log fasting glucose (mg dl^{-1})). Hyperinsulinemia was defined as ⩾ 10 IU ml^{-1} (ref. 44) and high IR was defined, according to the proposal by *Keskin et al.*,[45] as ⩾ 3.2; however, given the low prevalence of IR in this sample (1.7% in girls and 6.9% in boys), we used fasting insulin and HOMA-IR ⩾ 75th percentile of the sample. Low quantitative insulin sensitivity check index was defined as ⩽ 25th percentile of the sample distribution.

Other variables

Seasonality. season of VD assessment was classified as summer (December 21 to March 20); fall (March 21 to June 20); Winter, (June 21 to September 20); and spring (September 21 to December 20).[46]

Tanner staging. two health professionals (one per each sex) specially trained by a pediatric endocrinologist classified Tanner staging of the children by physical exam (palpation in girls and orchidometer in boys).[47,48]

Statistical analyses

Data are presented as means and s.d. (s.d. = *z*-score). Variables with non-normal distributions were log transformed. Differences by sex were evaluated using student's *t*-test for continuous variables or X^2/fisher test for dichotomous variables. Linear regression models using standardized coefficients (X- mean/s.d. of the sample) were used to compare associations between serum 25(OH)D and the different adiposity e IR indicators (beta coefficient: and 95% confidence interval (CI)); adjusted for age, seasonality, and adiposity (only IR model). Interactions by sex were all significant ($P < 0.05$); thus, results are presented by sex. To determine optimal cut-offs to assess the association between serum 25(OH)D and high adiposity and IR we used receiver operating characteristic curves to estimate area under the curve (AUC), sensitivity, specificity and the maximum youden index (sensitivity- (1-specificity)), and the point with shortest distance from the point (0, 1) $((1 - \text{sensitivity})^2 + (1 - \text{specificity})^2)$.[49] These cut-offs were then used to define optimal VD and assess the associations between suboptimal VD and high adiposity and IR using logistic regression models adjusted for age, seasonality, and adiposity (IR model). Statistical analyses were conducted using Stata 11.0 (StataCorp LP, Lakeway, TX, USA).

RESULTS

General characteristics by sex are presented in Table 1. A total of 435 prepubertal children (~53% girls) with an average age of ~6 years in girls and ~8 years in boys were enrolled in the study. Mean serum 25(OH)D was slightly above 30 ng ml^{-1} with concentrations being marginally lower among boys (31.2 in boys vs 32.9 ng ml^{-1} in girls, $P = 0.06$). Almost a third of the girls presented concentration of 25(OH) D < 30 ng ml^{-1} (26.3% 30-20 and 5.6% below 20 ng ml^{-1}), while 50% of the boys were below the standard VD cut-offs (39.9% 30-20 and 10.3% below 20 ng ml^{-1}). Children evaluated during fall and winter had lower 25(OH)D concentrations (31.0 ± 8.6 ng ml^{-1}, $n = 381$), than children evaluated during spring or summer (34.6 ± 11.4 ng ml^{-1}, $n = 54$) (*P* for seasonality differences < 0.05).

Excess weight (BAZ > 1) was prevalent in this sample, particularly among boys (~55% in boys and ~35% in girls, *P*-value < 0.05). Mean BF% (by skinfolds) was ~30% in both sexes; with slightly lower value when assessed by BIA. Central obesity (WC > 75 Pc) was also high compared with the US reference ~43% in both sexes, $P > 0.05$. High insulin or high HOMA-IR was

Table 1. General characteristics of 435 prepubertal Chilean children, by sex

Characteristics	*Girls* n = *232* *X, s.d.*	*Boys* n = *203* *X,* s.d.	P-*value*[a]
Age (years)	6.3, 0.6	8.0, 1.3	< 0.001
VD			
25-Hydroxyvitamin D [25(OH)D] (ng ml^{-1})	32.9, 8.2	31.2, 10.1	0.06
Insufficiency VD (⩾20, < 30 ng ml^{-1}) (%, *n*)	26.3 (61)	39.9 (81)	< 0.001
Deficiency VD (< 20ng ml^{-1}) (%, *n*)	5.6 (13)	10.3 (21)	0.07
Total Adiposity			
Weight (kg)	24.5, 4.2	32.1, 7.5	< 0.001
Height (cm)	120.4, 5.2	131.3, 8.4	< 0.001
BMI: kg m^{-2}	16.8, 2.0	18.4, 2.8	< 0.001
Height for age s.d.[b]	0.14, 0.9	0.29, 0.9	0.08
BMI for age s.d.[b]	0.65, 0.9	1.14, 1.2	< 0.001
BAZ (⩾1, < 2 s.d.) (%, n)[Y]	24.9 (58)	30.5 (62)	< 0.001
BAZ (⩾ 2 s.d.) (%, *n*)[Y]	9.9 (23)	24.1 (49)	< 0.001
Body fat % skinfolds GOCS[c]	26.5, 4.5	29.9, 7.7	< 0.001
Body fat % bioimpedance (BIA)[d]	24.1, 3.7	24.0, 5.2	0.85
Body fat % 3C model[e]	27.9, 7.9	30.9, 6.9	< 0.001
Central Adiposity			
WC (cm)[f]	57.7, 5.8	63.8, 8.1	< 0.001
WC ⩾75th percentile NHANES (%, *n*)[f]	43.1 (100)	43.3 (88)	0.96
WC/Height (cm)[f]	0.48, 0.04	0.48, 0.05	0.12
WC/height > 0.5 (%, *n*)	25.9 (60)	32.5 (66)	0.13
Truncal fat (mm)[g]	25.7, 10.5	36.4, 21.6	< 0.001
Insulin Resistance			
Fasting glucose (mg dl^{-1})[h]	89.2, 6.7	92.6, 6.7	< 0.001
Fasting glucose ⩾ 100 mg dl^{-1} (%, *n*)[i]	1.7 (4)	12.3 (25)	< 0.001
Fasting Insulin (IU ml^{-1})[h]	5.5, 1.6	7.5, 2.6	< 0.001
Fasting insulin (⩾ 10 μg dl^{-1}) (%, *n*)[i]	2.6 (6)	14.8 (30)	< 0.001
HOMA-IR[i,h]	1.2, 0.4	1.7, 0.7	< 0.001
HOMA-IR (⩾ 3.2) (%, *n*)[j]	1.7 (4)	6.9 (14)	< 0.001
QUICKI index	0.16, 0.05	0.15, 0.08	< 0.001

Abbreviations: 3C, three-component; BIA, bioelectrical impedance measurements; BMI, body mass index; GOCS, Growth and Obesity Chilean Cohort Study; HOMA-IR, homeostasis model assessment of IR; IR, insulin resistance; NHANES, National Health study in EEUU; QUICKI, quantitative insulin sensitivity check index; VD, vitamin D; WC, waist circumference. [a]Differences between the sexes were estimated using *t*-test, X^2-tests, or fisher tests, $P < 0.05$. [b]Based on Word Health Organization Growth References 2007. [c]Skinfolds equation of body fat from GOCS, Body fat (%) = (1.825*BAZ) + (0.7825*Triceps Skinfold) + (0.3072*Biceps Skinfold) + 15.558. [d]Bioimpedance (BIA) using Tanita BC-418 MA. [e]Three-components model, subsample $n = 283$, girls $n = 100$, boys $n = 183$. [f]WC, NHANES III Mexican-Children: cut-off ⩾75th percentile (girls at 6 years: 60.4 cm; and boys at 8 years: 66.2 cm). [g]Truncal fatness = calculated by summing abdominal, suprailiac and subscapular skinfold thicknesses.[43] [h]Variables not normally distributed were log transformed. [i]Hyperglycemia was defined as ⩾ 100 mg dl^{-1}.[44] [j]HOMA. QUICKI.[45]

virtually non-existent among girls (< 3% for both indexes) while more prevalent among boys (~15% and ~ 7%, respectively; *P* for sex differences < 0.05).

Serum 25-Hydroxyvitamin D associations with adiposity and insulin resistance

Standardized coefficients for the associations of 25(OH)D and adiposity and IR indicators are presented in Table 2. Serum 25(OH)D was inversely associated with all indicators of total and central adiposity, but the effect size was lower when using indicators based on skinfolds measurements (–0.18 and 0.02 in girls and boys, respectively). 25(OH)D was also inversely associated with IR indicators and positively associated with the quantitative insulin sensitivity check index insulin sensitivity index in both sexes, even after adjusting by BMI. Overall, effect sizes were weak to moderate (~0.3 for adiposity and IR indicators).

Table 2. Association between 25-Hydroxyvitamin D concentrations, adiposity (total and central) and insulin resistance in 435 prepubertal Chilean children by sex

	Girls (n = *232*) *β (95% CI)*	*Boys* (n = *203*) *β (95% CI)*
Total Adiposity[a]		
z Score BMI by age[b]	− 0.37 (−0.49, − 0.25)*	− 0.19 (10.30, − 0.08)*
z Body fat % skinfolds[c]	− 0.18 (−0.31, − 0.05)*	0.02 (−0.05, 0.08)
z Body fat % BIA[d]	− 0.34 (−0.46, − 0.21)*	− 0.31 (−0.44, − 0.18)*
z Body fat % 3C model[e]	− 0.36 (−0.49, − 0.23)*	− 0.16 (−0.31, − 0.01)*
Central Adiposity[a]		
z WC (cm)[f]	− 0.34 (−0.46, − 0.22)*	− 0.27 (−0.41, − 0.14)**
z WC/height (cm)[f]	− 0.33 (−0.44, − 0.21)*	− 0.20 (−0.33, − 0.08)**
z Truncal fatness (mm)[g]	− 0.34 (−0.46, − 0.22)*	− 0.30 (−0.44, − 0.16)*
Insulin resistance[h]		
z Fasting insulin (μg dl^{-1})[i]	− 0.35 (−0.48, − 0.22)*	− 0.33 (−0.45, − 0.21)*
z HOMA-IR[j,i]	− 0.30 (−0.43, − 0.16)*	− 0.29 (−0.42. − 0.17)*
z QUICKI index[j]	0.37 (0.19, 0.55)*	0.29 (0.17, 0.42)*

Abbreviations: 3C, three-component; BIA, bioelectrical impedance measurements; BMI, body mass index; CI, confidence interval; GOCS, Growth and Obesity Chilean Cohort Study; HOMA-IR, homeostasis model assessment of IR; IR, insulin resistance; NHANES, National Health study in EEUU; QUICKI, quantitative insulin sensitivity check index; VD, vitamin D; WC, waist circumference. z Standardized coefficient (X-mean/s.d. of the sample). [a]Model 1: Multiple regression analysis adjusted by sex, age and seasons $^*P < 0.001$, $^{**}P < 0.05$. [b]z Score BMI by age (BAZ) based on Word Health Organization Growth Standards 2007. [c]Skinfolds equation for body fat % from GOCS. [d]Bioimpedance (BIA) using Tanita BC-418 MA. [e]3C, subsample $n = 283$, girls $n = 100$, boys $n = 183$. [f]WC. [g]Truncal fatness = calculated by summing abdominal, suprailiac and subscapular skinfold thicknesses.[43] [h]Model 2: multiple regression analysis adjusted by sex, age, seasons and BMI by age $^*P < 0.001$, $^{**}P < 0.05$. [i]Variables not normally distributed were log transformed. [j]HOMA-IR; QUICKI.[45]

Cut-offs for optimal VD based on adiposity and IR outcomes

As is shown in Table 3, the cut-offs of serum 25(OH)D that best predicted (maximum specificity and sensitivity) higher adiposity and IR was ~ 30 ng ml^{-1} in both sexes, considering different indicators of adiposity and IR (see also Supplementary Figures S1 and S2).

Associations between suboptimal VD and high adiposity and IR

Table 4 presents the results for logistic models assessing associations between suboptimal VD (defined based on the study cut-off: < 30 ng ml^{-1}), high adiposity and IR. Boys with suboptimal VD had almost twice the risk of having high adiposity (that is, odds ratio (OR) = 2.0 (1.0, 3.9) for obesity and 2.2 (1.2, 4.0) for central obesity), while girls had almost three times greater risk (that is, OR = 4.8 (1.9, 12.1) for obesity and 2.4 (1.4, 4.3) for central obesity). Boys and Girls with suboptimal VD have almost three

Table 3. Cut-offs of 25-Hydroxyvitamin D associated with indicators of adiposity and insulin resistance in prepubertal Chilean Children

Indicators	*AUC (95%)*	*Sensitivity*	*Specificity*	*Younden index*[a]	*Cut-off 25(OH)D*[a]
Girls n = 232					
BAZ (⩾ 2 s.d.)[b]	0.75, (0.66 –0.84)	61.0	82.6	0.434	31.3
BF % (BIA) ⩾ 75th percentile[c]	0.69, (0.62 – 0.77)	68.4	63.8	0.322	30.9
WC ⩾ 75th percentile[d]	0.65, (0.58 – 0.72)	60.0	62.0	0.218	31.9
Truncal fatness ⩾ 75th percentile[e]	0.64, (0.57 – 0.72)	60.3	64.8	0.251	31.9
Fasting Insulin ⩾ 75th percentile[f]	0.71, (0.59 – 0.83)	83.4	55.6	0.390	28.0
HOMA-IR ⩾ 75th percentile[g]	0.72, (0.60 – 0.83)	83.0	53.8	0.368	28.0
QUICKI ⩽ 25th percentile[g]	0.76, (0.65 – 0.87)	83.3	60.9	0.442	28.0
Boys n = 203					
BAZ (⩾ 2 s.d.)[b]	0.66, (0.58 –0.75)	62.3	59.2	0.275	28.9
BF % (BIA) ⩾ 75th percentile[c]	0.71, (0.64 – 0.78)	62.0	73.1	0.349	30.5
WC ⩾ 75th percentile[d]	0.66, (0.58 – 0.74)	63.5	63.6	0.271	29.7
Truncal fatness ⩾ 75th percentile[e]	0.65, (0.57 – 0.72)	60.4	68.6	0.290	30.4
Fasting Insulin ⩾ 75th percentile[f]	0.74, (0.66 – 0.82)	73.0	69.1	0.421	27.5
HOMA-IR ⩾ 75th percentile[g]	0.74, (0.67 – 0.82)	73.2	70.4	0.436	27.5
QUICKI ⩽ 25th percentile[g]	0.72, (0.64 – 0.80)	71.9	70.0	0.419	27.5

Abbreviations: AUC, area under curve; BF, body fat; BIA, bioelectrical impedance measurements; BMI, body mass index; HOMA-IR, homeostasis model assessment of IR; NHANES, National Health study in EEUU; QUICKI, quantitative insulin sensitivity check index; WC, waist circumference. [a]Youden index (sensitivity-(1-specificity)), and the point with shortest distance from the point (0, 1) ($(1 - \text{sensitivity})^2+(1 - \text{specificity})^2$).[49] [b]z Score BMI by age (BAZ) based on Word Health Organization Growth Standards 2007. [c]Bioimpedance (BIA) using Tanita BC-418 MA. Cut-off ⩾ 75 percentile: girls 25.8% BF and boys 28.9% BF. [d]WC, NHANES III percentiles Mexican-Children: cut-off ⩾ 75th percentile girls at 6 years: 60.4 cm; boys at 8 years: 66.2 cm[42] WC/height, cut-off > 0.5. [e]Truncal fatness = calculated by summing abdominal, suprailiac and subscapular skinfold thicknesses ⩾ 75 percentile.[43] [f]Fasting insulin ⩾ 75 percentile: girls 5.6 μg dl^{-1}, boys 9.3 μg dl^{-1}. [g]HOMA-IR ⩾ 75 percentile: girls: 1.25, boys: 2.2. QUICKI ⩽ 25 percentile: girls 0.35, boys: 0.37.

Table 4. OR of the association between suboptimal vitamin D (< 30 ng ml^{-1}) and indicators of total and central adiposity and insulin resistance in 435 prepubertal Chilean children by sex

	Girls (n = *232*) *OR (95% CI)*	*Boys* (n = *203*) *OR (95% CI)*
Total adiposity[a]		
Obesity BAZ (⩾ 2 s.d.)[b]	4.8 (1.9, 12.1)*	2.0 (1.0, 3.9)**
BF % BIA ⩾ 75[h] percentile[c]	3.6 (1.9, 6.6)**	3.0 (1.7, 5.4)*
BF % 3C model ⩾ 75[h] percentile[d]	8.1 (2.7, 24.1)*	2.8 (1.1, 7.1)**
Central adiposity[a]		
WC ⩾ 75[h] percentile[e]	2.4 (1.4, 4.3)*	2.2 (1.2, 4.0)**
WC/height > 0.5 cm[e]	3.1 (1.7, 5.9)*	2.0 (1.2, 3.5)**
Truncal fatness ⩾ 75th percentile[f]	2.8 (1.5, 5.1)*	1.8 (1.0, 3.3)
Insulin resistance[g]		
Fasting insulin ⩾ 75 percentile[h]	3.3 (1.4, 8.2)*	3.4 (1.6, 7.0)*
HOMA-IR ⩾ 75 percentile[i]	2.9 (1.2, 7.1)**	3.3 (1.6, 7.0)*
QUICKI ⩽ 25 percentile[i]	3.7 (1.4, 9.7)*	3.1 (1.5, 6.7)*

[a]Model 1: logistic regression analysis adjusted by, age and seasons *$P < 0.001$, **$P < 0.05$. [b]*z*-score BMI by Age (BAZ) based on Word Health Organization Growth Standards 2007. [c]Bioimpedance (BIA) using Tanita BC-418 MA. Cut-off ⩾ 75 percentile: girls 25.8% BF and boys 28.9% BF. [d]3C model, subsample $n = 283$, girls $n = 100$, boys $n = 183$. ⩾ 75th percentile: girls 36.1 BF% and boys 33.8 BF%. [e]WC, NHANES III percentiles Mexican-Children: cut-off ⩾ 75th percentile in girls at 6 years: 60.4 cm; and boys at 8 years: 66.2 cm[42] WC/Height, cut-off > 0.5. [f]Truncal fatness = calculated by summing abdominal, suprailiac and subscapular skinfold thicknesses.[37] [g]Model 2: logistic regression analysis adjusted by age, seasons, BMI by age *$P < 0.001$, **$P < 0.05$. [h]Fasting insulin ⩾ 75 percentile: girls 5.6 μg dl^{-1}, Boys 9.3 μg dl^{-1}. [i]HOMA-IR ⩾ 75 percentile: girls: 1.25, boys: 2.2. QUICKI ⩽ 25 percentile: girls: 0.35, boys: 0.37.[45]

times more IR risk than children with optimal VD concentrations even after adjusting for BMI (that is, OR = 3.3 (1.4, 8.2); OR = 3.4 (1.6, 7.0) for hyperinsulinism and 2.9 (1.2, 7.1), OR = 3.3 (1.6, 7.0) for IR in girls and boys, respectively).

DISCUSSION

Serum 25(OH)D was inversely related to total and central adiposity using both simple techniques (i.e. BAZ, BIA, WC, WC/H and truncal fatness) or the gold standard method (3C model) in prepubertal Chilean children. Moreover, 25(OH)D was also inversely associated with IR, even after adjusting by total adiposity. For the study sample, a serum 25(OH)D of < 30 ng ml^{-1} was the best predictor of total and central adiposity and of IR. Children with suboptimal VD had two to three times greater risk of obesity, high BF% and central adiposity; and 3–4 times greater risk for IR.

Serum 25-hydroxyvitamin D associations with body fat

We found that associations between 25(OH)D and simple adiposity indicators varied from −0.16 to −0.37, (all *P*-value < 0.05); these results were further confirmed using a robust method such as 3C model based on plethysmography and deuterium measurements (OR: −0.21 (−0.32, −0.09)). These results are in agreement with the hypothesis of the migration of VD to adipose tissue (AT),[9,50] and/or with the emerging hypothesis of AT as a target for the active form of VD (1,25-hydroxyvitamin D).[2,6] This indicates a need to further study the role of VD given the current obesity epidemic experienced by children worldwide.

Serum 25-hydroxyvitamin D associations with insulin resistance

In concordance with previous evidence,[14–17,25–27] we observed an inverse relation between 25(OH)D concentration, fasting insulin and HOMA-IR; and, a direct relationship between 25(OH)D concentrations and insulin sensitivity (quantitative insulin sensitivity check index), after adjusting for adiposity. We believe these results are of special interest during the prepubertal period because suboptimal VD can be considered as an additional stressor to the physiologic IR that accompanies pubertal progression.[51]

Cut-offs of serum 25-hydroxyvitamin D that best reflect adiposity (total and central) and insulin resistance in prepubertal children

There is an ongoing debate on the cutoff values that best define optimal VD when considering the metabolic effects of VD.[35]

We found that serum 25(OH)D is closely associated with (total and central) adiposity; AUC ranges from 0.64 to 0.75 depending on the indicator and IR with an AUC ranging from 0.71 to 0.76. The relationships between these estimates are stronger than what has been previously reported. A study in Irani adolescents reported 11.6 ng ml^{-1} of serum 25(OH)D value as a cut-off based on IR with an AUC of 0.59 (95% CI: 0.52–0.66 for HOMA-IR > 2.1).[52] A study in Korean adolescents showed for abdominal obesity (WC ⩾ 90th percentile for age and sex) an AUC of 0.58 (95% CI: 0.51–0.65) with a cutoff of 17.6 ng ml^{-1} of serum 25(OH)D.[53]

Differences between results from different studies might be explained partially by the effect of puberty on body composition and IR, which could affect the relation between VD and IR in adolescents groups.[23,30–32] The optimal cut-off of serum 25(OH)D ~ 30 ng ml^{-1} suggests that our study is concordant with the cut-off suggested by the US Endocrine Society Task Force based on bone health observations:[54] (a) in postmenopausal women intestinal absorption of calcium is maximized above 32 ng ml^{-1} of 25-OHD,[55] and (b) when 25(OH)D concentration reaches a nadir between 30-40 ng ml^{-1} the concentration of parathyroid hormone decreases.[56] Interestingly, a cut-off of 30 ng ml is also in line with results from a meta-analyses that shows that pregnant women with serum 25(OH)D < 30 ng ml^{-1} present higher risk of gestational diabetes (pooled odds 1.49, 95% CI: 1.18–1.89), pre-eclampsia (pooled odds 1.79, 95% CI: 1.25–2.58) and small for gestational age infants (pooled odds 1.85, 95% CI: 1.52–2.26).[57] Additionally, a recent meta- analyses showed that serum 25(OH)D concentrations < 30 ng ml^{-1} were associated with higher 'all cause' mortality even after adjusting for age ($P < 0.01$).[58] Thus, the well accepted cut-off of 30 ng ml^{-1} of 25(OH)D for bone related outcomes may also be applicable to extraskeletal outcomes.

Associations between suboptimal VD and high adiposity and IR we observed that children with suboptimal 25(OH)D concentrations (< 30 ng ml^{-1}) had two and three times greater risk for obesity, high BF% and central adiposity; and 3–4 times greater risk of IR. Effects tended to be higher in girls than in boys. We are not certain what may account for the sex differences however dimorphism of body composition during onset of puberty may account in part for this. In boys, testosterone concentrations increases fat free mass while in girls, estrogens concentrations induce fat redistribution towards the extremities.[59] Further research in other life stages is needed to clarify this issue.

Our study is not exempt from limitations. Its cross-sectional design does not allow us to claim causality/directionality; however further follow-up of this cohort may provide some insight on the directions of these associations. At this early age there is also a lack of consensus about the adequate cut-points to define limits for normal adiposity and IR. We used several available cut-offs from the WHO multicenter study,[40] from the National Health study in EEUU (NHANES III) and from the American Academy of Pediatrics that involved Hispanic population;[42,44,45] and if not available, we used as a cut-off-point the ⩾ 75th percentile of the sample.

This study also has several strengths. We use a number of indicators of total and central adiposity, even including a gold standard method allowing us to assess the consistency of our finding; this was also true for the case of IR indicators. In addition, to our best knowledge, this is the first study that examines extensively the serum 25(OH)D thresholds as a marker of VD status based on different total and central adiposity and IR indicators in a large sample of children; moreover, the longitudinal nature of our study will allow us to confirm the validity of these findings for other long-term outcomes.

CONCLUSION

Serum 25(OH)D was inversely associated with adiposity (total and central) and IR indicators in prepubertal Chilean children. The traditional cutoff of VD sufficiency (⩾30 ng ml^{-1}) may also be adequate to assess obesity and IR related metabolic risks associated to VD in this age group. These findings highlight the importance of preventing obesity and suboptimal VD from early ages to avoid complications associated with IR and skeletal maturation during puberty.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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