Prepubertal Adiposity, Vitamin D Status, and Insulin Resistance

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abstract

OBJECTIVE: To evaluate the following from prepuberty to the puberty-onset: (1) changes in serum 25-hydroxyvitamin-D (25[OH]D), adiposity, and insulin resistance (IR); (2) the effect of prepubertal adiposity on serum 25(OH)D changes; and (3) the combined effect of prepubertal obesity and suboptimal-25(OH)D on IR at puberty-onset.

METHODS: A total of 426 prepubertal children (~54% girls) were followed during pubertal-onset assessing before and after puberty-onset serum 25(OH)D, adiposity (BMI and waist circumference) and IR indicators (homeostasis-model-assessment of IR [HOMA-IR]). Associations were tested using multiple and logistic regression models adjusted by age, gender, and seasonality.

RESULTS: At puberty-onset, mean serum 25(OH)D decreased (32.2 ± 8.9 Tanner I vs 25.2 ± 8.3 ng/mL Tanner II) and total and central obesity increased (BMI-for-age-*z*-score ≥2 SD [%]: 16.4 vs 22.1; waist-circumference ≥75th percentile [%]: 27.2 vs 37.1, all $P < .05$). Children with higher adiposity before puberty onset had higher risk of suboptimal-25(OH)D (<30 ng/mL) in Tanner II (ie, odds ratio = 2.7 [1.1–6.7] for obesity and 2.7 [1.4–5.5] for central-obesity) after adjusting for relevant covariates. Children with higher adiposity and suboptimal-25(OH)D before puberty-onset had higher HOMA-IR compared with their counterparts in Tanner II (HOMA-IR: 2.8 [2.5–3.1] if central-obese and suboptimal-25[OH]D vs 2.1 [1.9–2.3] no central-obesity and optimal-25[OH]D).

CONCLUSIONS: We found that serum 25(OH)D declined with puberty-onset, likely because of adiposity increase. Moreover, children with the combined condition of central-obesity and suboptimal-25(OH)D before puberty-onset had higher pubertal IR. These results highlight the need of ensuring adequate-25(OH)D status before pubertal-onset, particularly in obese children.

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Dr Cediel performed this work as part of his doctoral thesis in human nutrition at the University of Chile, conceptualized and designed the study, carried out the statistical analysis, and wrote the first draft; Dr Corvalán coordinated and supervised data collection, conceptualized and designed the study, and critically reviewed the manuscript; Drs López de Romaña and Mericq conceptualized the study, supervised data collection, and critically reviewed the manuscript; Dr Uauy conceptualized and designed the study, contributed to interpreting the data, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: In adults, vitamin D is associated with adiposity and insulin resistance. Puberty is a period of physiologic adiposity and insulin resistance increases; however, it is unclear whether vitamin D also plays a role in these processes.

WHAT THIS STUDY ADDS: We show that serum 25 hydroxyvitamin D declines with puberty-onset and that children with central-obesity in conjunction with suboptimal 25 hydroxyvitamin D (<30 ng/mL) before puberty-onset have higher insulin resistance during puberty.

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The paradigm of vitamin D nutrition has changed over recent years, encompassing targets beyond bone health.[1] Vitamin D status has been linked to adiposity and insulin resistance (IR), particularly in adults.[2,3] Recently, we have observed in prepubertal Chilean children that serum 25-hydroxyvitamin-D (25[OH]D) was inversely associated with key indicators of adiposity (total and central) and IR, and that the conventional cutoff of optimal-25(OH)D status (≥30 ng/mL) is adequate to assess obesity and IR risk in this age group.[4] Other cross-sectional studies in children and adolescents also have shown a modest negative correlation between serum 25(OH)D and IR; however, the magnitude and direction of association seems to differ according to pubertal stage.[5,6]

The onset of puberty is a period of important metabolic changes.[7] The increase of steroid hormones, which promote sexual maturation, stimulate a concomitant increase in mean concentrations of growth hormone,[8] inducing the physiologic pubertal increase on IR. In terms of body composition, girls increase body fatness and boys primarily increase lean mass, and in both cases, the result is an increase in BMI.[9]

Vitamin D needs increase during puberty given the increases on bone accretion[10,11]; the risk of suboptimal-25(OH)D at puberty-onset is high given that the physiologic increase of body-fatness may decrease the bioavailability of 25(OH)D.[12] Thus, it is unclear whether 25(OH)D status interacts with the increase in adiposity augmenting IR; we hypothesize that independent changes in serum-25(OH)D concentration or adiposity (ie, suboptimal-25[OH]D or obesity per se) may result in an increased IR.[13]

To test this hypothesis, we studied prospectively 426 Chilean girls and boys from prepubertal stage to the onset of puberty and assessed the following: (1) changes in serum-25(OH)D, adiposity, and IR; (2) the effect of prepubertal adiposity on serum-25(OH)D changes; and (3) the combined effect of prepubertal obesity and suboptimal-25(OH)D on IR at puberty-onset.

METHODS

Study Design

Longitudinal follow-up study of 426 children (~54% girls) participating in the Growth and Obesity Chilean Cohort Study (GOCS) in Santiago, Chile (latitude 33°27′S). GOCS children are evaluated starting at 6 years of age twice per year; measurements include detecting the onset of puberty. A blood sample was taken to all girls before any sign of puberty-onset at ~6 years and to all boys at ~8 years. These measurements were repeated at the time pubertal-onset was first detected. Details of recruitment procedures and a detailed description of study design are elsewhere.[4,14] Thus, we briefly summarize the latter in the next paragraphs.

Subjects

GOCS is a cohort of low- to middle-income children born in Santiago in 2002 to 2003 (n = 1196, ~50% girls), born at term (37–42 weeks gestational age) with a birth weight ≥2500 g. We randomly selected a sample of 450 children (50% girls) from all children (GOCS) with prepubertal and pubertal-onset blood samples. We excluded children with a history of genetic or metabolic disease, those who reported receiving vitamin D supplements in the past 6 months (n = 7), and those who did not comply with overnight fasting (n = 8). Sample size was estimated to assess magnitude of associations previously reported assuming 80% power, a 2-tail significance $P < .05$, and considering a small effect size (~0.3).[15] 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 those children who were not included (all $P > .05$). 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.

Physical/Sexual Maturation

Previously trained professional dietitians classified Tanner stages in girls based on breast bud palpation and in boys based on volume of testes (using orchidometer).[7,16] A blood sample (~8 hours of fasting) was taken to all children at Tanner stages I and II. Serum dehydroepiandrosterone-sulfate (DHEAS) was measured by competitive specific-binding radioimmunoassay (Diagnostic System Laboratories, Webster, TX); inter- and intra-assay coefficients of variation for the assay were 5.1% and 3.5%, respectively. Serum insulinlike growth factor 1 (IGF-1) was measured by radioimmunoassay by using a commercial kit (Linco-Research Inc, St Charles, MO) (sensitivity 5 ng/mL: intra- and interassay coefficients of variation: 8.6% and 10.2%, respectively).[17] In each of the visits, skeletal-maturation was assessed in duplicate by using an ultrasound-transmission method (BonAge; Sunlight Co, Medical, Tel-Aviv, Israel).[18]

Vitamin D

A single professional obtained all blood samples early in the morning. Serum concentrations of 25(OH)D were measured in duplicate by using the Liaison-25(OH)D total-kit (DiaSorin, Inc, Stillwater, MN), which had inter- and intra-assay coefficients of variation of 11.5% and 6.3%, respectively. Optimal-25(OH)D was

defined as serum 25(OH)D ≥30 ng/mL (cutoff previously validated for metabolic outcomes on this sample).[4]

Adiposity

Subsequent to blood sampling, a registered dietician assessed weight, height, WC, and skinfold thickness by using standardized procedures. Weight was measured with a portable electronic scale (Seca-770; Seca, Hamburg, Germany) with precision of 0.1 kg. Height was measured with a portable stadiometer (Harpenden-603; Holtain-Ltd, Crosswell, United Kingdom) 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-W606 PM; Lufkin, Baltimore, MD) to the closest 0.1 cm. In addition, the indicator WC divided by height (WC/H) was calculated. Skinfold thickness was measured in triplicate with a Lange caliper to the nearest 0.5 mm. Intraobserver measurement error and the mean average bias of the observer were within the limits suggested by the World Health Organization in the Multi-center Growth Reference Study for all measurements.[19] Bioelectrical impedance measurements were measured by using a Tanita-BC-418 MA, 8 electrodes, hand-to-foot system (Tanita-Corporation, Tokyo, Japan), according to the manufacture's guidelines and at measurement frequency of 50 kHz (accuracy 0.1 kg). Body composition was estimated based on the equation available in the equipment. Details of procedures have been previously published.[4]

Serum-glucose concentrations were measured with an enzymatic colorimetric technique (HUMAN; Gesellschaftfür Biochemica und Diagnostica, Weisbaden, Germany), and serum-insulin concentrations were measured with a radioimmunoassay kit (Linco Research, Inc, St Charles, MO). All analyses were conducted at the Nutrition 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).

Adiposity and Metabolic Indicators

High total adiposity was defined based on BAZ (weight/height^2; kg/m^2) by using the World Health Organization (WHO-2007)[20] growth reference for children 5 to 19 years. Obesity was defined as BAZ ≥2 SD, and overweight as BAZ ≥1 SD. High body fat percentage was defined as ≥75th percentile of the distribution of the sample for bioelectrical impedance measurement. High central adiposity was defined by using the following: (1) WC-NHANES-III >75th percentile of Hispanic population specific by age and gender[21]; (2) WC/height as ≥0.5 cm; and (3) truncal-fatness (sum of abdominal, suprailiac, and subscapular skinfold thicknesses).[22]

IR was estimated from fasting insulin and glucose levels by using the homeostasis model assessment of insulin resistance (HOMA-IR), defined as fasting-glucose (mg/dL) × fasting-insulin (mU/mL)/405, which has been validated in nondiabetic children and adolescents.[23]

Seasonality

In Chile there are 4 distinct seasons: summer, December 21 to March 21; fall, March 21 to June 21; Winter, June 21 to September 21; and spring September 21 to December 21.[24] The date of 25(OH)D sampling served to classify seasonality.

Statistical Analyses

Data are presented as means (SDs). Variables with non-normal distributions were log-transformed. In the baseline (prepubertal stage), 435 children were considered (data have been used for cross-sectional analysis in previous publication).[4]

In this longitudinal study after performing the diagnostic tests, outliers, leverage, influence, homoscedasticity, and normal distribution of residuals, 9 children were classified as outliers and thus excluded; leaving 426 children (54% girls) with complete data for the longitudinal analysis (Fig 1). To evaluate the differences by gender and Tanner between variables, we used Student's *t* test or χ^2/Fisher's tests. To evaluate the effect of prepubertal adiposity on serum 25(OH)D changes or suboptimal 25(OH)D (<30 ng/mL) at onset of puberty, we used linear regression models (β-coefficient: 95% confidence interval) by using standardized coefficients,

$$\frac{\textit{Value in sample} - \textit{mean}}{\textit{standard deviation of the sample}}$$

or logistic-regression-models adjusting for age, gender, seasonality, and δ of time between measurements. To assess the combined effect of prepubertal obesity and suboptimal 25(OH)D on IR, we compared the average HOMA-IR concentrations at puberty-onset of 4 groups stratified based on vitamin D and nutritional status at prepuberty: (1) optimal-25(OH)D + normal weight, (2) optimal-25(OH)D + high total or central adiposity, (3) suboptimal-25(OH)D + normal weight, and (4) suboptimal 25(OH)D + high total or central adiposity, by using multiple regression models adjusted for age, gender, and seasonality. Interactions by gender were nonsignificant in all the analyses ($P > .05$); thus, results are presented for genders combined. Statistical analyses were conducted by using Stata-11.0 (StataCorp, College Station, TX). Graphics were designed by using demo-graph-prism (GraphPad Software, Inc, San Diego, CA). The associations were considered significant if $P < .05$.

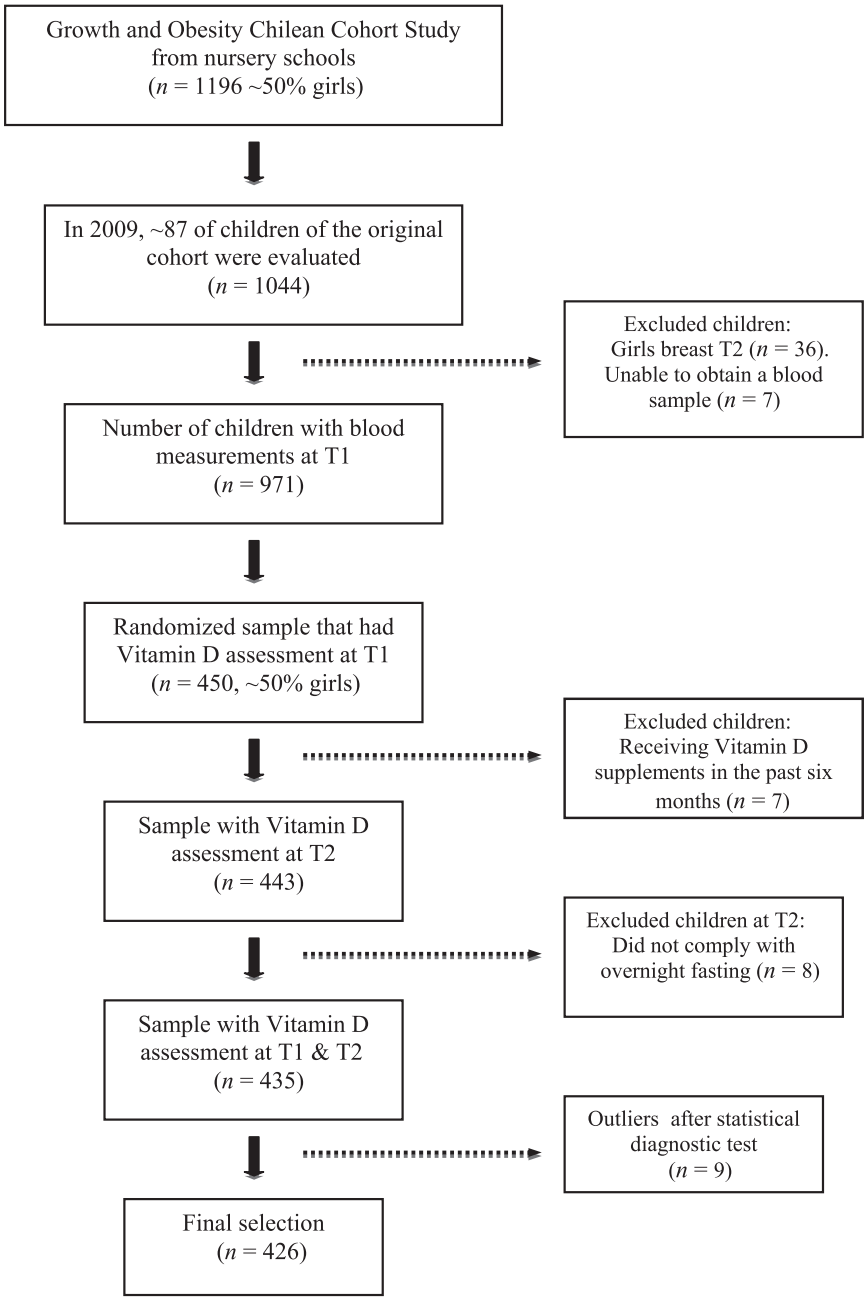

FIGURE 1
Flowchart of participants in the study.

RESULTS

General characteristics by gender and Tanner stage are presented in Table 1. A total of 426 children (~54% girls) were included in the study (mean age: girls ~6 years in Tanner I and ~9 years in Tanner II; boys: ~8 years in Tanner I and ~10 years in Tanner II). At puberty-onset, we observed an increase in the indicators of physical and hormonal maturation: bone age (Δ = 2.1 years), DHEAS (Δ = 32.3 μg/mL), and IGF-1 (Δ = 37.9 ng/mL).

Changes in Serum-25(OH)D From Prepuberty to Puberty-Onset

Serum 25(OH)D concentrations dropped significantly between prepuberty and puberty-onset (Δ = −6.9 ng/mL, Tanner I: 32.1 ± 9.2 vs Tanner II: 25.2 ± 8.3 ng/mL, $P < .05$), in both girls and boys (girls: Δ = −7.0 ng/mL vs boys Δ = −6.9 ng/mL) (Fig 2). In both Tanner stages, serum 25(OH)D concentrations were lower in boys (Δ = −1.9 ng/mL, median ± SD in boys 27.8 ± 9.8 vs girls 29.4 ± 8.9 ng/mL, $P < .05$), and lower during winter (median ± SD: winter 27.6 ± 9.3, autumn 28.5 ± 8.3, spring 29.7 ± 10.9, summer 33.1 ± 8.8ng/mL; analysis of variance, $P < .05$).

Changes in Adiposity From Prepuberty to Puberty-Onset

We observed an increase in indicators of adiposity, such as weight (Δ = 9.9 kg), BMI (Δ = 1.8 kg/m^2), fat mass (Δ = 3.1 kg), and obesity (BAZ ≥2 SD, Δ = 5.6%), as well as in central adiposity (WC [Δ = 7.4 cm], truncal fatness [Δ = 19.2 mm], and central obesity [WC: >75th percentile, Δ = 9.9%]). Changes were of similar magnitude for boys and girls ($P > .05$ for interaction), but boys presented higher level of total and central adiposity than girls in both Tanner stages ($P < .05$). Differences were also observed in height (Δ = 13.9 cm), and fat-free mass (Δ = 6.7 kg); however, *z*-score height-by-age was similar between Tanner stages in both genders (Table 1).

Changes in IR From Prepuberty to Puberty-Onset

In the transition between Tanner I and II, children experienced a significant increase in fasting insulin (Δ = 3.4 μg/dL) and IR (HOMA-IR: Δ = 0.8), with slightly higher values in boys than girls ($P > .05$ for gender interaction) likely due to their higher adiposity (Table 1).

Effect of Adiposity in Prepubertal Stage on Changes in Serum-25(OH)D in the Transition to Puberty

Longitudinal analysis showed an inverse association between adiposity (total and central) at Tanner I and changes in serum-25(OH)D between Tanner stages (Fig 3A). High total

TABLE 1 Characteristics of 426 Chilean Children at Onset of Puberty

	Total, *n* = 426		Δ	Girls, *n* = 231		Δ	Boys, *n* = 195		Δ
	Tanner I, Median ± SD	Tanner II, Median ± SD	Tanner I and II, Median ± SE	Tanner I, Median ± SD	Tanner II, Median ± SD	Tanner I and II, Median ± SE	Tanner I, Median ± SD	Tanner II, Median ± SD	Tanner I and II, Median ± SE
Age, y	7.1 ± 1.3	9.5 ± 1.2	2.4 ± 0.09*	6.3 ± 0.6	8.9 ± 0.9	2.6 ± 0.07*	8.0 ± 1.4	10.2 ± 1.03	2.2 ± 0.12*
25(OH)D, ng/mL	32.2 ± 8.9	25.2 ± 8.3	−6.9 ± 0.6*	32.9 ± 8.1	26.0 ± 8.3	−7.0 ± 0.8*	31.2 ± 9.6	24.3 ± 8.4	−6.9 ± 0.9*
Suboptimal vitamin D, <30 ng/mL, % (*n*)	39.7 (169)	79.1 (337)	39.4 (168)*	31.2 (72)	76.2 (176)	45 (104)*	49.7 (97)	82.6 (161)	32.8 (64)*
Maturation indicators									
IGF-1, ng/mL	196.1 ± 58.3	234.0 ± 67.5	37.9 ± 4.4*	176.7 ± 44.5	248.5 ± 72.9	71.8 ± 5.6*	216.7 ± 56.4	220.8 ± 64.2	4.1 ± 6.2*
DHEAS, μg/mL	44.7 ± 30.9	77.0 ± 42.8	32.3 ± 2.6*	34.1 ± 19.5	64.4 ± 32.3	30.3 ± 2.5*	57.4 ± 36.7	93.3 ± 48.7	35.9 ± 4.5*
Bone age, y	8.4 ± 1.6	10.5 ± 1.4	2.1 ± 0.10*	7.5 ± 0.9	9.8 ± 1.2	2.3 ± 0.09*	9.5 ± 1.6	11.4 ± 1.1	1.9 ± 0.1*
Anthropometric variables									
Weight, kg	27.8 ± 6.8	37.7 ± 9.1	9.9 ± 0.6*	24.5 ± 4.2	34.4 ± 7.5	9.9 ± 0.6*	31.8 ± 7.2	41.7 ± 9.3	9.9 ± 0.8*
Height, cm	125.2 ± 8.5	139.1 ± 7.7	13.9 ± 0.6*	120.4 ± 5.2	135.6 ± 6.3	15.2 ± 0.5*	130.9 ± 8.1	143.2 ± 7.2	12.3 ± 0.8*
BMI, kg/m^2	17.5 ± 2.5	19.3 ± 3.3	1.8 ± 0.2*	16.8 ± 2.1	18.6 ± 3.0	1.8 ± 0.2*	18.4 ± 2.7	20.2 ± 3.4	1.8 ± 0.3*
BAZ	0.87 ± 1.1	0.99 ± 1.2	0.12 ± 0.08	0.66 ± 0.9	0.79 ± 1.1	0.12 ± 0.09	1.11 ± 1.2	1.25 ± 1.2	0.14 ± 0.1
BMI by age ≥2 SD, % (*n*)[a]	16.4 (70)	22.1 (94)	5.6 (24)**	9.9 (23)	13.4 (31)	3.5 (8)	24.1 (47)	32.3 (63)	8.2 (16)
z-score height by age	0.19 ± 0.9	0.21 ± 0.9	0.01 ± 0.06	0.14 ± 0.8	0.12 ± 0.9	0.01 ± 0.08	0.27 ± 0.9	0.31 ± 0.9	0.04 ± 0.09
WC, cm,[a]	60.3 ± 7.4	67.7 ± 9.4	7.4 ± 0.6*	57.7 ± 5.8	64.8 ± 8.3	7.1 ± 0.7*	63.5 ± 7.8	71.2 ± 9.4	7.7 ± 0.9*
WC/height, cm	0.48 ± 0.05	0.49 ± 0.06	0.004 ± 0.004	0.48 ± 0.04	0.48 ± 0.05	0.001 ± 0.004	0.48 ± 0.05	0.49 ± 0.06	0.01 ± 0.06**
Central obesity, WC, % (*n*)[a, 21]	27.2 (116)	37.1 (158)	9.9 (42)**	21.2 (49)	30.7 (71)	9.5 (22)**	34.4 (67)	44.6 (87)	10.3 (20)**
Truncal fatness, mm	30.3 ± 17.1	49.5 ± 26.0	19.2 ± 1.5*	25.7 ± 10.5	46.9 ± 24.4	21.2 ± 1.7*	35.8 ± 21.3	52.6 ± 27.6	16.8 ± 2.5*
Fat mass, %[a]	24.0 ± 4.4	25.6 ± 5.7	1.6 ± 0.3*	24.1 ± 3.7	26.2 ± 4.9	2.1 ± 0.4*	23.9 ± 5.1	24.9 ± 6.4	1.0 ± 0.6
Fat mass, kg/m^2	6.9 ± 2.8	9.9 ± 4.4	3.1 ± 0.3*	6.0 ± 1.9	9.3 ± 3.7	3.3 ± 0.3*	7.9 ± 3.4	10.8 ± 4.9	2.9 ± 0.4*
Fat-free mass, kg/m^2	20.9 ± 4.3	27.7 ± 5.4	6.7 ± 0.3*	18.5 ± 2.5	25.2 ± 4.1	6.7 ± 0.3*	23.8 ± 4.2	30.7 ± 5.1	6.8 ± 0.5*
Metabolic biomarkers									
Fasting glucose, mg/dL	90.7 ± 6.9	90.7 ± 8.1	0.04 ± 0.5	89.2 ± 6.7	89.7 ± 7.7	0.6 ± 0.7	92.5 ± 6.7	91.8 ± 8.6	0.8 ± 0.8
Fasting insulin, μg/dL	6.3 ± 2.2	9.7 ± 5.3	3.4 ± 0.3*	5.5 ± 1.4	8.5 ± 3.4	2.9 ± 0.2*	7.2 ± 2.6	11.2 ± 6.7	3.9 ± 0.5*
HOMA-IR	1.4 ± 0.5	2.2 ± 1.3	0.8 ± 0.07*	1.2 ± 0.3	1.9 ± 0.8	0.7 ± 0.06*	1.7 ± 0.6	2.6 ± 1.6	0.9 ± 0.1*
QUICKI	0.16 ± 0.007	0.16 ± 0.04	−0.002 ± 0.00	0.16 ± 0.005	0.15 ± 0.008	−0.009 ± 0.00*	0.16 ± 0.07	0.16 ± 0.06	−0.007 ± 0.004

[a] Cutoff for suboptimal vitamin D <30 ng/mL. BMI by age ≥2 SD, body fat ≥75th percentile by bioimpedancia (Tanita BC-418 MA), WC (NHANES III in Mexican boys: Cutoff ≥75th percentile, Fernandez 2004). WC/height ≥0.5 cm. Hyperinsulinism was defined as fasting insulin ≥75th percentile of sample (Tanner I: 6.7, Tanner II: 11.2 μg/dL). IR was defined such as HOMA-IR: fasting glucose*Fasting insulin/405 ≥75th percentile of the sample (Tanner I: 1.5, Tanner II: 2.6). Baja insulin sensibility was defined such as Quantitative Insulin Sensitivity Check Index (QUICKI): (1/log [fasting insulin] + log [fasting glucose]). Differences between Tanner stages in each gender were estimated by using *t* test or χ^2 tests.

* $P < .001$.

** $P < .05$.

and central-adiposity at Tanner I increased 2 to 3 times the risk of suboptimal-25(OH)D (<30 ng/mL) at Tanner II, even after adjusting by gender, age, seasonality, and δ of time between Tanner-stages (Fig 3B).

Combined Effect of Prepubertal Obesity and Suboptimal-25(OH)D on IR at Puberty-Onset

We observed that those children with obesity (β: 0.37; 95% [0.11–0.64]), as well as those with suboptimal-25(OH)D (β: 0.57; 95% [0.29–0.85]) presented higher levels of HOMA-IR at puberty-onset compared with their counterparts, even after adjusting for serum-25(OH)D (obesity model) and adiposity (vitamin D models), suggesting an independent effect of obesity and suboptimal-25(OH)D. Higher HOMA-IR concentrations at puberty-onset were observed in prepubertal children with the combined condition of central obesity and suboptimal-25(OH)D ($P < .05$); a similar trend was observed using other adiposity indicators (Table 2). Interactions between adiposity and 25(OH)D were nonsignificant ($P > .05$).

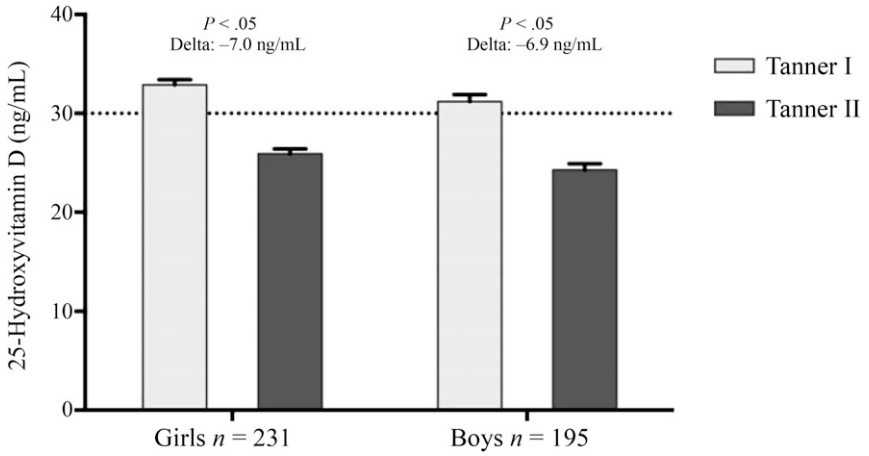

FIGURE 2
Serum 25(OH)D changes (mean, SE) in the transition between prepubertal stage and the onset of puberty. Both girls and boys presented a significant decrease in serum 25(OH)D (dashed line shows 25[OH]D at 30 ng/mL, which is considered optimal) in the transition to puberty ($P < .05$).

DISCUSSION

To our knowledge, this is the first study to examine the relationship between serum-25(OH)D, adiposity, and IR during puberty-onset. In a population with high prevalence of obesity (16.4%) and suboptimal-25(OH)D (39.7%), we found increases in adiposity and IR during this period, as expected. However, ~80% in children at Tanner stage II had suboptimal-25(OH)D. We also observed that being obese before puberty predicts lower 25(OH)D at the time of puberty-onset. In addition, the combination of prepubertal central obesity and suboptimal-25(OH)D predicts higher HOMA-IR concentrations over this period.

Changes in Adiposity, IR, and Serum-25(OH)D During Puberty-Onset

In agreement with the literature,[9] we observed an increase in fat mass in both genders at onset of puberty, with higher increase of total adiposity in girls (fat mass %: girls: Δ = 2.1 ± 0.4; boys: Δ = 1.0 ± 0.6) and slightly higher increase of central adiposity in boys (central obesity %: girls: Δ = 9.5%; boys: Δ = 10.3%), attributable to the action of sex steroid hormones.[25] Additionally,

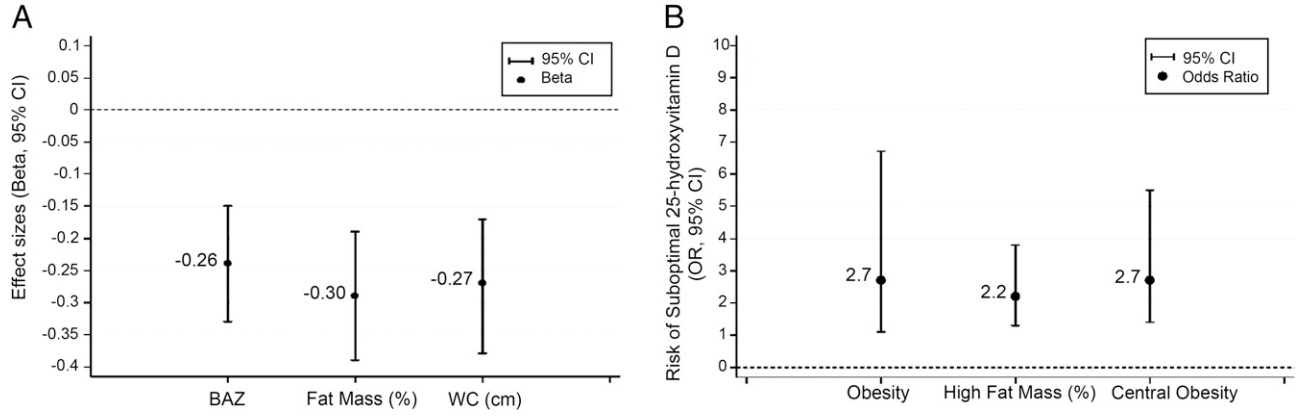

FIGURE 3
A, Standardized-coefficients (β, 95% confidence interval) for δ of change in serum 25(OH)D between Tanner I and II per sample-specific 1 SD in total-adiposity (BAZ, fat-mass) and central-adiposity (waist circumference [WC]) indicators in prepubertal stage (Tanner I), after adjusted by 25(OH)D, gender, age, seasonality and δ of time between Tanner I and II. B, Risk of suboptimal 25(OH)D at puberty-onset related with adiposity in prepuberty (Tanner I), using logistic regression models adjusting by gender, age, seasonality and δ of time between measurements. Suboptimal 25(OH)D: <30 ng/mL. Obesity: BAZ ≥2 SD. High Fat Mass (%): ≥75th percentile of the sample. Central-Obesity: waist-circumference (NHANES-III, Hispanic-children: ≥75th percentile).

we observed an increase of IR (~53%) in the transition to puberty (HOMA-IR: Δ = 0.8; Tanner I: 1.4 ± 0.5 vs Tanner II: 2.2 ± 1.3). These results are in line with data from previous cross-sectional studies (HOMA-IR: Tanner I: 1.5 ± 1.0 vs Tanner III: 2.5 ± 1.6)[26] and with other longitudinal observations. Goran and Gower[27] and Hannon et al[28] noted a 32% and 50% reduction on insulin sensitivity in the transition from Tanner I to Tanner III, respectively. We observed a significant decrease on serum 25(OH)D in the same period (Δ =–6.9ng/mL) that resulted in an increase in the prevalence of suboptimal-25(OH)D (Δ = ~40%), particularly in girls (girls: Δ = ~45% and boys: Δ = ~33%). Similarly, Buyukinan et al[6] showed in a cross-sectional analysis in obese children, a significant rate of 25(OH)D deficiency (<20 ng/mL) in the pubertal group (77.6%, *n* = 45) compared with that in the prepubertal group (43.7%, *n* = 21), *P* < .05.

TABLE 2 Prepubertal Suboptimal 25(OH)D (<30 ng/mL) and Adiposity (Total and Central) on IR (HOMA-IR) at Onset of Puberty (Tanner II) in 426 Children

	n	HOMA-IR, Mean[a]	Range 95% CI
Overweight[b]			
Optimal 25(OH)D/normal weight	171	2.0	1.8–2.2[c]
Optimal 25(OH)D/overweight	86	2.3	2.1–2.6[c,d]
Suboptimal 25(OH)D/normal weight	70	2.0	1.7–2.2[c]
Suboptimal 25(OH)D/overweight	99	2.5	2.3–2.8[d]
Obesity[b]			
Optimal 25(OH)D/no obese	229	2.1	1.9–2.3[c]
Optimal 25(OH)D/obese	28	2.3	1.8–2.7[c,d]
Suboptimal 25(OH)D/no obese	127	2.2	2.0–2.4[c]
Suboptimal 25(OH)D/obese	42	2.7	2.3–3.0[d]
Central obesity[b, 21]			
Optimal 25(OH)D/no central obesity	204	2.1	1.9–2.3[c]
Optimal 25(OH)D/central obesity	53	2.3	2.0–2.6[c]
Suboptimal 25(OH)D/no central obesity	106	2.0	1.8–2.2[c]
Suboptimal 25(OH)D/central obesity	63	2.8	2.5–3.1[d]

[a] Margins means and 95% confidence intervals (CI). Multiple regressions adjusted by gender, age, seasonality, and δ of time between Tanner I and Tanner II. Not sharing a common superscript letter is significantly different from each other at *P* < .05.

[b] Cutoff for suboptimal 25(OH)D (<30 ng/mL). For overweight: BMI by age ≥1 SD. For obesity: BMI by age ≥2 SD. For central obesity: WC (NHANES III in Hispanic boys: Cutoff ≥75th percentile, Fernandez 2004). IR was defined such as HOMA-IR: fasting glucose*fasting insulin/405.

Effects of Prepubertal Adiposity on Serum-25(OH)D Changes During Puberty-Onset

Total and central adiposity were inversely related to serum 25(OH)D changes during puberty-onset and with higher risk of suboptimal-25(OH)D (<30 ng/mL). We also found that the effect of adiposity on serum-25(OH)D was low to moderate, in agreement with our results from this same cohort at 7 years of age.[4] There has been some discussion on directionality of the adiposity and serum-25(OH)D relationship.[29] Our results are consistent with studies in adults who underwent a reduction in weight or visceral adiposity and show a significant increase in serum concentrations of 25(OH)D.[30,31] These results are consistent with the hypothesis of a potential sequestration/dilution of serum-25(OH)D by adipose tissue.[12] Consequently, if the availability of serum-25(OH)D in plasma and tissues decreases at puberty-onset, the maximum growth potential, bone mineral density, and skeletal muscle function may be affected, given the rise in the utilization of 1,25-dihydroxyvitamin D (1, 25[OH]2D).[10,11] In addition, we must consider the effect of the greater demand for calcium to ensure bone growth in this period (1300 mg/d of calcium required for 9- to 18-year-old children compared with 1000 mg/d for those 4 to 8 years).[32]

Combined Effects of Obesity and Suboptimal-25(OH)D on IR at Onset of Puberty

We found that adiposity and suboptimal-25(OH)D have independent effects on IR at puberty-onset. It is well accepted that obesity is associated with a decrease in peripheral glucose uptake that predisposes to IR condition.[33] In this same cohort, we have previously shown that excessive gain in BMI predicted a higher cardio-metabolic risk.[22,34] Our work demonstrates that prepubertal adiposity (whole body or central distribution) predicts higher HOMA-IR levels at puberty-onset.

Additionally, our data show that low serum-25(OH)D concentrations may be considered as an additional stressor of the physiologic IR that accompanies pubertal progression. Those children with suboptimal-25(OH)D showed higher IR in comparison with their counterparts even after adjusting by covariates. The biological mechanisms by which 1,25(OH)2D influenced the IR in children and adolescents remains to be unraveled. The evidence suggests that 1,25(OH)2D might improve peripheral uptake of glucose through the regulation of intracellular calcium pool, which is essential for insulin-mediated intracellular processes. Also, 1,25(OH)2D may act in the attenuation of inflammation and/or in the regulation of synthesis/secretion of insulin by pancreatic-β-cells.[35]

It has also been reported that in obese children, pancreatic β-cell insulin secretion may be unable to respond to the increased demands imposed by puberty, resulting in inadequate regulation of blood glucose levels.[36] We here suggest that this dysregulation may be augmented in children with the combined condition of central obesity and suboptimal-25(OH)D status. These

results suggest the importance of preventing obesity and vitamin D inadequacy before pubertal-onset to avoid the consequences of IR, metabolic derangements, and growth disturbances during puberty.[13] The American Society for Adolescent Health and Medicine,[37] in its 2013 position paper, recommended vitamin D supplementation even in adolescents without vitamin D deficiency to ensure adequate bone acquisition during puberty. We here propose that vitamin D concentrations would also be relevant for metabolic status during this period, particularly in obese children.[37]

Our study is not exempt of limitations; there is a lack of consensus about the adequate cutoff values (ie, adiposity, IR, and 25(OH)D status) at this early age. We used several available cutoffs of adiposity from the World Health Organization multicenter study[20] and the National Health study in the United States of America (NHANES-III) that involved Hispanic population[21,38], and considered several indicators of adiposity to assess the consistency of our findings. In this sample, we found a low prevalence of abnormal IR (HOMA-IR >3.2; 13.4% in Tanner II),[38] thus it was difficult to perform dichotomous analysis. However, the design of the study with a large sample size allowed us to make a good approximation of IR risk in the various groups. We used a cutoff for serum 25(OH)D ≥30 ng/mL to define 25(OH)D-sufficiency, as previously validated for metabolic outcomes on this sample.[4] This work does not include data for the full sample of the primary (GOCS) study. However, the data of this study do not differ in age, BAZ, *z*-score height-to-age, or WC relative to children not included (details in the Methods section). This study also has several strengths. We followed a large number of children with minor losses in the sample size and we were also able to determine with good accuracy the onset of puberty by using recommended clinical methods (palpation of the breast bud and testes development). To the best of our knowledge, this is the first study that examines the relationship between serum-25(OH)D and adiposity and their effect on physiologic IR during the onset of puberty, taking into account potential confounders, such as gender, age, and seasonality, in a large sample of both girls and boys. Furthermore, the longitudinal nature of our study will allow us to confirm the effects of these associations in later stages of puberty.

CONCLUSIONS

In a sample of children with high adiposity and suboptimal-25(OH)D, we observed an important decline in serum 25(OH)D with puberty-onset. This decline may be at least partially explained by the physiologic adiposity increase of puberty, and is relevant because the combination of central obesity and suboptimal-25(OH)D increases pubertal IR. These results highlight the need to ensure adequate-25(OH)D status by increasing vitamin D intake and sun exposure before puberty, particularly in obese children.

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ABBREVIATIONS

BAZ: *z*-score BMI for age
DHEAS: dehydroepiandrosterone sulfate
GOCS: Growth and Obesity Chilean Cohort Study
HOMA-IR: homeostasis model assessment of insulin resistance
IGF-1: insulinlike growth factor-1
IR: insulin resistance
WC: waist circumference
1,25(OH)2D): 1,25-dihydroxyvitamin D
25(OH)D: serum 25-hydroxyvitamin D

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