Vitamin D status in infancy and cardiometabolic health in adolescence

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ABSTRACT

Background: Vitamin D deficiency is associated with obesity-related conditions, but the role of early life vitamin D status on the development of obesity is poorly understood.

Objectives: We assessed whether serum 25-hydroxyvitamin D [25(OH)D] at age 1 y was related to metabolic health through adolescence.

Methods: We quantified serum 25(OH)D in samples obtained at age 1 y from 306 participants in a cohort study in Santiago, Chile. Anthropometry was performed at ages 5, 10, and 16/17 y. At 16/17 y, we determined body composition using DXA and quantified metabolic parameters in a blood sample. We examined the associations of infancy 25(OH)D with BMI-for-age *z*-score (BMIZ) at ages 5, 10, and 16/17 y; with percentage fat and percentage lean body mass at age 16/17 y; and with a metabolic syndrome (MetS) score and its components at age 16/17 y.

Results: Infancy 25(OH)D was inversely associated with BMIZ in childhood. Every 25-nmol/L difference in 25(OH)D was related to an adjusted 0.11 units lower BMIZ at age 5 y (95% CI: −0.20, −0.03; $P = 0.01$) and a 0.09 unit lower BMIZ change from ages 1 to 5 y (95% CI: −0.17, −0.01; $P = 0.02$). Also, every 25-nmol/L 25(OH)D in infancy was associated with an adjusted 1.3 points lower percentage body fat mass (95% CI: −2.2, −0.4; $P = 0.005$) and an adjusted 0.03 units lower MetS score (95% CI: −0.05, −0.01; $P = 0.01$) at age 16/17 y, through inverse associations with waist circumference and the HOMA-IR.

Conclusions: Serum 25(OH)D at age 1 y is inversely associated with childhood BMIZ, percentage body fat at age 16/17 y, and a MetS score at age 16/17 y. Intervention studies are warranted to examine the effects of vitamin D supplementation in early life on long-term cardiometabolic outcomes. *Am J Clin Nutr* 2020;00:1–9.

Keywords: vitamin D, infancy, metabolic syndrome, body mass index, obesity, insulin resistance

Introduction

Childhood overweight and obesity are serious global public health problems, including in Latin America (1). They contribute to a growing prevalence of metabolic disturbances in early life, such as insulin resistance (2) and dyslipidemia (3). These metabolic abnormalities, frequently defined as the metabolic syndrome (MetS), track into adulthood and increase the risk of cardiovascular disorders (4, 5). It is therefore essential to identify modifiable exposures that can impact cardiometabolic health early in life.

Vitamin D deficiency (VDD) is another major challenge globally, even in countries with abundant sun exposure year-round (6). Although the vitamin D receptor is expressed in most systems of the human body, the functional consequences of low circulating vitamin D concentrations beyond musculoskeletal health remain a matter of debate (7), particularly at early ages.

VDD might increase adiposity and impair metabolic health either directly through adipocyte differentiation or indirectly through its effects on regulation of calcium homeostasis (8, 9). Epidemiological research indicates that low vitamin D serostatus is associated with obesity (10). Most prior studies have been cross-sectional, and their interpretation is limited by reverse causation because fat-soluble vitamin D could be sequestered out of the blood and stored in adipose tissue (11). Furthermore, both VDD and obesity could relate to aspects of poor nutrition. Previous research has primarily focused on adults. In children, a few studies have examined intrauterine (12–15) or middle-childhood (16, 17) exposure to low vitamin D serostatus in

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Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Supplemental Tables 1–4 and Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: BMIZ, BMI-for-age *z*-score; IDA, iron deficiency anemia; INTA, Institute of Nutrition and Food Technology; MetS, metabolic syndrome; RCT, randomized controlled trial; SES, socioeconomic status; VDD, vitamin D deficiency; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin D.

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relation to cardiometabolic end points, but its role at other ages is unknown.

We used prospectively collected data to evaluate the associations between vitamin D serostatus in infancy (age 1 y) and measures of metabolic health through age 16/17 y, including body composition and MetS, in the context of a cohort of participants recruited as infants from low- and middle-income neighborhoods in Santiago, Chile.

Methods

Study population

We analyzed prospectively collected data from a cohort study of Chilean participants who were enrolled as infants in an investigation of iron status and neurodevelopment, and who were followed through adolescence; a cardiovascular risk study was added in adolescence. A detailed description of the iron study design has been published previously (18). In brief, infants aged 4–6-mo were recruited from low- and middle-income communities in Santiago, Chile, between 1991 and 1996. Eligible participants were singleton infants, born at term with uncomplicated vaginal births, weighing ≥3 kg, and free of major health complications. Infants without iron deficiency anemia (IDA) at baseline were enrolled in a clinical trial of iron supplementation through random assignment to high- or low-dose iron or usual nutrition until 12 mo of age. Infants with IDA were treated with iron and enrolled in an observational study of neurodevelopment, along with a group of nonanemic controls. There were 1798 infants enrolled, of whom 1790 had a visit at 1 y of age. Among them, 49%, 62%, and 61% were followed for anthropometric and developmental outcomes at ages 5, 10, and 16 y, respectively (**Supplemental Figure 1**). The 5-y follow-up was purposely targeted to the high- and no-iron supplementation subgroups only, because of an unanticipated budget restriction. In further follow-up waves, 73% of attrition was due to household moves or inability to locate participants, whereas only 19% was related to participation refusal or parental work schedule conflict. The cardiometabolic health study was conducted in a subset of 679 participants aged 16/17 y (median age 16.8 y) (19), who had the most complete data from previous assessments, preferably including follow-up at 5 y of age. For the vitamin D study, we selected a random group of participants among those who had a stored serum sample available at age 1 y (baseline) plus ≥1 additional visit through the developmental study at ages 5, 10, or 16 y; or an assessment through the cardiometabolic health study at age 16/17 y (Supplemental Figure 1). When participants with a baseline sample were assessed both through the developmental study at 16 y and the cardiometabolic health study at 16/17 y, we selected the cardiometabolic study visit. Compared with children excluded from the vitamin D substudy, those included were more likely to be male and to have been in the no-iron supplementation group in infancy, had longer exclusive breastfeeding, and had higher BMI for age (**Supplemental Table 1**). There were no substantial differences with respect to maternal education, socioeconomic status (SES), or metabolic parameters in adolescence.

The institutional review boards of the University of Chile Institute of Nutrition and Food Technology (INTA), the University of Michigan, and the University of California, San Diego, approved all study procedures. Parents provided written informed consent for their children to participate; children additionally provided written assent beginning at 10 y of age.

Data collection

Birth weight in grams, birth length in centimeters, and gestational age in weeks were recorded from hospital records. Gestational age was determined by the date of the mother's last menstrual period. At enrollment, study personnel collected information from parents on household socioeconomic indicators. They also collected information on breastfeeding duration from mothers at weekly study visits, which took place from enrollment until the infant was 12 mo old. These data included the dates of the first bottle feeding and of the last breastfeeding. For infants who had already been bottle-fed at enrollment, mothers were asked to recall the date of the first bottle feeding. In this population, breast milk was not fed by bottle; therefore, bottle feeding was equivalent to giving infant formula or cow milk.

Anthropometric measurements were collected at INTA by trained physician investigators using standardized techniques. At 1 y of age, weight was measured unclothed to the nearest 10 g, and length was measured to the nearest millimeter using a recumbent length board. At ages 5, 10, and 16 y, weight was measured to the nearest 100 g using a Seca scale, and height was measured to the nearest millimeter using a Holtain stadiometer. All measures were obtained in duplicate and averaged. On rare occasions when the difference between the first 2 exceeded 0.3 kg or 0.5 cm, a third measurement was taken and all 3 were averaged.

In the subset of participants in the adolescent cardiometabolic health study at 16/17 y, we measured weight and height following the same procedures; waist circumference (WC) was measured to the nearest millimeter at the highest point of the iliac crest using an inextensible metric tape. Blood pressure was assessed in the nondominant arm using a standard mercury sphygmomanometer in the morning after 15 min at rest, as recommended by the National High Blood Pressure Education Working Group (20); the mean of second and third measurements was used for analysis. Next, we performed body composition assessments with use of DXA (Lunar Prodigy Corp) that quantified percentage total fat mass, truncal fat mass, and total lean mass. A blood sample was obtained after a 12-h overnight fast for analysis of metabolic biomarkers.

Laboratory methods

Within 2 y of completing infancy data collection (late 1990s), aliquots of serum obtained at age 1 y were transported frozen to the University of Michigan where they were cryostored at −80°C until the time of analysis. Total 25-hydroxyvitamin D [25(OH)D] was quantified at Heartland Assays using the DiaSorin LIAISON 25-OH Vitamin D Total assay (Diasorin, Inc) (21). This method uses a direct competitive chemiluminescence immunoassay that is cospecific for 25-hydroxyvitamin D-3 and D-2. The assay has a sensitivity of 6.26 nmol/L and inter-and intra-assay CVs of 11.2% and 8.1%, respectively; recovery of endogenous 25(OH)D is 100% (22).

Cardiometabolic risk biomarkers at age 16/17 y were quantified in the fasting blood samples collected through the

adolescence cardiometabolic health study. Serum triglycerides and HDL cholesterol were determined using the dry chemical method (Vitros; Johnson & Johnson, Clinical Diagnostics Inc); total glucose was quantified using an enzymatic-colorimetric test (QCA S.A.); and insulin was measured using RIA (DCP Diagnostic Products Corp) with intra-assay CV of 5.1% and interassay CV of 7.1% for 14.4 μUI/mL, and a sensitivity of 1.2 μUI/mL. We also quantified hormones related to appetite and metabolism. Serum leptin, ghrelin, and adiponectin were measured via RIA using kits with high sensitivity (23) from Linco Research, Inc; orexin-A was measured using a kit from Peninsula Laboratories, Inc.

Definition of outcomes

We used available anthropometric data obtained at the 1-, 5-, and 10-y developmental evaluations. To represent the 16/17-y point, we used data from the cardiometabolic health study among their participants, or from the 16-y developmental evaluations otherwise. BMI was calculated as kg/m^2. We estimated sex- and age-standardized z-scores for BMI (BMIZ) at age 1 y using the WHO Child Growth Standards for children aged <5 y (24); and, at ages 5, 10, and 16/17 y, using the WHO Growth Reference for children aged 5–19 y (25). The primary outcome was change in BMIZ from age 1 y to 5, 10, and 16 y. In addition, among participants in the cardiometabolic health study, we considered percentage fat, lean, and truncal fat masses at age 16/17 y, based on results from the DXA scan.

Secondary outcomes encompassed MetS and its components as previously reported (19). We calculated a continuous metabolic score at 16/17 y for participants in the cardiometabolic health study, as previously recommended for children and adolescents (26) and implemented in this population (27). This score was based on WC, serum triglycerides, HDL cholesterol, mean arterial pressure, and insulin resistance assessed using HOMA-IR (28). We first calculated a score for each of these components by regressing log-transformed values of each component on sex and log-transformed age using linear regression and obtaining standardized residuals. The overall metabolic score was then calculated as the mean of the component scores, using the negative of HDL. Higher values of this score indicate poorer metabolic health. Last, we assessed the metabolic hormones leptin, ghrelin, and adiponectin, in addition to orexin-A at age 16/17 y, as continuous outcomes.

Definition of exposures

The exposure of interest was 25(OH)D concentration (in nanomoles per liter) at age 1 y. We evaluated 25(OH)D both continuously and according to categories of vitamin D status to allow for nonlinear associations with the outcomes. There is no consensus on a definition of VDD in infants according to 25(OH)D concentrations (29). Cutpoints previously used include <50 nmol/L (30) and <75 nmol/L (31), but 25(OH)D concentrations associated with adverse clinical outcomes can depend on ethnicity and other factors. We thus categorized serum 25(OH)D as <50 nmol/L, 50 to <75 nmol/L, or ≥75 nmol/L to facilitate comparability between studies. We did not use the cutpoint that the National Academy of Medicine recommends to define VDD [25(OH)D <30 nmol/L] because there were very few infants under it (32). We further overcame the lack of a consistent VDD definition in infancy by presenting estimates per unit difference in 25(OH)D when there was evidence of linearity in the associations.

Covariates

Covariates were sociodemographic and anthropometric characteristics measured in infancy. We categorized birth length and weight as average or large for gestational age, as recommended by the INTERGROWTH 21st standards for newborn size (33). Large-for-gestational-age was defined as ≥90th percentile; we did not consider a category of small-for-gestational-age because birth weight ≥3 kg was an eligibility criterion for recruitment and there were no children ≤10th percentile for birth weight. However, 5 children with birth length ≤10th percentile were classified as average-for-gestational-age. We categorized breastfeeding as <6 mo, ≥6 mo of mixed bottle/breastfeeding, or ≥6 mo of exclusive breastfeeding. Iron supplementation was categorized as low-dose, high-dose, or none. Maternal education was also considered categorically as incomplete elementary, complete elementary, incomplete secondary, complete secondary, or postsecondary education. Finally, we measured SES using the modified Graffar index, consisting of 13 items related to family structure, education and employment, crowding and housing condition, and ownership of assets (34). The index ranges from 0 to 65, where higher values indicate lower SES; we categorized this variable into quintiles for analysis.

Data analysis

The final analytic sample consisted of 306 children for whom information was available on both serum 25(OH)D and ≥1 follow-up assessment. First, we examined the associations between baseline sociodemographic characteristics and 25(OH)D. We compared mean ± SD 25(OH)D by categories of these characteristics and estimated the statistical significance of the differences in linear regression models using serum 25(OH)D as a continuous outcome. We also estimated prevalence ratios and 95% CI of 25(OH)D <50 nmol/L across levels of each characteristic using generalized estimating equations with the binomial distribution. For ordinal variables, we assessed linear trend using the Wald test; for the breastfeeding variable, we conducted a test of general association using the χ^2 score statistic.

The primary analytic strategy for BMIZ consisted of modeling average growth curves by categories of vitamin D status with use of mixed models with restricted cubic splines (35). These models do not require the same number of measurements per child during follow-up; thus, all available measurements were included. Spline terms for age were constructed by placing 4 knots at the 1-, 5-, 10-, and 16/17-y points of the distribution, respectively. BMIZ was the continuous outcome in each model, whereas predictors included infancy 25(OH)D categories, linear and 2 spline terms for age, interaction terms between vitamin D categories and the age terms, and adjustment covariates (sex, maternal education, breastfeeding, and the SES Graffar index). Random effects were specified for the intercept and the linear term for age, to account for the correlation of within-child

measurements in the estimation of variances. From these models, we estimated adjusted differences in BMIZ change from age 1 y to ages 5, 10, and 16 y between infancy vitamin D status categories. In supplemental analyses, we estimated adjusted differences in BMI change from age 1 y to ages 1, 5, and 16 y following an analogous approach. Also, we examined the prevalence of BMIZ categories < -1, -1 to 1, 1 to <2, or ≥ 2 at 16/17 y by vitamin D status categories in infancy.

Analyses of continuous outcomes assessed at 16/17 y (body composition, the metabolic score and its components, and hormones) included 227 participants in the adolescence cardiometabolic health study who had infancy vitamin D measurements. We compared the distribution of each outcome by categories of vitamin D status using means ± SD. Crude and adjusted mean differences and 95% CI were estimated with use of linear regression models. In addition, we estimated the associations per 25-nmol/L difference in 25(OH)D, assuming linearity. Adjustment variables included sex, maternal education, breastfeeding, SES (Graffar index), and BMIZ at baseline (age 1 y). The models for body composition variables were also adjusted for decimal age at body composition assessment to decrease extraneous variability.

For all models, including mixed splines and multivariable linear regression, we specified empirical SEs, because they are robust to heteroscedasticity and nonnormality (36). P values <0.05 were considered statistically significant. All analyses were conducted using Statistical Analysis Software version 9.4 (SAS Institute).

Results

The mean ± SD age of infants was 12.8 ± 2.8 mo; 57.5% of infants were boys. Mean ± SD 25(OH)D concentration was 80.7 ± 33.1 nmol/L; the prevalence of 25(OH)D <50 nmol/L was 11.1%. Serum 25(OH)D concentration at age 1 y was not significantly related to the sociodemographic, neonatal, or nutritional characteristics examined (**Table 1**).

Vitamin D and BMI change and body composition through age 16/17 y

Vitamin D status categories in infancy were not significantly related to BMIZ after adjustment; nevertheless, continuous 25(OH)D concentrations were inversely associated with BMIZ at 5 y of age (**Table 2**, **Supplemental Figure 2**). Every 25 nmol/L difference in 25(OH)D was associated with an adjusted 0.11 units lower BMIZ at age 5 y (95% CI: −0.20, −0.03; $P = 0.01$) and with 0.09 units less BMIZ change from age 1 y (95% CI: −0.17, −0.01; $P = 0.02$). Similarly, every 25 nmol/L difference in serum 25(OH)D was associated with an adjusted 0.18 kg/m^2 lower BMI at age 5 y (95% CI: −0.34, −0.03; $P = 0.02$) and with 0.15 kg/m^2 less BMI change from age 1 y (95% CI: −0.30, −0.01; $P = 0.04$) (**Supplemental Table 2**, **Supplemental Figure 3**). At 16/17 y, the proportion (percentage) of children in BMIZ categories < -1, -1 to 1, 1 to <2, or ≥ 2 was, respectively, 7.1, 52.5, 23.9, and 16.4. Prevalence (percentage) of BMIZ < -1 in the 25(OH)D ≥75 nmol/L compared with <50 nmol/L categories was, respectively, 10.2 and 3.0; whereas prevalence (percentage) of BMIZ >2 was 16.1 and 24.2 (**Supplemental Table 3**).

Infancy 25(OH)D <50 nmol/L was associated with an adjusted mean 3.9 points higher percentage fat mass (95% CI: 0.3, 7.5; $P = 0.03$) at age 16/17 y, compared with 25(OH)D ≥75 nmol/L (**Table 3**); similarly, every 25 nmol/L difference in 25(OH)D was associated with a mean 1.3 point lower body fat percentage (95% CI: −2.2, −0.4; $P = 0.005$). Although percentage lean or truncal fat mass did not differ significantly between vitamin D status categories after adjustment, every 25 nmol/L difference in 25(OH)D was related to an adjusted mean 1.4 point higher percentage lean mass (95% CI: 0.4, 2.3; $P = 0.005$) and to a 1.0 point lower percentage truncal mass (95% CI: −1.7, −0.4; $P = 0.002$) at age 16/17 y.

MetS score at age 16/17 y

Neither the overall MetS score nor its components at age 16/17 y differed significantly between vitamin D status categories (**Table 4**); nonetheless, every 25 nmol/L difference in 25(OH)D in infancy was related to an adjusted 0.03 units lower overall MetS score (95% CI: −0.05, −0.01; $P = 0.01$) and a 0.02 units lower WC score (95% CI: −0.03, −0.002; $P = 0.02$). There was a borderline statistically significant inverse association between 25(OH)D as a continuous exposure and the HOMA-IR score (−0.06 per 25 nmol/L; 95% CI: −0.12, −0.001; $P = 0.045$).

Appetite and metabolic hormones

Mean ghrelin and adiponectin concentrations at 16/17 y were higher in the 25(OH)D ≥75 nmol/L compared with the <50 nmol/L category; however, the associations were not statistically significant (**Supplemental Table 4**).

Discussion

In this prospective cohort study of Chilean children, vitamin D serostatus at age 1 y was inversely associated with BMIZ change in childhood and with body fat mass percentage at age 16/17 y in a linear manner. In addition, vitamin D concentrations in infancy were inversely related to the MetS score at age 16/17 y, primarily through inverse associations with WC and HOMA-IR.

Most previous studies of vitamin D serostatus and adiposity were cross-sectional (10). Because vitamin D is fat-soluble and can be sequestered out of the blood into adipose tissue (11), prospective studies are necessary to minimize reverse-causation bias. Our findings are largely consistent with evidence from longitudinal studies of vitamin D status and body composition in children, although previous investigations have not focused on exposure in infancy. In the Generation R cohort, severe maternal VDD (25[OH]D <25 nmol/L) was associated with higher fat and lower lean mass percentages in offspring at age 6 y (14). In the Southampton Women's Survey, 25(OH)D at 34 wk gestation as a continuous exposure was positively associated with the offspring's fat mass at birth, but inversely associated with fat mass at ages 4 and 6 y (12). In the Bogotá School Children Cohort, 25[OH]D <50 nmol/L at ages 5–12 y was positively related to BMI and WC change over a median 30 mo of follow-up (16). Also, in Brazilian children aged <10 y, 25(OH)D <75 nmol/L was positively related to BMI change over a median of 4.6 y of follow-up in those with a fat mass and obesity-associated (FTO)

TABLE 1 Mean (± SD) serum 25(OH)D concentrations and prevalence of 25(OH)D <50 nmol/L at age 1 y by categories of sociodemographic variables in infants from Santiago, Chile[1]

		25(OH)D concentration, nmol/L			25(OH)D <50 nmol/L		
Characteristic	*n*	Mean ± SD	Mean difference (95% CI)[2]	*P*	Prevalence (%)	Prevalence ratio (95% CI)[3]	*P*
Sex				0.65[4]			0.84[4]
Female	130	81.7 ± 39.1	Reference		11.5	1.00	
Male	176	79.9 ± 27.9	−1.8 (−9.7, 6.0)		10.8	0.94 (0.49, 1.77)	
Birth length				0.30			0.73
Average for gestational age[5]	206	79.2 ± 29.2	Reference		10.7	1.00	
Large for gestational age	100	83.8 ± 39.9	4.6 (−4.1, 13.3)		12.0	1.12 (0.58, 2.18)	
Birth weight				0.14			0.82
Average for gestational age	220	79.0 ± 33.6	Reference		11.4	1.00	
Large for gestational age	86	84.9 ± 31.5	5.9 (−2.0, 13.9)		10.5	0.92 (0.45, 1.89)	
Breastfeeding				0.17[6]			0.57[6]
Breastfeeding < 6 mo	107	79.1 ± 23.8	Reference		8.4	1.00	
Mixed bottle/breastfeeding ≥6 mo	101	86.7 ± 45.0	7.6 (−2.2, 17.4)		10.9	1.29 (0.56, 2.99)	
Exclusive breastfeeding ≥6 mo	92	76.8 ± 26.4	−2.3 (−9.3, 4.7)		13.0	1.55 (0.68, 3.51)	
Missing	1						
Iron supplementation				0.18			0.23
None	169	78.3 ± 32.1	Reference		11.8	1.00	
High dose	97	84.6 ± 37.6	6.3 (−2.6, 15.1)		10.3	0.87 (0.43, 1.78)	
Low dose	26	86.5 ± 23.8	8.2 (−2.0, 18.4)		3.8	0.33 (0.05, 2.32)	
Missing	14	70.9 ± 22.5			21.4		
Maternal education				0.13 (trend)[7]			0.40 (trend)[7]
Incomplete elementary	58	81.2 ± 23.5	Reference		12.1	1.00	
Complete elementary	50	75.6 ± 25.7	−5.6 (−14.8, 3.7)		14.0	1.16 (0.44, 3.08)	
Incomplete secondary	101	76.0 ± 27.1	−5.2 (−13.2, 2.7)		11.9	0.98 (0.41, 2.36)	
Complete secondary	68	83.8 ± 29.6	2.6 (−6.6, 11.8)		7.4	0.61 (0.20, 1.82)	
Postsecondary	29	97.2 ± 67.0	16.0 (−8.7, 40.7)		10.3	0.86 (0.24, 3.07)	
Graffar index[8]				0.50 (trend)			0.17 (trend)
Q1 (high SES)	56	85.9 ± 42.3	Reference		5.4	1.00	
Q2	54	78.9 ± 38.9	−7.0 (−22.0, 8.0)		9.3	1.73 (0.43, 6.88)	
Q3	69	79.6 ± 28.4	−6.4 (−19.2, 6.5)		15.9	2.98 (0.87, 10.15)	
Q4	62	78.2 ± 26.4	−7.7 (−20.5, 5.1)		9.7	1.81 (0.47, 6.89)	
Q5 (low SES)	65	81.1 ± 29.5	−4.8 (−17.9, 8.3)		13.8	2.58 (0.74, 9.08)	

[1]Q, quartile; SES, socioeconomic status; 25(OH)D, 25-hydroxyvitamin D.
[2]From linear regression models with vitamin D as the outcome and indicator variables for levels of each characteristic as predictors. Robust estimates of variance were specified in each model.
[3]From generalized estimating equations with the binomial distribution, the log-link, and robust variances.
[4]Wald test.
[5]Includes 5 children who were short for gestational age.
[6]χ^2 score statistic.
[7]Wald test for a variable representing ordinal categories of the characteristic that was introduced as a continuous covariate in regression models with vitamin D or 25(OH)D <50 nmol/L as the outcome.
[8]Index of SES that includes number of people in the home, presence of the father, head of household's education level and employment, home ownership, type and size of housing, running water supply, ownership of household appliances, and crowding (34). Higher values indicate lower socioeconomic status.

protein gene variant (37). Evidence from intervention studies is mixed. In a randomized controlled trial (RCT) in Lebanese girls aged 10–17 y, supplementation with 1400 or 14,000 IU/wk vitamin D-3 resulted in increased lean body mass after 1 y (38). By contrast, in 2 RCTs in children aged 9–15 y, supplementation with 300–800 IU/d vitamin D-3 for ≤6 mo did not affect weight or BMI (39). Also, in a school-based RCT in 10 y-old Chinese girls, milk fortification with calcium plus ∼130 IU/d vitamin D-3 for 2 y was not significantly related to BMI change compared with calcium alone (40). Results from RCTs could have varied due to differences in age, pubertal status, duration and dose of the intervention, and baseline prevalence of low vitamin D serostatus. The long-term effects of vitamin D in infancy have not been examined in RCTs; thus, our results are not directly comparable with those available from intervention studies.

Our study extends the understanding of the potential role of vitamin D on body composition by focusing on exposure during infancy and assessing long-term outcomes. The mechanisms through which vitamin D might affect adiposity are not completely understood. The vitamin D receptor is expressed in adipocytes (41); vitamin D might suppress key proteins involved in adipocyte differentiation, such as lipoprotein lipase, sterol-regulatory element-binding protein 1, and peroxisome proliferator–activated receptor γ (42, 43). In addition, vitamin

TABLE 2 Estimated change in BMI-for-age *z*-score from ages 1 to 16 y according to serum 25-hydroxyvitamin D [25(OH)D] concentrations at age 1 y in children from Santiago, Chile

	25(OH)D, nmol/L			
Estimated BMI-for-age *z*-score[1]	<50 ($n = 34$)	50 to <75 ($n = 118$)	≥75 ($n = 154$)	Per 25-nmol/L difference (95% CI)
Age 1 y				
Mean ± SE	0.91 ± 0.18	0.99 ± 0.08	0.74 ± 0.08	
Adjusted difference (95% CI)[2]	0.19 (−0.19, 0.56)	0.21 (−0.01, 0.43)	Reference	−0.02 (−0.09, 0.05)
Age 5 y				
Mean ± SE	1.42 ± 0.21	1.16 ± 0.11	0.87 ± 0.11	
Adjusted difference (95% CI)[2]	0.56 (0.11, 1.01)	0.26 (−0.05, 0.56)	Reference	−0.11 (−0.20, −0.03)
Change between ages 1-5 y				
Mean (± SE)	0.50 ± 0.21	0.17 ± 0.10	0.13 ± 0.10	
Adjusted difference (95% CI)[2]	0.38 (−0.08, 0.83)	0.05 (−0.24, 0.34)	Reference	−0.09 (−0.17, −0.01)
Age 10 y				
Mean ± SE	1.44 ± 0.19	1.21 ± 0.10	0.99 ± 0.10	
Adjusted difference (95% CI)	0.47 (0.05, 0.89)	0.19 (−0.09, 0.47)	Reference	−0.08 (−0.17, 0.01)
Change between ages 1-10 y				
Mean ± SE	0.53 ± 0.21	0.23 ± 0.11	0.25 ± 0.10	
Adjusted difference (95% CI)	0.28 (−0.18, 0.74)	−0.02 (−0.31, 0.28)	Reference	−0.06 (−0.15, 0.02)
Age 16 y				
Mean ± SE	1.15 ± 0.21	0.87 ± 0.09	0.65 ± 0.10	
Adjusted difference (95% CI)	0.51 (0.05, 0.97)	0.18 (−0.08, 0.45)	Reference	−0.06 (−0.16, 0.03)
Change between ages 1-16 y				
Mean ± SE	0.24 ± 0.26	−0.12 ± 0.10	−0.09 ± 0.11	
Adjusted difference (95% CI)	0.32 (−0.23, 0.88)	−0.03 (−0.32, 0.26)	Reference	−0.04 (−0.13, 0.05)

[1] According to the WHO Child Growth Standards for children aged <5 y (24) and the WHO Growth Reference for children aged 5–19 y (25).

[2] From linear mixed models with BMI-for-age *z*-score as the outcome and linear and cubic spline terms for age, vitamin D status indicators, and interaction terms for age and vitamin D categories as predictors. Adjusted estimates included as covariates sex, maternal education, breastfeeding, and the socioeconomic status Graffar index. Complete case analysis $n = 305$.

D might affect mitochondrial uncoupling (44, 45) and apoptosis of adipocytes through activation of calcium-dependent protease enzymes (46). Of note, the association of vitamin D serostatus with BMIZ change was restricted to age 5 y. Some of the differences in BMI distribution by vitamin D status in infancy could have persisted through age 16/17 y; for example, the adolescence prevalences of BMIZ >1 to 2 and ≥2, cutpoints that conventionally define overweight and obesity, were higher in children with 25(OH)D <50 nmol/L than in those with 25(OH)D ≥75 nmol/L. However, the prevalence

TABLE 3 Body composition at age 16/17 y according to serum 25(OH)D concentrations at age 1 y in children from Santiago, Chile[1]

	25(OH)D, nmol/L			
Body compartment	< 50	50 to < 75	≥ 75	Per 25-nmol/L difference (95% CI)
Percentage fat mass				
n	30	98	97	
Mean (± SD)	31.7 ± 11.1	29.6 ± 10.4	26.7 ± 11.5	
Unadjusted difference (95% CI)[2]	5.0 (0.4, 9.5)	2.9 (−0.2, 5.9)	Reference	−1.6 (−3.0, −0.1)
Adjusted difference (95% CI)[3]	3.9 (0.3, 7.5)	1.8 (−0.6, 4.3)	Reference	−1.3 (−2.2, −0.4)
Percentage lean body mass				
n	30	98	97	
Mean (± SD)	64.9 ± 11.1	67.1 ± 10.7	70.2 ± 11.9	
Unadjusted difference (95% CI)	−5.2 (−9.8, −0.7)	−3.1 (−6.3, 0.0)	Reference	1.6 (0.1, 3.2)
Adjusted difference (95% CI)	−4.2 (−7.8, 0.6)	−2.1 (−4.6, 0.4)	Reference	1.4 (0.4, 2.3)
Percentage truncal fat mass				
n	30	98	97	
Mean (± SD)	51.3 ± 5.2	50.1 ± 5.2	49.7 ± 6.1	
Unadjusted difference (95% CI)	1.6 (−0.5, 3.8)	0.4 (−1.2, 2.0)	Reference	−0.9 (−1.5, −0.2)
Adjusted difference (95% CI)	1.7 (−0.4, 3.8)	1.2 (−0.4, 2.8)	Reference	−1.0 (−1.7, −0.4)

[1] Measured with DXA. 25(OH)D, 25-hydroxyvitamin D.

[2] From linear regression models with each body compartment as the outcome and indicator variables for vitamin D categories as predictors. Robust variances were specified in all models.

[3] From multivariable linear regression models adjusted for sex, maternal education, breastfeeding, Graffar index, BMI *z*-score at age 1 y (baseline), and age at body composition measurement.

TABLE 4 Metabolic syndrome score and its components at age 16/17 y according to serum 25-hydroxyvitamin D [25(OH)D] concentrations at age 1 y in children from Santiago, Chile[1]

Metabolic syndrome score component	25(OH)D, nmol/L			Per 25-nmol/L difference (95% CI)
	<50	50 to <75	≥75	
Overall score				
n	30	99	98	
Mean (± SD)	0.04 ± 0.26	0.02 ± 0.21	−0.03 ± 0.20	
Unadjusted difference (95% CI)[2]	0.07 (−0.03, 0.16)	0.04 (−0.01, 0.10)	Reference	−0.03 (−0.05, 0.00)
Adjusted difference (95% CI)[3]	0.07 (−0.03, 0.17)	0.06 (0.00, 0.11)	Reference	−0.03 (−0.05, −0.01)
Waist circumference score				
n	30	99	98	
Mean (± SD)	0.02 ± 0.16	0.01 ± 0.12	−0.02 ± 0.15	
Unadjusted difference (95% CI)	0.04 (−0.02, 0.10)	0.03 (−0.01, 0.06)	Reference	−0.02 (−0.03, −0.002)
Adjusted difference (95% CI)	0.03 (−0.03, 0.09)	0.03 (−0.01, 0.06)	Reference	−0.02 (−0.03, −0.002)
Triglycerides score				
n	30	99	98	
Mean (± SD)	0.00 ± 0.48	0.06 ± 0.44	−0.06 ± 0.40	
Unadjusted difference (95% CI)	0.06 (−0.13, 0.25)	0.13 (0.01, 0.24)	Reference	−0.03 (−0.09, 0.02)
Adjusted difference (95% CI)	0.08 (−0.11, 0.27)	0.15 (0.03, 0.26)	Reference	−0.04 (−0.09, 0.02)
HDL cholesterol score				
n	30	99	98	
Mean (± SD)	−0.01 ± 0.29	−0.02 ± 0.25	0.02 ± 0.23	
Unadjusted difference (95% CI)	−0.03 (−0.14, 0.08)	−0.04 (−0.11, 0.02)	Reference	0.02 (0.00, 0.05)
Adjusted difference (95% CI)	−0.03 (−0.14, 0.08)	−0.04 (−0.11, 0.02)	Reference	0.02 (0.00, 0.05)
Mean arterial pressure score				
n	30	99	98	
Mean (± SD)	0.00 ± 0.08	−0.01 ± 0.08	0.00 ± 0.08	
Unadjusted difference (95% CI)	0.00 (−0.03, 0.03)	−0.01 (−0.03, 0.01)	Reference	0.00 (−0.01, 0.01)
Adjusted difference (95% CI)	0.00 (−0.03, 0.03)	−0.01 (−0.03, 0.01)	Reference	0.00 (−0.01, 0.00)
HOMA-IR score				
n	30	99	98	
Mean (± SD)	0.16 ± 0.63	−0.01 ± 0.59	−0.04 ± 0.61	
Unadjusted difference (95% CI)	0.20 (−0.05, 0.45)	0.03 (−0.14, 0.20)	Reference	−0.05 (−0.11, 0.01)
Adjusted difference (95% CI)	0.20 (−0.05, 0.45)	0.07 (−0.09, 0.23)	Reference	−0.06 (−0.12, −0.001)

[1]Component scores (waist circumference, serum triglycerides, serum HDL cholesterol, mean arterial pressure, and HOMA-IR) were created by regressing each log-transformed component on sex and log-transformed age in linear regression models and obtaining standardized residuals. The overall score was calculated as the average of the 5 component scores, using the negative of the HDL cholesterol score.

[2]From linear regression models with each metabolic syndrome component as the outcome and indicator variables for vitamin D categories as predictors. Robust variances were specified in all models.

[3]From multivariable linear regression models adjusted for maternal education, breastfeeding, Graffar index, and BMI *z*-score at age 1 y (baseline).

of BMIZ < −1, a cutpoint for underweight, was lower in the 25(OH)D <50 nmol/L than in the ≥75 nmol/L category; thus, an effect of high vitamin D on underweight cannot be discarded.

We found an inverse relation between infancy 25(OH)D and MetS in adolescence, through WC and HOMA-IR. Prospective studies of vitamin D and metabolic health in children are scant. In the Avon Longitudinal Study, maternal 25(OH)D was not consistently related to metabolic outcomes through adolescence (15); however, serum 25(OH)D in middle childhood was positively associated with HDL cholesterol and inversely related to fasting insulin at age 15 y (17). In addition, an RCT in obese adolescents aged 14 y found that supplementation with 4000 IU/d vitamin D-3 for 6 mo resulted in significant reductions in HOMA-IR and the leptin-to-adiponectin ratio (47).

The pathways through which vitamin D could impact MetS are unclear (48). Vitamin D might decrease insulin resistance through improved β-cell function, insulin action, and reduced systemic inflammation; these effects might be mediated through calcium homeostasis (41, 49). Vitamin D might also improve glucose uptake when it is inhibited by free fatty acids (50) and block signaling pathways in skeletal muscle that mediate diet-related metabolic impairments (51).

Our study has important strengths. The prospective design allowed us to assess the temporal association between vitamin D status and BMI change, minimizing reverse causation bias. Very few previous studies have had a comparably long follow-up of participants through adolescence; and none focused on exposure to low vitamin D serostatus in infancy. We collected data from children in a region where childhood obesity and metabolic abnormalities are increasing public health challenges. We had an opportunity to describe the vitamin D status of infants from Chile in the 1990s, a period of accelerated economic development in that country that could have influenced the incidence of chronic disease risk factors.

Some limitations should also be noted. First, because we only measured 25(OH)D at age 1 y, we could not assess the role of changes in this exposure during childhood. Baseline vitamin D

status might represent cumulative exposure throughout follow-up. A study in adults that measured 25(OH)D repeatedly for ≤14 y found a moderate within-subject correlation, suggesting that a single baseline measurement can provide a reasonable estimate of long-term exposure (52). If this were the case in our population, we could not necessarily attribute a potential effect of vitamin D to exposure during infancy. Second, any characteristics that affect both vitamin D in infancy and the development of adiposity in childhood, and which are not consequences of infancy vitamin D status, could have confounded the associations. Outdoor physical activity is one example and we were unable to adjust for. Notwithstanding, it is unlikely that physical activity could influence vitamin D status during infancy. Still, we cannot rule out residual confounding by other unmeasured characteristics that might affect both infancy vitamin D and cardiometabolic health, including genetics. Third, generalizability could be restricted to settings with comparable distributions of vitamin D serostatus and cardiometabolic outcomes. Finally, there were some differences in measured characteristics between children included in the vitamin D substudy and the rest of the cohort, partly due to attrition. Bias could have affected the results if selection into the vitamin D substudy was a common effect of *1*) the exposure [serum 25(OH)D concentrations] or a cause of the exposure, and *2*) the outcome (e.g., BMI, metabolic score) or a cause of the outcome (53). Differences in breastfeeding duration—a possible cause of exposure—and BMI (an outcome) between children included in compared with excluded from the vitamin D substudy could illustrate a potential selection bias scenario; nevertheless, the direction and magnitude of bias cannot be consistently predicted without further assumptions.

In conclusion, vitamin D serostatus in infancy was inversely associated with both the development of adiposity and MetS by age 16/17 y. Randomized intervention studies are warranted to ascertain the effect of improving vitamin D status early in life on cardiometabolic health throughout adolescence.

The authors' responsibilities were as follows—JG: analyzed the data; EV, KSF: designed the research; SG, BL, RB: conducted the research; JG, EV: wrote the paper and had primary responsibility for final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

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