Determinants of 25(OH)D Sufficiency in Obese Minority Children: Selecting Outcome Measures and Analytic Approaches

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Objective To highlight complexities in defining vitamin D sufficiency in children.

Study design Serum 25-(OH) vitamin D [25(OH)D] levels from 140 healthy obese children age 6 to 21 years living in the inner city were compared with multiple health outcome measures, including bone biomarkers and cardiovascular risk factors. Several statistical analytic approaches were used, including Pearson correlation, analysis of covariance (ANCOVA), and "hockey stick" regression modeling.

Results Potential threshold levels for vitamin D sufficiency varied by outcome variable and analytic approach. Only systolic blood pressure (SBP) was significantly correlated with 25(OH)D (r = -0.261; P = .038). ANCOVA revealed that SBP and triglyceride levels were statistically significant in the test groups [25(OH)D <10, <15 and <20 ng/mL] compared with the reference group [25(OH)D >25 ng/mL]. ANCOVA also showed that only children with severe vitamin D deficiency [25(OH)D <10 ng/mL] had significantly higher parathyroid hormone levels (Δ = 15; P = .0334). Hockey stick model regression analyses found evidence of a threshold level in SBP, with a 25(OH)D breakpoint of 27 ng/mL, along with a 25(OH)D breakpoint of 18 ng/mL for triglycerides, but no relationship between 25(OH)D and parathyroid hormone.

Conclusions Defining vitamin D sufficiency should take into account different vitamin D–related health outcome measures and analytic methodologies. *(J Pediatr 2011;158:930-4)*.

Measurement of serum 25-hydroxyvitamin D [25(OH)D] is used to assess vitamin D status. Traditionally, determination of vitamin D sufficiency was based primarily on the prevention of rickets in children or osteopenia in adults.[1,2] Serum calcium, phosphate, alkaline phosphatase, and parathyroid hormone (PTH) levels are frequently measured as indicators of adequate serum 25(OH)D concentration when evaluating bone health. In particular, an inverse relationship between serum 25(OH)D and PTH levels has been observed. In adults, the serum concentration of 25(OH)D considered to indicate sufficiency (30 ng/mL) is based on the threshold level at which a higher 25(OH) D level has no further effect on PTH level.[3-5] The current vitamin D sufficiency level for children of >20 ng/mL defined by the Pediatric Endocrine Society[1,6] is also based mainly on the relationship of serum 25-(OH)D level and the prevention or treatment of rickets.

The identification of vitamin D receptors in different tissues led to the discovery of many nonskeletal roles for 1,25-dihydroxyvitamin D, including immune modulation and antiproliferative and antineoplastic activities.[7] Low vitamin D levels also have been linked to dyslipidemia, hypertension, and diabetes mellitus.[8-10]

Epidemiologic surveys indicate that 25(OH)D levels are generally low in the US population,[4,10-13] especially in obese people.[14,15] The significance of these results is not clear, however. Is the interpretation of a serum 25(OH)D level the same in a child as in an adult, or in an obese person compared with a lean person, or in all ethnic or racial groups? Stated alternatively and germane to this study, because vitamin D is a fat-soluble vitamin believed to be stored in fat cells,[16,17] does serum 25(OH)D level mean the same in obese and nonobese children? What health outcome measures should be used to determine the optimal serum 25(OH)D concentration? To date, few studies in children have attempted to determine the threshold of 25(OH)D for nonskeletal effects.[18]

Once possible health outcomes of vitamin D status are selected for investigation, how should the data be analyzed? Most clinical studies of obese children are observational, with only correlations calculated. Analogous to choosing health outcome measures of the effects of vitamin D, should other analytic approaches be considered?

The present study aimed to define vitamin D sufficiency in obese minority youths. We used several methods to evaluate the relationship between 25(OH)D level and various skeletal and nonskeletal measures, including cardiovascular disease risk factors such as hypertension and hyperlipidemia, in a group of obese Hispanic and African-American children and adolescents living in the inner city.

25(OH)D	25-(OH) vitamin D
ANCOVA	Analysis of covariance
BMI	Body mass index
PTH	Parathyroid hormone
QUICKI	Quantitative Insulin Sensitivity Check Index
SBP	Systolic blood pressure
TG	Triglycerides

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Methods

This is a cross-sectional retrospective data analysis approved by the Montefiore Medical Center's Institutional Review Board. Subjects were referred to our Pediatric Endocrine Clinic for evaluation of obesity. For this study, the inclusion criteria were obesity and age between 6 and 21 years. Those with a chronic disease that affects vitamin D absorption and metabolism or any underlying disease that could cause hypertension were excluded. Patients with type 2 diabetes mellitus were excluded as well.

Anthropomorphic and bone-related biochemical measures, including calcium, phosphate, alkaline phosphatase, PTH, and 25(OH)D levels, and obesity-related metabolic measures were collected for each patient (Table I). Obesity was defined as body mass index (BMI) ≥95th percentile for age and sex. BMI was calculated as weight in kilograms divided by height in meters squared. This value then was converted to a percentile and a *z* score using standard charts based on age and sex.[19] Insulin resistance was based on the Quantitative Insulin Sensitivity Check Index (QUICKI), calculated as follows:

$$\text{QUICKI} = 1/[\log (I_0 * G_0)],$$

Where I_0 is fasting plasma insulin level in μU/mL and G_0 is fasting plasma glucose level in mg/dL. A QUICKI value <0.34 indicates insulin resistance.[20]

All biochemical tests were performed in the Montefiore Medical Center's clinical laboratory (Bronx, New York). Serum PTH was determined by a two-site chemiluminescent enzyme-labeled immunometric assay (IMMULITE 2000; Siemens, Los Angeles, California) that detects intact PTH (1 to 84) with intra-assay and interassay variations of 4.2%-5.7% and 6.2% to 8.3%, respectively. 25(OH)D was measured by a chemiluminescent assay (Liaison; DiaSorin, Stillwater, Minnesota) with intra-assay and interassay variations of 2.9% to 5.5% and 6.3% to 12.9%, respectively, and a lower limit of detection of 7 ng/mL.

Statistical Analysis

Several analytical approaches were used to examine the relationships between 25(OH)D and the measures of interest. The distribution of serum 25(OH)D levels was plotted (Figure 1; available at www.jpeds.com), and Lowess smoothed curves[21] were graphed to examine selective 25(OH)D–variable relationships (Figure 2). To assess for linear associations, we calculated Pearson product moment correlations between 25(OH)D levels and anthropomorphic measures and with biochemical variables (Table I). To explore whether specific breakpoints in serum 25(OH)D level in relation to changing values of the dependent variable of interest could be estimated, we began by performing analysis of covariance (ANCOVA) comparing bone and metabolic measures between individuals with low 25(OH)D levels (test groups) and those with normal 25(OH)D levels (reference group). Because there were a limited number of participants with a 25(OH)D level >25 ng/mL (n = 23) and none with a level >45 ng/mL, the reference group was defined as participants with a 25(OH)D level >25 ng/mL. This threshold is arbitrary but convenient in the sense that it gives us an adequate number of subjects in the reference group. To establish comparison groups, we varied the cutoff of 25(OH)D levels for test groups as follows: 25(OH)D <10 (group 1a), <15 (group 1b), <20 (group 1c), or ≤25 ng/mL (group 1d). The vitamin D information, however, is complicated by the fact that the assay used to measure it lacks analytic sensitivity below the level of 7 ng/mL. These values, showing up in our

Table I. Patient characteristics and simple correlation of bone markers and metabolic variables with serum 25(OH)D level

Variable	n	Mean ± SD	Range	*r*/25(OH)D	*P*	Normal range
Age, years	140	13.9 ± 3.2	6.2-21	-0.161	NS	NA
Height, cm	140	158.5 ± 12.6	122-182	-0.184	NS	NA
Weight, kg	140	88.2 ± 26.3	29.1-172.9	-0.199	NS	NA
BMI	140	34.5 ± 7.4	18.4-55.7	-0.158	NS	NA
BMI *z* score	138	2.23 ± 0.43	1.05-3.25	-0.091	NS	NA
SBP, mm Hg	139	118.4 ± 13.2	84-164	-0.261	.038	100-140
DBP, mm Hg	139	65.8 ± 8.5	41-98	-0.175	NS	60-90
25(OH)D, ng/mL	140	18.2 ± 8.1	5.6-44.9	1	NA	30-100
Total cholesterol, mg/dL	97	165.0 ± 35	75-257	-0.137	NS	100-169
HDL-C, mg/dL	97	42.3 ± 12.3	24-94	0.020	NS	40-59
LDL-C, mg/dL	97	98.9 ± 30.5	28-191	-0.110	NS	0-99
TG, mg/dL	97	117.4 ± 67.2	34-360	-0.212	NS	<130
HbA1c, %	124	5.3 ± 0.38	4.5-6.4	-0.044	NS	4.2-5.9%
Insulin (fasting), μU/mL	90	23.8 ± 13.4	4.69-64.8	0.023	NS	0-17
Glucose (fasting), mg/dL	111	86 ± 9.93	60-120	-0.024	NS	60-100
QUICKI	90	0.31 ± 0.027	0.264-0.386	0.087	NS	≥0.34
PTH, pg/mL	140	41.7 ± 20.9	11.9-127	-0.104	NS	10-65
Alkaline phosphatase, U/L	133	174.7 ± 111	29-516	0.021	NS	100-390
Calcium, mg/dL	134	9.7 ± 0.42	8.5-10.8	0.156	NS	8.5-10.5
Phosphorous, mg/dL	123	4.3 ± 0.69	2.9-6.2	0.087	NS	3.0-4.5
Magnesium, μg/dL	96	2.05 ± 0.20	1.5-2.9	-0.015	NS	0.8-2.1

DBP, diastolic blood pressure; *HbAlc*, hemoglobin A1c; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *NA*, not applicable.