Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial

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ABSTRACT

Background: Obese adolescents are at a greater risk of vitamin D deficiency because vitamin D is thought to be sequestered by excess adipose tissue. Poor vitamin D status has been associated with a higher prevalence of the metabolic syndrome, type 2 diabetes, or both in adults and adolescents.

Objective: The objective was to determine in obese adolescents the efficacy and safety of 4000 IU vitamin D3/d and whether subsequent increased circulating concentrations of 25-hydroxyvitamin D [25(OH)D] are associated with improved markers of insulin sensitivity and resistance and reduced inflammation.

Design: Obese adolescent patients [n = 35; mean ± SD age: 14.1 ± 2.8 y; BMI (in kg/m2): 39.8 ± 6.1; 25(OH)D: 19.6 ± 7.1 ng/mL] were recruited from the University of Missouri Adolescent Obesity Clinic and were randomly assigned to receive either vitamin D3 (4000 IU/d) or placebo as part of their standard care. Anthropometric measurements, inflammatory markers (IL-6, TNF-α, C-reactive protein), adipokines (leptin, adiponectin), fasting glucose, fasting insulin, and HOMA-IR values were measured at baseline and at 2 follow-up visits (3 and 6 mo).

Results: After 6 mo, there were no significant differences in BMI, serum inflammatory markers, or plasma glucose concentrations between groups. Participants supplemented with vitamin D3 had increases in serum 25(OH)D concentrations (19.5 compared with 2.8 ng/mL for placebo; P < 0.001), fasting insulin (−6.5 compared with +1.2 µU/mL for placebo; P = 0.026), HOMA-IR (−1.363 compared with +0.27 for placebo; P = 0.033), and leptin-to-adiponectin ratio (−1.41 compared with +0.10 for placebo; P = 0.045). Inflammatory markers remained unchanged.

Conclusion: The correction of poor vitamin D status through dietary supplementation may be an effective addition to the standard treatment of obesity and its associated insulin resistance. This trial was registered at clinicaltrials.gov as NCT00994396.

INTRODUCTION

Studies in obese adults provide strong evidence that body fat mass is inversely related to serum 25-hydroxyvitamin D [25(OH)D] concentrations (1). Although the pediatric data are not as voluminous, a few reports indicate that obese children also have significantly lower vitamin D status compared with their lean peers (2). It is thought that the observed lower serum concentrations of 25(OH)D in individuals with excess adiposity are likely due to this fat-soluble vitamin’s preferred deposition in fat tissue, thus decreasing its bioavailability (3).

More than one-third of US adults and almost 17% of US youth are obese (4). Despite this alarming prevalence, the 2011 Institute of Medicine’s (IOM’s) vitamin D recommendations do not take into account the reduced bioavailability associated with obesity (5), which may further put the obese, including adolescents, at risk of vitamin D inadequacy (6).

Mounting research suggests that improving the vitamin D status of individuals with poor or marginal status may have significant benefits involving several health outcomes, including those related to obesity (7, 8). Epidemiologic studies in adults show that lower vitamin D intakes are associated with a higher prevalence of type 2 diabetes (9). Cross-sectional studies have shown inverse associations between circulating 25(OH)D concentrations and elevated fasting glucose and insulin, whereas others have established a similar inverse relation with pancreatic β cell function and a positive association with insulin sensitivity (10). There remains, however, a lack of prospective randomized clinical trials of vitamin D supplementation on obesity-associated conditions in the young. The objectives of the study described herein were to determine the efficacy and safety of 4000 IU vitamin D3/d [IOM Tolerable Upper Intake Level] and whether subsequent increased circulating concentrations of 25(OH)D improved markers of insulin sensitivity/resistance and reduced inflammation in obese adolescents.

SUBJECTS AND METHODS

Participants

From November 2009 until January 2011, obese adolescents were recruited from the University of Missouri Adolescent Obesity Clinic to take part in a study investigating the effects of vitamin D supplementation on metabolic health factors related to obesity. The clinic uses a multidisciplinary approach in managing overweight
adolescents and their associated comorbidities; patients were between the ages of 9 and 19 y and were at least at the 85th percentile for BMI (11). All potential participants were identified by the clinic physician; consent and screening were conducted by other study staff. Exclusion criteria included the following: 1) use of vitamin D supplements other than a general multivitamin, 2) the use of medication affecting vitamin D metabolism, 3) the use of a tanning bed or undergoing UV light therapy, 4) use of oral hypoglycemic agents, 5) previously diagnosed hepatic or renal disorders, 6) pregnancy, 7) or the use of tobacco or alcohol. Study protocols were approved by the University of Missouri Health Sciences Institutional Review Board; written consent was provided by participants’ legal guardians, and written assent was provided by all participants.

Study design
This 6-mo, randomized, double-blind, placebo-controlled trial consisted of 3 data collection points: at baseline and 2 subsequent follow-up visits at 3 and 6 mo. On enrollment, participants were randomly assigned, with the use of a random number generator, to 1 of 2 groups by the University of Missouri Hospital Pharmacy Investigational Drug Service Division. Treatment group assignment and pill distribution were blinded to both the investigators and participants. To minimize possible age- and development-related differences in primary outcomes, randomization was stratified into 3 age groups (9–12 y, 13–15 y, 16–19 y). Permuted blocking of participants. To minimize possible age- and development-related differences in primary outcomes, randomization was stratified into 3 age groups (9–12 y, 13–15 y, 16–19 y). Permuted blocking of group size 4 was used within each age group so that equal numbers were assigned to treatment and placebo groups after randomly assigning each set of participants.

Adolescents in both the placebo and vitamin D supplementation groups were asked to continue with standard care, which consisted of quarterly visits with the clinic physician and dietitian. They were also asked to avoid the use of tanning beds for the duration of the study.

Placebo and vitamin D pills
Vitamin D3 and placebo were provided by Reliance Private Label Supplements. The vitamin D pills contained 2000 IU vitamin D3 and the placebo pill contained soy oil but were otherwise indistinguishable. The University Hospital Pharmacy stored and distributed all pills. Ninety-day supplies (180 pills) were dispensed at the baseline and 3-mo visits. Participants were instructed to consume 2 pills daily. To monitor compliance, participants were provided with a calendar to record daily pill consumption and were also instructed to return unused pills along with the calendars at the follow-up visits.

Outcomes

Anthropometric measurements
Height, to the nearest 0.5 cm, and weight, to the nearest 0.1 kg, were obtained at the clinic. From these values, BMI was calculated as weight (in kg) divided by height (in m) squared. Waist circumference was determined at the umbilicus, with a measuring tape parallel to the floor according to the standards established by the NIH. BMI z scores were calculated on the basis of 2000 normative data by using Epi-Info software (version 3.5.3) from the CDC (12).

Questionnaires
The following 4 questionnaires were administered to all participants at the baseline visit: a one-page sun-exposure questionnaire developed for this study to assess tanning bed use, outdoor sun exposure, and sunscreen use; a Fitzpatrick skin typing questionnaire (17); the Harvard Youth/Adolescent Questionnaire, a self-administered semiquantitative food-frequency questionnaire assessing habitual intake (13); and the Harvard Youth/Adolescent Activity Questionnaire, a self-administered activity questionnaire designed for children aged 9–18 y (14). The Harvard Youth/Adolescent Questionnaire and the Harvard Youth/Adolescent Activity Questionnaire were also administered at the 6-mo visit.

Serum collection and analyses
Serum 25(OH)D concentrations were measured by using ELISA (Immunodiagnostik AG; intraassay CV = 7.0%). Serum concentrations of IL-6 (intraassay CV = 7.8%), TNF-α (CV = 4.3%), C-reactive protein (CRP; intraassay CV = 3.8%), and leptin (intraassay CV = 3.0%) were also measured by using ELISA (R&D Systems). Total adiponectin and high-molecular-weight adiponectin were measured by using a multimeric ELISA (ALPCO Diagnostics; intraassay CVs = 5.4 and 5.0, respectively).

Fasting plasma glucose, insulin, serum calcium, and percentage of glycosylated hemoglobin were determined by the hospital laboratory as part of routine care. From these, the HOMA-IR and the Quantitative Insulin Sensitivity Check Index (QUICKI), which examine the complex yet normally tightly controlled relation between insulin and glucose, were calculated as follows:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (μU/mL)} \times \text{fasting glucose (mg/dL)}}{22.5}
\]

\[
\text{QUICKI} = 1 \div \log(\text{fasting insulin (μU/mL)}) + \log(\text{fasting glucose (mg/dL)})
\]

Statistical analysis
To determine whether increasing serum concentrations of 25(OH)D would improve the insulin insensitivity and inflammation associated with obesity, we needed to test whether after 6 mo, subjects who received treatment (vitamin D group) experienced significantly greater increases in circulating 25(OH)D concentrations than those in the placebo group. Based on the literature, the SD of this outcome measure is ~8 units (ng/mL); we wanted to detect a difference of ≥10 units (15). By using a 2-sample t test with a 2-sided alternative and a significance level of 0.05, a sample size of 17 participants would have 94% power to detect such a difference. This same sample size would still have 80% power to detect a smaller difference of 8 units, corresponding to an effect size of 1.0 (where effect size is defined as the difference in group means divided by the SD of the outcome variable). Our goal was to recruit 26 participants per group to allow for some loss to follow-up.

Baseline characteristics of all enrolled participants were compared by using either independent t tests (continuous) or chi-square tests (categorical). After assumptions were checked, between-group changes from baseline were analyzed for each outcome variable by repeated-measures ANOVA (PROC MIXED; SAS Institute), with treatment (between), time (within), and
treatment × time interaction serving as independent variables. Evaluable analyses were performed on primary outcome variables [25(OH)D, glucose, insulin, adipokines, and inflammatory markers] for all participants completing at least one of the follow-up visits (3 or 6 mo) (16). No imputations of missing data were made. In the case of non-normally distributed residuals for outcome variables, log transformations were applied before statistical testing and subsequently back transformed. To verify accuracy of primary analysis, secondary per-protocol analyses were also conducted by using only data from participants who completed all 3 visits and yielded results similar in magnitude and direction (not reported). Hypotheses were specified a priori; therefore, adjustments for multiple comparisons were not made (17).

Associations between baseline and 6-mo changes were conducted by simple linear regression by using absolute change in 25(OH)D as the predictor variable and absolute change in outcome as the dependent variable. Values are represented as means ± SEM, except where indicated. *P < 0.05 was considered significant for all tests. All statistical analyses were carried out by using SAS 9.22 statistical software (SAS Institute).

RESULTS

Attrition and compliance

Forty-four participants were enrolled in the study, and 35 made at least one return visit (Figure 1). There was no statistical difference in attrition between groups (*P = 0.825). Participants took their assigned pills ~81% of the days, with no differences in the level of compliance between the vitamin D and placebo groups (81% and 82%, respectively; *P = 0.743). As indicated by the pill calendars, no participants in either group missed >3 consecutive days during the study.

Baseline characteristics

No differences were observed between groups for any measurements at baseline (Table 1). The baseline serum 25(OH)D concentration in individuals whose baseline visit occurred in the winter/spring was significantly less than in those who began in the summer/fall (17.5 ± 7.1 ng/mL compared with 21.8 ± 6.9 ng/mL, respectively; *P = 0.046). There were no significant differences in serum 25(OH)D status between Fitzpatrick skin-tone scale group means (*P = 0.486).

Anthropometric measurements, dietary intakes, and physical activity

BMI and waist circumference were not significantly different between groups at 3 or 6 mo. Energy and nutrient intakes between the groups at baseline and 6-mo follow-up visits were also not significantly different (Supplemental Table 1 under “Supplemental data” in the online issue). Total hours of reported physical activity per week between the groups were similar at baseline (~9.5 h; *P = 0.808) and at 6 mo (~9.2 h; *P = 0.735).

FIGURE 1. Study sample recruitment and testing flowchart. *Evaluable analyses were conducted for all primary and secondary outcome variables by mixed-model ANOVA using data from all participants who completed the baseline visit and at least one follow-up visit (3 or 6 mo). appt., appointment.
TABLE 1  
Baseline characteristics of all participants randomly assigned to groups

<table>
<thead>
<tr>
<th></th>
<th>All (n = 44)</th>
<th>Vitamin D (n = 21)</th>
<th>Placebo (n = 23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>50</td>
<td>52</td>
<td>48</td>
<td>0.763</td>
</tr>
<tr>
<td>Age (y)</td>
<td>14.2 ± 2.6</td>
<td>14.6 ± 2.3</td>
<td>13.9 ± 2.4</td>
<td>0.360</td>
</tr>
<tr>
<td>African American (%)</td>
<td>30</td>
<td>33</td>
<td>26</td>
<td>0.599</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>119.3 ± 11.9</td>
<td>122.5 ± 13.4</td>
<td>117.7 ± 13.2</td>
<td>0.268</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39.2 ± 5.9</td>
<td>39.5 ± 5.1</td>
<td>38.9 ± 6.7</td>
<td>0.769</td>
</tr>
<tr>
<td>BMI score</td>
<td>2.53 ± 0.25</td>
<td>2.54 ± 0.24</td>
<td>2.54 ± 0.28</td>
<td>0.987</td>
</tr>
<tr>
<td>Serum 25(OH)D (ng/mL)</td>
<td>19.4 ± 7.3</td>
<td>19.2 ± 6.3</td>
<td>19.6 ± 7.9</td>
<td>0.865</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>89.6 ± 8.4</td>
<td>90.7 ± 9.2</td>
<td>88.6 ± 7.6</td>
<td>0.411</td>
</tr>
<tr>
<td>FPI (µU/L)</td>
<td>24.6 ± 12.5</td>
<td>24.6 ± 12.1</td>
<td>24.5 ± 13.1</td>
<td>0.982</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.5 ± 2.9</td>
<td>5.5 ± 2.7</td>
<td>5.5 ± 3.2</td>
<td>0.992</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.306 ± 0.024</td>
<td>0.304 ± 0.020</td>
<td>0.308 ± 0.03</td>
<td>0.631</td>
</tr>
</tbody>
</table>

1P values were derived from independent t tests (continuous) or chi-square tests (categorical) comparing 2 groups. 
There were no significant differences between treatment groups. FPG, fasting plasma glucose; FPI, fasting plasma insulin; 
QUICKI, Quantitative Insulin-Sensitivity Check Index; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin D.

2Mean ± SD (all such values).

Vitamin D status and serum calcium concentrations

At baseline, the mean (±SD) serum 25(OH)D concentration in all participants was 19.4 ± 7.3 ng/mL (Figure 2). By using the cutoff designations as discussed at the vitamin D workshop and elaborated on by Grant and Holick (based on concentrations associated with several health outcomes), all but 2 adolescents were deficient (<20 ng/mL) or insufficient (20–30 ng/mL) (18–20). Within 3 mo of supplementation, serum 25(OH)D concentrations increased such that none of the participants in the vitamin D group were in the category of deficient, and by 6 mo, 93% had 25(OH)D concentrations considered to be sufficient. In contrast, the 25(OH)D concentrations in the placebo group did not increase significantly from baseline to 3 or 6 mo. These changes were independent of season. Serum calcium concentrations remained unchanged in both groups throughout the study and were well within the normal range at each time point (Figure 2).

Insulin resistance and sensitivity

After adjustment for covariates (baseline value of outcome variable, age, sex, waist circumference, and BMI), neither the changes in fasting plasma glucose nor fasting plasma insulin were significantly different at 3 mo (see Supplemental Figure 1 under “Supplemental data” in the online issue). However, after 6 mo, there was a significant reduction in fasting plasma insulin between the treatment groups, whereas fasting glucose remained unchanged. In addition, there were no observed between-group changes from baseline in glycosylated hemoglobin at either 3 mo (P = 0.321) or 6 mo (P = 0.241). However, adolescents who received vitamin D had significant improvements in both HOMA-IR and QUICKI compared with placebo at 6 mo but not at 3 mo (see Supplemental Figure 1 under “Supplemental data” in the online issue).

Inflammatory markers

After adjustment for the covariates (baseline value of outcome variable, age, sex, waist circumference, and BMI), there were no between-group changes from baseline in CRP, IL-6, or TNF-α after 3 mo (see Supplemental Figure 2 under “Supplemental data” in the online issue). Likewise, these 3 inflammatory markers remained unchanged after 6 mo (placebo compared with control).

Adipokines and adipokine ratios

After adjustment for covariates (baseline value of outcome variable, age, sex, waist circumference, and BMI), serum leptin, adiponectin, and the leptin to adiponectin ratio were unchanged from baseline after 3 mo (see Supplemental Figure 3 under “Supplemental data” in the online issue). However, after 6 mo, only the serum leptin to adiponectin ratio was significantly lower in the vitamin D group compared with the placebo group (Table 2). The associations between 6-mo change in serum 25(OH)D concentration and 6-mo change in HOMA-IR and the change in the ratio of leptin to adiponectin, as well as the extent to which the classifications of vitamin D status at 6 mo were consistent with these associations, are shown in Figure 3. Simple linear regression showed a significant inverse relation between the baseline to the 6-mo change in 25(OH)D concentration and the change in the leptin to adiponectin ratio over the same period. No such relation was seen between the change in HOMA-IR and change in 25(OH)D concentration.

DISCUSSION

Our findings show that supplementing obese adolescents with 4000 IU cholecalciferol/d safely increases their 25(OH)D...
concentrations to a level at which the impaired glucose metabolism and insulin resistance associated with obesity are attenuated. Participants who received vitamin D had significant improvements in 2 widely used surrogate markers of insulin resistance and sensitivity (HOMA-IR and QUICKI, respectively), as well as a third more recently proposed marker, the ratio of leptin to total adiponectin (21). However, we did not find evidence to support our hypothesis that increasing vitamin D status reduces the inflammation that commonly accompanies the progression from obesity to impaired glucose metabolism.

In this study, the prevalence of vitamin D deficiency/insufficiency among participants at baseline was consistent with other studies in obese populations (2, 22). The mean total vitamin D intake of the participants was ~250 IU/d, which is below the IOM’s recommendation of 400 IU/d (6). However, it is worth noting that these recommendations assume minimal exposure to sunlight. The observation that season of recruitment had a significant effect on baseline vitamin D status [those recruited during the winter/spring months had significantly lower circulating concentrations of 25(OH)D than those who enrolled during summer/fall; \( P = 0.048 \)] suggests that skin synthesis of vitamin D was a significant contributor to vitamin D status in these teens. This is in agreement with reports of US intakes that showed that no child or adult receives the recommended vitamin D dose from dietary sources alone (23, 24).

The prevalence of vitamin D insufficiency observed in obese populations is thought to be partly due to the decreased bioavailability of the vitamin to body cells from skin and dietary sources because of its preferred deposition in body fat compartments (3). It has been estimated that nonobese adults require a 100-IU intake to increase serum concentrations of 25(OH)D by ~1.0 ng/mL (25), whereas obese adults require twice the dose to see an equivalent response (3). Our results in obese adolescents are similar to these estimates in adults, because 4000 IU/d produced a mean increase of 19.5 ng/mL or 1 ng/mL for every 205 IU ingested.

HOMA-IR and QUICKI are common clinical indexes used to assess insulin resistance and sensitivity from fasting laboratory values (26, 27). In obese children, HOMA-IR values greater than 3.2 are often considered insulin resistant (28), whereas QUICKI values <0.328 are considered insulin resistant (26). At baseline, our participants had values of 5.5 ± 2.9 and 0.306 ± 0.020, respectively, and the vitamin D group showed significant decreases
from baseline in HOMA-IR and increases in QUICKI. The reduction in HOMA-IR observed in this study is similar to results involving metformin (29). By comparison, metformin is reported to reduce the HOMA-IR score by ~2 units in obese children; in our study, vitamin D decreased the score by ~1.5 units. Remarkably, this improvement in insulin resistance was independent of changes in body weight.

The proposed mechanisms for vitamin D’s role in improving insulin resistance/sensitivity in obesity include reducing inflammation (30) and enhancing peripheral/hepatic uptake of glucose through both direct and indirect means (31–33) and through regulation of glucose-mediated synthesis/secretion of insulin by pancreatic β cells (34–36). Our inflammatory marker data do not support the first mechanism. CRP, IL-6, and TNF-α were slightly elevated in our participants compared with lean controls found in the literature, but we saw no significant changes in our treatment group (37, 38). However, we were most likely inadequately powered to detect biologically relevant changes in these 3 markers.

In exploring the other mechanisms, there was a greater decrease in fasting insulin (28.1%) than fasting glucose (6.1%) relative to baseline. This implies that the improvements in insulin resistance that resulted from enhancing vitamin D status were primarily mediated through decreases in circulating insulin concentrations. Given the fasting state of our participants, it is likely that the decreases in circulating insulin concentration indicate improved glycemic control. Although it remains possible that vitamin D plays a role in the regulation of insulin secretion, we do not have direct evidence that this is occurring here. The absence of change in any of the insulin resistance/sensitivity outcomes between baseline and 3 mo suggests that the improvements observed at 6 mo were either gradual or occurred only after serum 25(OH)D reached an effective concentration. This interesting finding on the delayed effects of vitamin D status on insulin sensitivity was also observed in the only other known randomized controlled trial of 4000 IU vitamin D supplementation in individuals with insulin resistance (7).

The ratio of leptin to adiponectin has recently been proposed as a potential clinical tool for the assessment of insulin resistance (32, 33). Oda et al (21) found that this ratio was more strongly correlated with results from a hyperinsulinemic/euglycemic clamp, considered the gold standard of assessing insulin resistance, than were HOMA-IR or QUICKI methods. Leptin and adiponectin are 2 adipocyte-derived cytokines that have been independently associated with glucose metabolism. Adiponectin has been shown to have strong insulin-sensitizing properties (39, 40). Lin et al (41) reported that elevated serum adiponectin strongly predicts lower insulin resistance. Likewise, leptin also has several metabolically beneficial roles such as appetite suppression, increased glucose uptake, and regulation of insulin secretion, which occurs through a bidirectional feedback loop termed the adipoinsular axis (42, 43). Paradoxically, obesity produces a state of hyperleptinemia that leads to leptin resistance (44).

Our results showing significant decreases in the leptin to adiponectin ratio from baseline to 6 mo suggest that vitamin D either directly or indirectly influences circulating concentrations of adipokines independent of adiposity, because there were no changes in BMI z score or waist circumference in our participants. Although the fasting leptin concentrations in our participants remained elevated compared with lean controls reported in the literature (45), the reduction in leptin accounted for the majority of the change seen in the leptin to adiponectin ratio, which, along with the large changes seen in fasting insulin, may indicate modulation of the adipoinsular axis by 1,25-dihydroxyvitamin D.

One of the most remarkable and novel findings of this study is that the participants who had increases in serum 25(OH)D concentrations considerable enough to raise their classification status to sufficient had the most improved response in the serum leptin to adiponectin ratio. In contrast, this observation was not made with the HOMA-IR data, where there was no association between 6-mo change in 25(OH)D concentration and the change in HOMA-IR. This is further evidence that vitamin D’s effects on fasting insulin concentrations in the obese may be modulated at multiple sites of the adipoinsular axis. The exact mechanisms by which vitamin D works within the axis remain unknown, and further investigation is warranted.

The primary limitation of our study was sample size because it was powered to detect a biologically relevant change in serum...


REFERENCES


