Hypovitaminosis D is associated with insulin resistance and β cell dysfunction\textsuperscript{1–3}

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ABSTRACT

Background: Although the role of vitamin D in type 2 diabetes is well recognized, its relation to glucose metabolism is not well studied.

Objective: We investigated the relation of 25-hydroxyvitamin D [25(OH)D] concentrations to insulin sensitivity and β cell function.

Design: We enrolled 126 healthy, glucose-tolerant subjects living in California. Insulin sensitivity index (ISI) and first- and second-phase insulin responses (1stIR and 2ndIR) were assessed by using a hyperglycemic clamp.

Results: Univariate regression analyses showed that 25(OH)D concentration was positively correlated with ISI ($P < 0.0001$) and negatively correlated with 1stIR ($P = 0.0045$) and 2ndIR ($P < 0.0001$). Multiple regression analyses confirmed an independent correlation between 25(OH)D concentration and ISI ($P = 0.0007$). No independent correlation was observed between 25(OH)D concentration and 1stIR or 2ndIR. However, an independent negative relation of 25(OH)D concentration with plasma glucose concentration was observed at fasting ($P = 0.0258$), 60 min ($P = 0.0011$), 90 min ($P = 0.0011$), and 120 min ($P = 0.0007$) during the oral-glucose-tolerance test. Subjects with hypovitaminosis D (<20 ng/mL) had a greater prevalence of components of metabolic syndrome than did subjects without hypovitaminosis D (30% compared with 11%; $P = 0.0076$).

Conclusions: The data show a positive correlation of 25(OH)D concentration with insulin sensitivity and a negative effect of hypovitaminosis D on β cell function. Subjects with hypovitaminosis D are at higher risk of insulin resistance and the metabolic syndrome. Further studies are required to explore the underlying mechanisms.


KEY WORDS Diabetes mellitus, insulin sensitivity, β cell function, glucose metabolism, insulin resistance, vitamin D, hypovitaminosis D, metabolic syndrome

INTRODUCTION

Serum 25-hydroxyvitamin D [25(OH)D] concentrations are largely determined by environmental factors, mainly through vitamin D intake and ultraviolet exposure (1). The concentration of 25(OH)D, but not that of 1,25-dihydroxyvitamin D, defines nutritional vitamin D status (2, 3). Vitamin D deficiency is a risk factor for hypertension, type 1 diabetes, and various cancers (4). Most tissues have not only vitamin D receptors, but also the hydroxylase enzyme that is required to convert 25(OH)D to the active form, 1,25-dihydroxyvitamin D (4). Therefore, vitamin D can affect tissues that are not involved in calcium homeostasis and bone metabolism.

Hypovitaminosis D has long been suspected as a risk factor for glucose intolerance. The 25(OH)D concentration was lower in patients with type 2 diabetes than in the nondiabetic control subjects ($5, 6$). A high prevalence of hypovitaminosis D was noted in women with type 2 diabetes ($7$). The 25(OH)D concentrations were lower in patients at risk for diabetes than in those who were not at risk for diabetes ($8$). Furthermore, hypovitaminosis D was associated with impaired insulin secretion in a population at high risk for diabetes ($8$). Hyperresponsive insulin secretion after a glucose challenge has been found in older men with hypovitaminosis D ($9$). Therefore, vitamin D could play a role in the pathogenesis of type 2 diabetes, by affecting either insulin sensitivity or β cell function, or both.

However, the interaction of vitamin D with insulin sensitivity and β cell function has not been examined in a group of well-defined subjects. Because abnormal glucose tolerance could adversely affect insulin sensitivity and β cell function ($10$), we investigated the relation of 25(OH)D concentration to insulin sensitivity and β cell function as assessed by the hyperglycemic clamp technique in glucose-tolerant subjects.

SUBJECTS AND METHODS

Subjects

Through an advertisement in the campus newspaper of the University of California, Los Angeles, School of Medicine, healthy subjects who received no medical treatment were invited to undergo a screening test after an overnight fast. The screening included an oral-glucose-tolerance test (OGTT) with 75 g glucose and a brief physical examination as previously described ($11$). Only those subjects who had normal glucose tolerance (fasting plasma glucose: <110 mg/dL; interval plasma glucose: <200 mg/dL; and 2-h plasma glucose: <140 mg/dL) and were...
normotensive (blood pressure: <140/90 mm Hg) were invited to return for an assessment of β cell function and insulin sensitivity with the use of a 3-h hyperglycemic clamp technique. This study enrolled 126 subjects of various racial or ethnic backgrounds: 34 were Asian American, 11 were African American, 54 were white, and 27 were Mexican American (Table 1). The subjects were 73 women and 53 men with a mean (± SD) age of 26 ± 6 y, a body mass index (BMI; in kg/m$^2$) of 24.7 ± 4.2, and a waist-to-hip ratio (WHR) of 0.79 ± 0.07.

The study was approved by the institutional review board of this institution. Written informed consent was obtained from each participant before he or she entered the study.

Assessment of insulin sensitivity and β cell function

Hyperglycemic clamps were performed as described previously (11). After fasting overnight and resting in the General Clinical Research Center, participants received a bolus of 50% dextrose solution based on their body surface area (11.4 g/m$^2$) at time zero. Continuous infusion of 30% dextrose solution was started 15 min later at variable rates, which were adjusted every 5 min on the basis of the prevailing plasma glucose concentrations to maintain a plasma glucose concentration of ≈180 mg/dL until 180 min. The first-phase insulin response (1stIR) was defined as the sum of the plasma insulin concentrations at 2.5, 5.0, 7.5, and 10 min of the clamp experiment, and the second-phase insulin response (2ndIR) was defined as the average plasma insulin concentration during the last hour (120–180 min) of the clamp process, when plasma insulin concentrations are expected to plateau. The insulin sensitivity index (ISI) was calculated by dividing the average glucose infusion rate during the last hour of each clamp process [(μmol/L)·m$^{-2}$·min$^{-1}$] by the average plasma insulin concentration (pmol/L) during the same interval. The CV for steady state plasma glucose concentrations was 5.6 ± 2.3%.

Definition of the metabolic risk

The risk factors for the metabolic syndrome were defined according to the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (12). They are waist circumference >102 cm in men and 88 cm in women; a serum triacylglycerol concentration of ≥150 mg/dL; HDL-cholesterol concentration of <40 mg/dL in men and <50 mg/dL in women; blood pressure of ≥130/85 mm Hg; or a plasma glucose concentration of ≥110 mg/dL (12). Because this study enrolled only normotensive, glucose-tolerant subjects, none of the participants had a plasma glucose concentration >110 mg/dL, systolic blood pressure >140 mm Hg, or diastolic pressure >90 mm Hg.

Laboratory assays

Plasma glucose, insulin, and lipid concentrations were assayed as previously described (11). The 25(OH)D concentration was determined from a fasting sample by using an enzyme-binding protein assay (Alpco Diagnostics, Windham, NH) with intraassay and interassay CVs of 11%. Hypovitaminosis D was defined as a 25(OH)D concentration <20 ng/mL (13–15).

Statistical analysis

Differences in continuous variables among the groups of subjects were tested with one-factor analysis of variance and corrected with Bonferroni’s post hoc test when appropriate. Differences in proportions were evaluated by using a chi-square test. Continuous variables that failed the normality test were logarithmically transformed before analysis. To examine the influence of confounding variables, multivariate analysis with stepwise regression was used. Backward stepwise regression with α values of 0.10 was used to exclude variables that had little or no influence on the trait under analysis. SYSTAT for WINDOWS software (version 10.0; SPSS Inc, Chicago) was used for statistical analysis. $P<0.05$ was considered significant.

RESULTS

Although only glucose-tolerant subjects were enrolled in this study, there was a wide range in ISI [1.3632–17.9944 (μmol/L)·m$^{-2}$·min$^{-1}$·(pmol/L)$^{-1}$], 1stIR (465–7415 pmol/L), and 2ndIR (104–1567 pmol/L). Even though none of the studied subjects had clinical evidence of hypovitaminosis D, 47 subjects had 25(OH)D concentrations <20 ng/mL. Ethnic differences in 25(OH)D were noted (Table 1). Of the Asian American, African American, white, and Mexican American subjects, 47%, 54%, 26%, and 41%, respectively, had 25(OH)D concentrations <20 ng/mL. Sex and age had no effect on 25(OH)D concentration ($P = 0.3255$ and $P = 0.4917$, respectively), and season had a marginal effect on 25(OH)D concentration ($P = 0.0729$).
tween 25(OH)D concentration and BMI (diastolic blood pressure. We observed an inverse relation be-
25(OH)D concentration had no interaction with either systolic or glucose concentrations was investigated (Interaction of 25(OH)D with clinical features
WHR had no independent effect on 25(OH)D concentration. 0.0086) and BMI (0.0008).

For multivariate analyses of systolic and diastolic blood pressure, BMI, waist to-hip ratio, those factors were not included as covariates, respectively.

For multivariate regression analysis showed that 15% of the variation in 25(OH)D concentration was found with fasting plasma glucose concentration (P = 0.0777) or 30-min postchallenge plasma glucose concentration (P = 0.1386). After consideration of age, sex, ethnicity, BMI, WHR, systolic and diastolic blood pressure, 25(OH)D concentration, and season as potential covariates, multivariate analysis confirmed the independent and negative correlation of 25(OH)D concentration with fasting, 60-, 90-, and 120-min postchallenge plasma glucose concentrations (Table 2).

Relation of 25(OH)D to insulin sensitivity
We found a positive correlation of 25(OH)D concentration with ISI (Figure 2A). Because various factors could affect ISI, we performed multivariate regression analyses and included the potential covariates of age, sex, ethnicity, BMI, WHR, systolic and diastolic blood pressure, 25(OH)D concentration, and season. As shown in Table 3, 25(OH)D concentration was a highly significant and independent predictor for ISI; along with sex, BMI, diastolic blood pressure, age, and ethnicity, it accounted for 42% of the variation in ISI. These results show an independent and positive correlation between 25(OH)D concentration and ISI.

Relation of 25(OH)D to β cell function
In this glucose-tolerant population, ISI was inversely correlated with 1stIR (P < 0.0001, r = -0.5860) and 2ndIR (P < 0.0001, r = -0.7612). Thus, because ISI was positively correlated with 25(OH)D in this population, we observed that both

### Table 2
Regression analysis of the effect of 25-hydroxyvitamin D on the subjects’ clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
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<td></td>
<td>Coefficient</td>
<td>P</td>
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<td>NS</td>
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<tr>
<td>Diastolic blood pressure</td>
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<td>NS</td>
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<tr>
<td>BMI</td>
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<td>0.0045</td>
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<td>Waist-to-hip ratio</td>
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<tr>
<td>Lipid profile</td>
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<tr>
<td>Triacylglycerol</td>
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<td>NS</td>
</tr>
<tr>
<td>Total cholesterol</td>
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<td>0.0246</td>
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<tr>
<td>HDL cholesterol</td>
<td>1.3312</td>
<td>NS</td>
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<tr>
<td>LDL cholesterol</td>
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<td>0.0126</td>
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<tr>
<td>Plasma glucose concentration</td>
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<tr>
<td>At fasting</td>
<td>-0.0967</td>
<td>NS</td>
</tr>
<tr>
<td>At 30 min</td>
<td>-0.2395</td>
<td>NS</td>
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<tr>
<td>At 60 min</td>
<td>-0.6415</td>
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<td>At 90 min</td>
<td>-0.6946</td>
<td>0.0011</td>
</tr>
<tr>
<td>At 120 min</td>
<td>-0.5196</td>
<td>0.0007</td>
</tr>
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</table>

*r* Covariates considered were sex, age, ethnicity, season, systolic and diastolic blood pressure, BMI, waist-to-hip ratio, and 25-hydroxyvitamin D. For multivariate analyses of systolic and diastolic blood pressure, BMI, and waist-to-hip ratio, those factors were not included as covariates, respectively.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Relation between serum 25-hydroxyvitamin D concentration and plasma glucose concentration at 60, 90, and 120 min during a standard 75-g oral-glucose-tolerance test. The skewed variable (25-hydroxyvitamin D) was logarithmically transformed to normality before univariate regression analysis.
First-phase insulin response (B: P tolerant subjects, the subtle effect of 25(OH)D concentration on tolerant subjects, plasma glucose concentration (Figure 1 and Table 2). In glucose-astolic blood pressure, BMI, waist to-hip ratio, and 25-hydroxyvitamin D. Insulin sensitivity index covariate entered Partial Dependent variable and sensitivity Multivariate analysis of the effect of 25-hydroxyvitamin D on insulin Table 3 and Table 2). These observations indicated that a low 25(OH)D concentration on either 1stIR or 2ndIR (Table 4), and 25(OH)D concentration was excluded from analysis for insignificant P values (P = 0.7781 and P = 0.9667, respectively).

Although 25(OH)D concentration had no independent effect on the measured β cell function (1stIR and 2ndIR) in glucose-tolerant subjects, the subtle effect of 25(OH)D concentration on β cell function was suggested by the relation of 25(OH)D to plasma glucose concentration (Figure 1 and Table 2). In glucose-tolerant subjects, β cells compensate for the prevailing insulin resistance to maintain plasma glucose concentration within a relatively narrow range. If 25(OH)D concentration had no effect on β cell function and if β cells compensated appropriately in those subjects with different 25(OH)D concentrations, we would observe no relation between plasma glucose concentration and 25(OH)D concentration. However, we did observe an inverted and independent relation of 25(OH)D concentration with plasma glucose concentrations at fasting, 60, 90, and 120 min (Figure 1 and Table 2). These observations indicated that a low 25(OH)D concentration had some effect on β cell function and prevented a proper compensatory insulin response that would keep the plasma glucose concentration similar to that in subjects with a higher 25(OH)D concentration. Therefore, subjects with a lower 25(OH)D concentration had decompensated β cell function, which resulted in a higher plasma glucose concentration than that in subjects with a higher 25(OH)D concentration. Furthermore, the effect of 25(OH)D on β cells is continuous, as shown in the regression lines in Figure 1. A lower 25(OH)D concentration has a more adverse effect on β cell function.

Relation of 25(OH)D to the metabolic syndrome
Because only glucose-tolerant subjects were enrolled in this study, none of the participants had fasting plasma glucose > 110 mg/dL. We defined those with ≥2 metabolic abnormalities defined by the Adult Treatment Panel III (12) as at risk of the metabolic syndrome. We found 14 subjects (30%) at risk for the metabolic syndrome among 47 subjects with hypovitaminosis D (11%) were at risk of the metabolic syndrome (P = 0.0097). These observations indicate that hypovitaminosis D is associated with increased risk of the metabolic syndrome.

![Graph showing the relationship between 25-hydroxyvitamin D concentration and insulin sensitivity index, first-phase insulin response, and second-phase insulin response](https://academic.oup.com/ajcn/article-abstract/79/5/820/4690192)

**TABLE 3**

Multivariate analysis of the effect of 25-hydroxyvitamin D on insulin sensitivity

<table>
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<th>Dependent variable and covariate entered</th>
<th>Partial r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity index</td>
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<td></td>
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<tr>
<td>Sex</td>
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<tr>
<td>25-Hydroxyvitamin D</td>
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<td>0.0007</td>
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<tr>
<td>BMI</td>
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<tr>
<td>Diastolic blood pressure</td>
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<td>0.0028</td>
</tr>
<tr>
<td>Age</td>
<td>0.1839</td>
<td>0.0105</td>
</tr>
<tr>
<td>Ethnicity</td>
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<td>0.0539</td>
</tr>
</tbody>
</table>

1 Covariates considered were sex, age, ethnicity, season, systolic and diastolic blood pressure, BMI, waist to-hip ratio, and 25-hydroxyvitamin D.

**TABLE 4**

Multivariate analysis of the effect of 25-hydroxyvitamin D on β cell function

<table>
<thead>
<tr>
<th>Dependent variable and covariate entered</th>
<th>Partial r</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>1st-phase insulin response</td>
<td>−0.5869</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2nd-phase insulin response</td>
<td>−0.7204</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin sensitivity index</td>
<td>−0.1476</td>
<td>0.0107</td>
</tr>
</tbody>
</table>

1 Covariates considered were sex, age, ethnicity, season, systolic and diastolic blood pressure, BMI, waist to-hip ratio, 25-hydroxyvitamin D, and insulin sensitivity index.
DISCUSSION

Our data show that, in glucose-tolerant subjects, 25(OH)D concentration has a positive relation to insulin sensitivity and a negative effect on β cell function. These relations are independent of confounding factors. Therefore, hypovitaminosis D is a risk factor for type 2 diabetes and the metabolic syndrome. Although there is to date no report on both of these associations in a single study such as the current study, separate reports have shown the association of hypovitaminosis D with insulin resistance (16) and β cell dysfunction (8).

Vitamin D status is usually assessed by measuring the serum 25(OH)D concentration. In Europe, there is a significant positive correlation between serum 25(OH)D concentration and latitude (17). Latitude determines the available sunlight exposure, which affects 25(OH)D concentration. Therefore, regional differences in 25(OH)D concentration are a well-recognized phenomenon (18). As a result, the reference ranges defined with the use of the regional population samples lead to different ranges of lower limits among various regions (19). The definition using the regional population samples did not reflect the true body need because hypovitaminosis D causes secondary hyperparathyroidism. Another approach to defining hypovitaminosis D is based on the relation of 25(OH)D and parathyroid hormone concentration (20). Although one study showed that serum intact parathyroid hormone held a stable plateau concentration at 36 pg/mL as long as the serum 25(OH)D concentration was >31 ng/mL (21), we chose a more conservative value of 20 ng/mL as the definition of hypovitaminosis D (4, 20).

One of the unique features of this study is the use of the hyperglycemic clamp. Although the gold standard for the measurement of insulin sensitivity is the use of the euglycemic clamp, the hyperglycemic clamp provides both insulin sensitivity and β cell function from a single procedure. Furthermore, insulin sensitivity measured by using a hyperglycemic clamp has an excellent correlation with insulin sensitivity measured by using a euglycemic clamp (22–24). Therefore, we chose the hyperglycemic clamp for this study, which allowed us to assess insulin sensitivity and β cell function.

Although we deduced the effect of β cell function from plasma glucose concentration and not from the measured 1stIR or 2ndIR, the published data strongly supported the association between hypovitaminosis D and β cell dysfunction. There is ample evidence in animal studies that vitamin D is essential for normal insulin secretion. Insulin secretion was impaired in the vitamin D–deficient pancreas, and it was improved by dietary vitamin D repletion (25–28). Vitamin D repletion improved glucose clearance and insulin secretion in vivo, independent of nutritional factors and prevailing plasma calcium and phosphorous concentrations (29). The de novo synthesis of numerous proteins decreases during the period of vitamin D deficiency and is gradually restored by vitamin D repletion in the islets of Langerhans in rats (30). Vitamin D not only facilitates the biosynthetic capacity of β cells but also accelerates the conversion of proinsulin to insulin (30). Vitamin D deficiency also reduced insulin turnover in rats (31). The effect of vitamin D on insulin secretion is also observed in humans. Vitamin D supplementation has been reported to improve insulin secretion in vitamin D–deficient and nondiabetic subjects (32) and in patients with type 2 diabetes (33). These reports suggest that vitamin D deficiency affects β cell function and that vitamin D supplementation improves β cell function.

As compared with the published data from both rodent and human studies, the effect of vitamin D on β cells is much more subtle in our populations. There are several explanations for the discrepancy. The studies in rodents were all performed in vitamin D–deprived animals (25, 26, 28). Therefore, those studies found much more profound β cell defects. All of the human studies included some subjects with diabetes, impaired glucose tolerance, or impaired fasting plasma glucose (8, 9), and those studies found obvious β cell dysfunction. In contrast, our sample set was very clean; the subjects were healthy, normotensive, and glucose tolerant and were taking no medications on a regular basis. None of the subjects had diabetes or impaired glucose tolerance. Furthermore, none of them had a fasting plasma glucose concentration >100 mg/dL. Therefore, the effect of vitamin D on β cell function is much more subtle in our study. Nevertheless, even after exclusion of subjects with obvious β cell dysfunction, we still observed the negative effect of hypovitaminosis D on β cell function.

As compared with evidence for the effect of hypovitaminosis D on β cell function, the evidence for the association of hypovitaminosis D with insulin sensitivity is quite limited. A positive relation between serum 25(OH)D concentration and insulin sensitivity was reported in a group of 34 men, including 7 subjects with diabetes (16). That study also found that serum 25(OH)D concentration was inversely associated with fasting insulin concentration (P < 0.05), 1-h and 2-h insulin concentrations (P < 0.05), and insulin area under the curve (P < 0.05) in 134 elderly nondiabetic men, independent of BMI, skinfold thickness, alcohol, smoking, and physical activity (9). These results suggest a positive association of 25(OH)D concentration with insulin sensitivity. Supplementation with vitamin D reduces the concentrations of serum free fatty acids in patients with type 2 diabetes (33), which further suggests an improvement in insulin sensitivity. Our study provides the first evidence of a positive association between 25(OH)D concentration and measured ISI in glucose-tolerant subjects.

The role of vitamin D in the metabolic syndrome is suggested by a recent report from the Coronary Artery Risk Development in Young Adults (CARDIA) Study, a population-based prospective study (34). In sampling 3157 black and white adults aged 18–30 y from 4 US metropolitan areas, it was observed that dairy consumption was inversely associated with the incidence of insulin resistance syndrome among overweight adults. Therefore, dairy consumption may reduce the risk of type 2 diabetes and cardiovascular disease. Subjects with the highest dairy consumption had a 72% lower incidence of the metabolic syndrome than did those with the lowest dairy intake. The role of vitamin D in insulin resistance syndrome has been the subject of speculation (35). However, 25(OH)D concentration was not reported in that population. Because milk is fortified with vitamin D in the United States, it is highly possible that vitamin D may play a central role in this association. This possibility is in accord with our observation that hypovitaminosis D is a risk factor for the metabolic syndrome.

To our knowledge, the current study is the first to show the relation of 25(OH)D concentration to insulin sensitivity and secretion by using a hyperglycemic clamp technique in a group of healthy, glucose-tolerant subjects. We also observed that hypovitaminosis D is a risk factor for the metabolic syndrome. Ex-
trapolation from the observations in the current study suggests that increasing 25(OH)D from 10 to 30 ng/mL can improve insulin sensitivity by 60%, from 3.8128 to 6.1176 (μmol/L · m² · min⁻¹ · (μmol/L)⁻²). This improvement in insulin resistance could potentially eliminate the burden on β cells and reverse abnormal glucose tolerance. Furthermore, the 60% improvement in insulin sensitivity that results from vitamin D treatment indicates that that treatment is more potent than either troglitazone or metformin treatment (54% and 13% improvement in insulin sensitivity, respectively; 36). The modest effect of vitamin D on insulin sensitivity in individual persons may translate into a dramatic effect in the population as a whole because of the high prevalence of hypovitaminosis D, which, in a large population, carries an attributable risk for type 2 diabetes and the metabolic syndrome. Although a review of the literature suggests non-calcium-mediated effects, the underlying molecular mechanism remains to be elucidated.

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KCC designed and implemented the study. KCC was responsible for recruiting the subjects, providing medical care during the study, evaluating the data, writing the manuscript, and organizing the figures and tables. KCC and AC were responsible for collecting and managing the data, assessing 25(OH)D and glucose, analyzing the laboratory data, managing the subjects and samples, and preparing the tables and figures and otherwise assisting in preparation of the manuscript. MFS performed the insulin assay. MFS and VLWG were involved in the design of the experiment, analysis of the data, and writing of the manuscript. None of the authors had a conflict of interest.

REFERENCES