Serum 25-hydroxyvitamin D and parathyroid hormone are independent determinants of whole-body insulin sensitivity in women and may contribute to lower insulin sensitivity in African Americans1–3

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ABSTRACT

Background: Circulating 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) concentrations have been shown to be associated with insulin sensitivity; however, adiposity may confound this relation. Furthermore, African Americans (AAs) have lower insulin sensitivity and 25(OH)D concentrations than do European Americans (EAs); whether these differences are associated in a cause-and-effect manner has not been determined.

Objectives: The objectives of this study were to examine the relation of 25(OH)D and PTH concentrations with whole-body insulin sensitivity and to determine whether lower 25(OH)D concentrations in AAs compared with EAs contribute to the lower insulin sensitivity of AAs relative to that of EAs.

Design: This was a cross-sectional study of 25 AA and 25 EA women. We determined the whole-body insulin sensitivity index (S_i) with an intravenous glucose tolerance test and minimal model estimation. Percentage body fat was determined with dual-energy X-ray absorptiometry, and intraabdominal adipose tissue (IAAT) was determined with computed tomography.

Results: Multiple linear regression analysis indicated that 25(OH)D and PTH concentrations were independent determinants of S_i [standardized β = 0.24 (P = 0.04) and −0.36 (P = 0.002), respectively] after adjustment for age, race, and IAAT. The mean ethnic difference in S_i decreased from 2.70 [10^−4 · min⁻¹(μIU/mL)] after adjustment for IAAT and percentage body fat to 1.80 [10^−4 · min⁻¹(μIU/mL)] after further adjustment for 25(OH)D and PTH concentrations.

Conclusions: 25(OH)D and PTH concentrations were independently associated with whole-body insulin sensitivity in a cohort of healthy women, which suggested that these variables may influence insulin sensitivity through independent mechanisms. Furthermore, ethnic differences in 25(OH)D concentrations may contribute to ethnic differences in insulin sensitivity. Am J Clin Nutr 2010;92:1344–9.

INTRODUCTION

Vitamin D may play a role in maintaining insulin sensitivity (1), the loss of which has been implicated in the progression of type 2 diabetes (2). Cross-sectionally, 25-hydroxyvitamin D [25(OH)D] concentrations are generally shown to be associated with insulin sensitivity (1, 3–6). However, because vitamin D may become sequestered in adipose tissue (7) and obesity is associated with vitamin D deficiency (8, 9) and insulin resistance (2), adiposity may be a confounder of these relations (10). Parathyroid hormone (PTH) concentrations are often elevated in vitamin D–deficient states (11) and may further confound the relation between 25(OH)D concentrations and insulin sensitivity because PTH has also been shown to be positively associated with insulin sensitivity (12, 13). Indexes of insulin sensitivity derived from fasting values of insulin and glucose, such as the homeostatic model assessment of insulin resistance (HOMA-IR), are commonly used to investigate the associations between 25(OH)D concentrations and insulin sensitivity (1). These indexes tend to reflect more hepatic insulin resistance (14) as opposed to whole-body insulin sensitivity, which encompasses both hepatic and peripheral insulin sensitivity (15). In addition, the accuracy of indexes such as HOMA-IR in estimating whole-body insulin sensitivity may vary by ethnicity and obesity status (16). To our knowledge, no previous study has examined the relation between 25(OH)D concentrations and a direct measure of whole-body insulin sensitivity after adjusting for a robust measure of adiposity and PTH concentrations.

African Americans (AAs) have a disproportionately greater risk of insulin resistance and type 2 diabetes than do European Americans (EAs) (17). The causes of ethnic differences in insulin sensitivity are not clear and have not been explained by differences in adiposity and body composition (17). AAs also generally have lower serum 25(OH)D concentrations than EAs (9). To our knowledge, it has not been examined whether lower serum 25(OH)D concentrations in AAs relative to those in EAs contribute to ethnic differences in insulin sensitivity.

The overall objective of this study was to examine the relations of serum 25(OH)D and PTH concentrations with insulin sensitivity, independent of adiposity, in healthy women by using

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SUBJECTS AND METHODS

Subjects

Subjects were 50 healthy, premenopausal and postmenopausal women of AA and EA ethnicity identified from 2 studies conducted at the University of Alabama at Birmingham (UAB). Exclusion criteria for both studies were type 1 or type 2 diabetes, polycystic ovary syndrome, disorders of glucose or lipid metabolism, use of medications known to affect body composition or glucose metabolism (including antihypertensive medications), tobacco use, or abnormal glucose tolerance. All premenopausal women had regular menstrual cycles. A portion of the premenopausal women (n = 13) had been previously subjected to a weight-loss intervention (18) and were in the 2-y follow-up phase of the study (2 y after weight loss). Subject selection for this substudy was based on a sufficient quantity of sera to assay 25(OH)D and PTH, as well as available insulin sensitivity, DXA, and CT measurements. The mean age, body mass index (BMI; in kg/m²), percentage body fat, intra-abdominal adipose tissue (IAAT), and insulin sensitivity index (SI) of this substudy did not significantly differ from the respective parent studies, with mean differences <10% of mean values from the parent studies. All subjects were recruited between February 2001 and February 2007. Both studies were approved by the UAB Institutional Review Board for Human Use, and written informed consent was obtained from all subjects before testing.

Protocol

The protocol for each study was previously reported (18–20). In brief, subjects were admitted as inpatients to the UAB General Clinical Research Center, and insulin sensitivity and body-composition testing was performed. Weight stability in the post-weight-loss women was ascertained before testing via a 4-wk weight-maintenance diet with weights checked 3 times/wk and food provided in the last 2 wk. Testing in all premenopausal women was conducted during the follicular phase of the menstrual cycle. Metabolic tests were conducted year round.

Insulin sensitivity testing

Insulin sensitivity was measured by using an insulin-modified intravenous glucose tolerance test and minimal modeling after a 12-h overnight fast, the details of which were previously described (19, 20). In brief, a catheter was placed in the antecubital space of both arms, and fasted blood samples were drawn for subsequent analyses of lipids and hormones. Glucose (50% dextrose) was injected intravenously at time zero, and multiple subsequent blood draws were taken over a period of 3–4 h. Insulin (0.02 U insulin/kg) was administered at time 20 relative to glucose administration. Samples were processed and stored at ~80°C until ready for analysis. SI was determined from insulin and glucose values entered into the MINMOD computer program (version 3; Richard N Bergman, University of Southern California, Los Angeles, CA) (15). This index has been validated against and correlates well with the gold-standard euglycemic clamp (21). HOMA-IR was calculated as follows:

\[
[\text{fasting insulin concentration (in } \mu\text{U/mL}) \times \text{fasting glucose concentration (in mmol/L})] / 22.5
\]

Laboratory analyses

Glucose, insulin, and lipid profiles were assayed in the UAB Metabolism Core Laboratory of the General Clinical Research Center, Clinical Nutrition Research Center, Diabetes Research Training Center, and Center for Clinical and Translational Science. Glucose was measured in 10 µL sera with an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics, Rochester, NY). The mean intra- and interassay CVs for glucose analysis in the Core Laboratory were 0.61% and 1.45%, respectively. Serum concentrations were determined in duplicate 100-µL aliquots with double-antibody radioimmunoassays (Linco Research Inc, St Charles, MO). The insulin assay has a sensitivity of 3.35 µU/mL in the Core Laboratory and mean intra- and interassay CVs of 3.49% and 5.57%, respectively. Serum 25(OH)D concentrations were assayed with a liquid chromatography-tandem mass spectrometry technique (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA). Values are reported as total serum 25(OH)D concentrations [including 25(OH)D2 and 25(OH)D3]. Vitamin D deficiency was defined as serum 25(OH)D concentrations <20 ng/mL, and vitamin D insufficiency was defined as serum 25(OH)D concentrations <30 ng/mL. Serum PTH concentrations were assessed by a 2-site immunochemiluminometric assay that detected intact PTH and the aminoterminally truncated PTH fragments, and serum calcium concentrations were determined by using spectrophotometry (Quest Diagnostics Nichols Institute). The PTH value for one subject was >3 SD above the mean and, therefore, was excluded from the analyses.

Body composition and fat distribution

Percentage body fat was determined by using DXA (Lunar Prodigy densitometer; GE Healthcare Lunar, Madison, WI) in the UAB Department of Nutrition Sciences. Subjects were scanned in light clothing and in the supine position with their arms to the sides. IAAT and subcutaneous abdominal adipose tissue were determined by using CT (23) with a HiLight/Advantage Scanner (General Electric, Milwaukee, WI) in the UAB Department of Radiology. Subjects were scanned in the supine position with their arms above their heads. A 5-mm scan was taken at the level of the umbilicus (approximately the L4–L5 intervertebral space) and later analyzed for the cross-sectional area (in cm²) of adipose tissue with a density-contour computer program (NIH Image; NIH, Bethesda, MD; http://rsb.info.nih.gov/nih-image/) with Hounsfield units for adipose tissue set at −190 to −30.
Statistical analyses

Descriptive characteristics are reported as means (±SDs). The distribution of all continuous variables was examined, and variables that deviated from a normal distribution were log_{10} transformed (fasting insulin concentrations, SI, 25(OH)D concentrations, IAAT, and HOMA-IR). Partial correlation analyses, with adjustment for ethnicity, were used to investigate the relations of 25(OH)D and PTH concentrations with indexes of insulin sensitivity. Potential interactions of ethnic group with 25(OH)D and PTH concentrations were investigated with multiple linear regression analysis (MLR). Interaction terms were not statistically significant for any indexes of insulin sensitivity (P > 0.18 for all); thus, data are reported with both ethnic groups pooled together. Forward stepwise MLR was used to explore the best independent predictors of the dependent variable SI. The following variables were tested as predictors of SI: race, IAAT, subcutaneous abdominal adipose tissue, age, percentage body fat, and serum concentrations of PTH, 25(OH)D, and calcium. A significance probability value of 0.15 was used as the criteria for entry into and exit out of the model. Variables observed to be significant in the stepwise analysis were subsequently used in the MLR analysis for SI. To explore the possibility of a threshold effect of vitamin D concentration on SI, MLR analyses were repeated in subjects characterized as vitamin D insufficient. Ethnic differences in continuous variables were determined with 2-group t tests. Ethnic differences in mean SI, adjusted for IAAT and percentage body fat, were determined with analyses of covariance and compared with means that were further adjusted for 25(OH)D and PTH concentrations. Statistical analyses were 2-tailed with a significance level of 0.05 and were performed with SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS

Descriptive and metabolic characteristics for all women and by ethnic group are presented in Table 1. A majority of the women were premenopausal (78%) and overweight or obese (53% of women with a BMI >25). Ages ranged from 18.9 to 67.4 y. The mean 25(OH)D concentration was 22.3 ± 13.7 ng/mL with 54% of women classified as vitamin D deficient, and 72% of women were classified as vitamin D insufficient. Two-group t tests indicated that AAs had lower 25(OH)D (P < 0.001) concentrations and SI (P < 0.001) than EAs and had greater fasting insulin (P = 0.001) concentrations, HOMA-IR (P = 0.005), and PTH concentrations (P = 0.07). 25(OH)D concentrations did not differ by the season of blood draw (P = 0.28 (analysis of variance)).

Both 25(OH)D and PTH concentrations were significantly associated with SI in ethnic group–adjusted partial correlation analyses, (r = 0.31, P = 0.03 and r = −0.37, P = 0.009, respectively). Neither 25(OH)D nor PTH concentrations were significantly associated with fasting glucose or fasting insulin concentrations or HOMA-IR (P > 0.05 for all). The correlation between PTH and 25(OH)D concentrations was r = −0.25 (P = 0.08). Age was significantly associated with PTH concentrations (r = 0.29, P = 0.04) but not with 25(OH)D concentrations (r = 0.02, P = 0.89). Variables identified in stepwise MLR analysis as significant independent predictors of SI, and subsequently used in MLR analyses, were race, IAAT, age, and PTH and 25(OH)D concentrations. The final model for the dependent variable SI is shown in Table 2A and indicates that both 25(OH)D and PTH concentrations had independent associations with SI. The linear relations of 25(OH)D and PTH concentrations with SI, which were adjusted for all other variables in the model, are depicted in Figure 1. Subset MLR analysis in subjects with vitamin D insufficiency only is provided in Table 2B. Ethnic group was removed from the reduced model because the majority of subjects were AAs (n = 21), and race was not a significant correlate.

The ethnic difference in SI, which was adjusted for IAAT and percentage fat, was 2.70 [10^{-4} · min^{-1}(µIU/mL)] (adjusted mean 5.04 ± 0.59 and 2.34 ± 0.21 [10^{-4} · min^{-1}(µIU/mL)] for EAs and AAs, respectively) as shown in Figure 2 (P for ethnic difference <0.001). Adjustment for 25(OH)D

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 50)</th>
<th>European Americans (n = 25)</th>
<th>African Americans (n = 25)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38.2 ± 13.1</td>
<td>39.1 ± 13.3</td>
<td>37.4 ± 13.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Premenopausal status [% (n)]</td>
<td>39 (78)</td>
<td>39 (76)</td>
<td>20 (80)</td>
<td>0.73</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 4.7</td>
<td>25.1 ± 3.9</td>
<td>27.7 ± 5.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Percentage body fat</td>
<td>39.0 ± 7.9</td>
<td>38.6 ± 7.2</td>
<td>39.4 ± 8.6</td>
<td>0.74</td>
</tr>
<tr>
<td>IAAT (cm²)</td>
<td>76.6 ± 43.5</td>
<td>81.4 ± 40.6</td>
<td>71.8 ± 46.6</td>
<td>0.25</td>
</tr>
<tr>
<td>SAAT (cm²)</td>
<td>278.1 ± 150.1</td>
<td>251.8 ± 140.0</td>
<td>305.4 ± 157.8</td>
<td>0.21</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>91.2 ± 9.3</td>
<td>91.2 ± 8.3</td>
<td>91.3 ± 10.4</td>
<td>0.98</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>22.3 ± 13.7</td>
<td>29.3 ± 13.5</td>
<td>15.4 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>44.5 ± 17.7</td>
<td>40.0 ± 16.7</td>
<td>49.2 ± 17.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.5 ± 0.5</td>
<td>9.6 ± 0.6</td>
<td>9.4 ± 0.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Fasting insulin (µIU/mL)</td>
<td>11.0 ± 5.0</td>
<td>8.6 ± 3.4</td>
<td>13.3 ± 5.3</td>
<td>0.001</td>
</tr>
<tr>
<td>SI [10^{-4} · min^{-1}(µIU/mL)]</td>
<td>4.3 ± 2.7</td>
<td>5.7 ± 2.7</td>
<td>2.8 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.5 ± 1.2</td>
<td>1.97 ± 0.84</td>
<td>3.06 ± 1.36</td>
<td>0.005</td>
</tr>
</tbody>
</table>

1 IAAT, intraabdominal adipose tissue; SAAT, subcutaneous abdominal adipose tissue; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; SI, insulin sensitivity index; HOMA-IR, homeostatic model assessment of insulin resistance.

² P for ethnic differences measured by a 2-group t test or a chi-square test.

³ Mean ± SD (all such values).

⁴ Log_{10} transformed for analysis.

⁵ n = 24.
concentrations reduced the ethnic difference to 1.98 \times 10^{-2} \text{IU/mL} (P \text{ for ethnic difference} = 0.003). Adjustment for both 25(OH)D and PTH concentrations reduced the ethnic difference in SI by 33% to 1.80 \times 10^{-2} \text{IU/mL} (\text{adjusted mean SI} = 4.48 \pm 0.55 \text{ and } 2.68 \pm 0.34 \times 10^{-2} \text{IU/mL}) for EAs and AAs, respectively; P \text{ for ethnic difference} = 0.008).

### DISCUSSION

This study aimed to investigate the relations of circulating 25(OH)D and PTH concentrations with insulin sensitivity by using direct measures of insulin sensitivity after accounting for robust measures of body composition and fat distribution. Results indicated that both 25(OH)D and PTH concentrations were independently associated with a measure of whole-body insulin sensitivity. In addition, the magnitude of lower insulin sensitivity in AAs was reduced by adjustment for 25(OH)D and PTH concentrations. These observations suggest that vitamin D and PTH may play independent roles in the regulation of metabolic health, and that vitamin D supplementation may be useful for minimizing ethnic disparities at risk of type 2 diabetes.

Several studies have shown 25(OH)D concentrations to be associated with insulin sensitivity and resistance (1); however, the majority of studies relied on proxy indexes of insulin sensitivity, used crude measures of adiposity as adjusting variables, and/or have not accounted for PTH. We show, for the first time to our knowledge, that 25(OH)D concentrations were positively associated with a robust measure of insulin sensitivity, independent of percentage body fat, IAAT, and PTH concentrations in a sample of AA and EA women. Our use of DXA-derived percentage body fat and CT-derived IAAT as covariates, as opposed to BMI, provided accurate adjustment for both body composition and abdominal fat accumulation. Nevertheless, our results are in accordance with studies by Lind et al (6) and Chiu et al (3) who showed that 25(OH)D concentrations were positively associated with clamp-derived measures of insulin sensitivity, independent of BMI, in middle-aged Swedish men and in relatively young men and women of varying ethnicity, respectively. However, this is in contrast to a recent study in middle-aged Italian men and women that reported that 25(OH)D concentrations were associated with euglycemic clamp-derived insulin sensitivity but not after adjustment for BMI (24). It is likely that differences in study populations lead to varying outcomes. The relation between 25(OH)D concentrations and SI was strengthened when only

### TABLE 2

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Variable estimate ± SEE</th>
<th>Partial $r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. All subjects ($n = 49$)$^1$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td>0.28 ± 0.13</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>IAAT</td>
<td>-0.56 ± 0.13</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.006 ± 0.002</td>
<td>0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>0.01 ± 0.003</td>
<td>0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>Ethnic group</td>
<td>0.11 ± 0.04</td>
<td>0.19</td>
<td>0.003</td>
</tr>
<tr>
<td>B. Vitamin D insufficient ($n = 35$)$^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td>0.90 ± 0.17</td>
<td>0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IAAT</td>
<td>-0.40 ± 0.15</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.007 ± 0.002</td>
<td>0.31</td>
<td>0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.006 ± 0.004</td>
<td>0.07</td>
<td>0.14</td>
</tr>
</tbody>
</table>

$^1$ 25(OH)D, 25-hydroxyvitamin D; IAAT, intraabdominal adipose tissue; PTH, parathyroid hormone. Variables that were log$_{10}$ transformed for analyses were SI, 25(OH)D, and IAAT. Ethnic group was coded such that European American = 0 and African American = 1. Vitamin D–insufficient subjects were those with 25(OH)D concentrations <30 ng/mL.

$^2$ $R^2 = 0.60, P < 0.001$.

$^3$ $R^2 = 0.62, P < 0.001$.
vitamin D insufficient subjects were analyzed (Table 2B). A comparison of the partial contribution of 25(OH)D to SI in the whole group compared with the vitamin D insufficient subset (9% compared with 48%) suggested that 25(OH)D may be more clinically relevant to insulin sensitivity in vitamin D–insufficient subjects.

In contrast to observations with whole-body insulin sensitivity, 25(OH)D were not significantly associated with HOMA-IR, to EAs. However, adjustment for 25(OH)D and PTH concentrations in AAs did not fully explain their lower SI relative to EAs. However, adjustment for 25(OH)D and PTH concentrations reduced the magnitude of the ethnic difference in SI, suggesting that 25(OH)D and PTH concentrations explain a portion of this difference. Lower 25(OH)D and elevated PTH concentrations in AAs are likely a result of decreased cutaneous vitamin D synthesis (33) and low vitamin D and calcium intakes (34–36). Vitamin D supplementation is a feasible, safe, and relatively inexpensive approach to elevate 25(OH)D concentrations in AAs (37). We previously showed that dietary vitamin D and calcium were positively associated with insulin sensitivity in AA women (38). Vitamin D and calcium interventions in AAs may thus help to reduce ethnic health disparities in insulin resistance and type 2 diabetes.

Major strengths of this study were the use of sophisticated, robust methodology to measure insulin sensitivity, fat distribution, and body composition. The predictors of SI identified in this study [25(OH)D and PTH concentrations, IAAT, age, and ethnicity] explained 60% of the variance in SI. In addition, measurement of both 25(OH)D and PTH concentrations provided some insight into the independent roles of these hormones on metabolic health. Limitations of the study were that we did not have measures of dietary intake or physical activity in a majority of the women and, thus, could not determine the influence of these factors, and the cross-sectional nature of this study limited our ability to infer cause-and-effect relations. Furthermore, the relatively small sample size in the current study may have limited our power to detect significant ethnic differences in the relations of 25(OH)D and PTH concentrations with metabolic outcomes. Given previous suggestions that relations of dietary vitamin D and 25(OH)D concentrations with metabolic variables may differ in ethnic groups (38, 39), further study by using an appropriate methodology and sufficient sample size is warranted.
In conclusion, circulating 25(OH)D and PTH concentrations were independently associated with whole-body insulin sensitivity and may be more closely related to peripheral rather than hepatic insulin sensitivity. Although vitamin D and PTH are closely connected in the regulation of skeletal health, they likely influence metabolic health through independent mechanisms. Furthermore, lower 25(OH)D and greater PTH concentrations in AAs compared with EAs may contribute to the ethnic disparities in insulin resistance and type 2 diabetes.

We thank Maryellen Williams and Cindy Zeng for their continued laboratory support.

The authors’ responsibilities were as follows—JAA and APA: conceived of the project and developed the overall research plan; JAA, APA, and BAG: designed the study and interpreted the analyses; JAA: analyzed the data and was responsible for overall drafting of the manuscript; GRH: provided statistical expertise; APA, GRH, and BAG: provided critical review of the manuscript; and all authors: contributed to data collection and read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES