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Evidence for Threshold Effects of 25-Hydroxyvitamin D on Glucose Tolerance and Insulin Resistance in Black and White Obese Postmenopausal Women[1,2]

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Abstract

We identified normal vs. abnormal 25-hydroxyvitamin D [25(OH)D] concentrations by examining the relation of 25(OH)D to non-bone–related measures (plasma glucose, insulin resistance, lipids, blood pressure, fitness, obesity, and regional adiposity) and asking whether there is a 25(OH)D concentration above and below which the relation between 25(OH)D and outcome changes. We examined the relation between 25(OH)D and outcome by race to see whether race-specific normal ranges are needed, and we examined the role of insulin-like growth factor-1 (IGF-1) in modulating the relation between 25(OH)D and outcome. In a cross-sectional study of 239 overweight and obese, sedentary postmenopausal women without diabetes (83 black, 156 white), outcome measures included plasma lipids, glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), IGF-1, parathyroid hormone (PTH), aerobic fitness, body composition, subcutaneous abdominal and visceral fat, and blood pressure. We identified threshold effects in the association between 25(OH)D and these variables using piecewise linear regressions. We found that 25(OH)D was inversely related to fasting glucose, fasting and 2-h insulin, HOMA-IR, visceral abdominal fat, percentage fat, PTH, and triglycerides. Evidence for a threshold effect of 25(OH)D was found for 2-h glucose, 2-h insulin, fasting insulin, and HOMA-IR. There was no evidence suggesting the need for race-specific normal 25(OH)D concentrations. IGF-1 modulated the relation between 25(OH)D and outcome but only below, and not above, a threshold 25(OH)D concentration. Our findings suggest a threshold effect of 25(OH)D on glucose–insulin metabolism such that 25(OH)D $\geq$ ~26 μg/L (65.0 pmol/L) supports normal glucose homeostasis and that the same cut point defining normal 25(OH)D concentration can be used in black and white women. This study was registered at clinicaltrials.gov as NCT01798030. J. Nutr. 144: 734–742, 2014.

Introduction

The measurement of 25-hydroxyvitamin D [25(OH)D][8] concentration and prescription of vitamin D supplements to patients found to have a low concentration have become common medical practice (1). Despite the accepted importance of correcting vitamin D deficiency (2), the 25(OH)D concentrations that should be considered normal remain controversial. Guidelines for interpreting 25(OH)D concentration have been established primarily from studies of bone metabolism. In 2011, the Endocrine Society defined a 25(OH)D concentration of 30 μg/L (75 nmol/L) (3) as the lower limit of normal, 21–29 μg/L (52.5–72.5 nmol/L) as insufficiency, and <20 μg/L (50 nmol/L) as deficiency. In the same year, the Institute of Medicine (IOM) defined 20 μg/L (50 nmol/L) as the lower limit of normal (4) and divided the abnormal 25(OH)D concentration range into 2 categories: *1*) insufficient, 12–19.9 μg/L (30–50 nmol/L); and *2*) deficient, <12 μg/L (<30 nmol/L). The difference between the Endocrine Society and IOM clinical guidelines is primarily the result of differing interpretations of the effects of 25(OH)D on measures of bone health (5). In addition to its well-known effects on bone and mineral metabolism, low 25(OH)D concentration has been associated with glucose intolerance (6,7), type 2 diabetes (8), insulin resistance (9), hypertension (10),

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[7] J.D.S. and T.S.V. contributed equally to this work.
[8] Abbreviations used: IGF-1, insulin-like growth factor-1; IOM, Institute of Medicine; I_0, fasting plasma insulin; PTH, parathyroid hormone; VO_2max, maximal aerobic capacity; 25(OH)D, 25-hydroxyvitamin D.
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hyperlipidemia (11), and cardiovascular disease (9,12). Therefore, in addition to reflecting the relation of 25(OH)D to bone health, guidelines for interpreting 25(OH)D concentration should take into account the relation of 25(OH)D to non-bone end points. A threshold, if one exists, defining 2 regions, 1 in which 25(OH)D is related to metabolic outcome and another in which there is no relation, could help define the normal range.

At least in part because of increased skin melanin, blacks are at higher risk of vitamin D deficiency than whites (13). Although 25(OH)D concentration is known to be less strongly associated with bone health in blacks compared with whites (14), little is known about race-related differences in the relation between 25(OH)D and non-bone–related physiologic variables. Individuals who are obese are at increased risk of vitamin D deficiency (15), type 2 diabetes (6), and cardiovascular disease (16). Therefore, obese individuals constitute an ideal population in which to study the association of 25(OH)D with measures of glucose tolerance, insulin resistance, and other risk factors for cardiovascular disease. Our study had 2 goals: *1*) to examine the association between 25(OH)D and measures of glucose tolerance, insulin resistance, and other cardiovascular risk factors in overweight and obese postmenopausal women; and *2*) to determine whether the associations differ by race.

Participants and Methods

Participants. We studied 239 (83 black, 156 white) (**Table 1**) generally healthy, overweight and obese (mean BMI: 33.0 kg/m^2; IQR: 29.3–35.8; range: 24.1–51.4), sedentary (performing <20 min of aerobic exercise per week), postmenopausal women (mean age, 59.7 y; IQR: 54–64; range: 46–78). Women were excluded if they: *1*) had diabetes [fasting plasma glucose concentration ≥ 126 mg/dL (7.0 mmol/L), or a 2-h plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L), or were taking medication for diabetes]; *2*) had a history of coronary artery disease; or *3*) were current smokers. The women previously participated in at least 1 of 8 NIH-funded, Institutional Review Board–approved studies conducted at the Baltimore Veterans Affairs Medical Center Geriatrics Research, Education, and Clinical Center from June 1995 through July 2009 (17–19). The data used in this report were obtained during baseline testing for the studies. At the time the data were gathered, the only intervention the participants received was 6–8 wk of an AHA diet (20) designed to stabilize weight and metabolic variables. The study reported here was approved by the University of Maryland Institutional Review Board.

Body composition [fat-free mass (lean mass + bone mass) and percentage fat] was measured by DXA. Over the time period of the 8 studies that contributed data to the present analysis, 3 DXA scanners were used (DPX-L, DPXIQ, and Prodigy; all manufactured by GE LUNAR Radiation). The scanners were cross-calibrated to ensure that they gave comparable measurements. Visceral and subcutaneous abdominal fat was determined from a single computed tomography image obtained at the level of the second and third lumbar vertebral body as previously

TABLE 1 Characteristics of participants by race[1]

		Black			White			Difference (black − white)		
Variable	*N*	*n*	Mean	SE	*n*	Mean	SE	Mean	SE	*P*
Vitamin D										
25(OH)D, *nmol/L*	239	83	48.0	2.26	156	62.1	1.62	−14.1	2.76	0.001
Age and fitness										
Age, *y*	239	83	58.5	0.69	156	60.3	0.55	−1.8	0.91	0.048
VO_2max, $(mL/kg) \cdot min^{-1}$	205	73	17.7	0.52	132	20.1	0.36	−2.4	0.62	0.001
Body composition										
BMI, kg/m^2	239	83	34.7	0.62	156	32.0	0.35	2.7	0.66	0.001
Waist, *cm*	215	71	99.4	1.42	144	96.0	0.90	3.4	1.62	0.040
Waist:hip ratio	214	70	0.82	0.01	144	0.82	0.01	0.001	0.010	0.962
Fat-free mass,[2] *kg*	239	83	47.5	0.73	156	44.3	0.41	3.2	0.77	0.001
Percentage fat, *%*	239	83	48.4	0.54	156	46.9	0.39	1.4	0.66	0.034
Visceral fat, cm^2	198	69	149.6	7.38	129	159.6	5.07	−10.0	8.78	0.255
Subcutaneous abdominal fat, cm^2	171	57	420.8	21.8	114	347.3	11.6	73.5	22.54	0.004
Visceral:total abdominal fat ratio	171	57	0.27	0.01	114	0.32	0.01	−0.05	0.01	0.001
Glucose–insulin										
Fasting glucose, *mmol/L*	236	81	5.29	0.05	155	5.30	0.03	−0.01	0.06	0.828
2-h glucose, *mmol/L*	235	80	7.45	0.21	155	7.05	0.15	0.40	0.25	0.112
ln fasting insulin, *ln(pmol/L)*	223	74	4.56	0.05	149	4.27	0.03	0.29	0.06	0.001
ln 2-h insulin, *ln(pmol/L)*	222	73	6.28	0.09	149	6.01	0.05	0.28	0.10	0.005
ln HOMA-IR, *ln[(mg/dL) · (mmol/L)]*	223	74	1.31	0.06	149	1.03	0.04	0.28	0.06	0.001
Hormones										
IPTH, *pg/mL*	238	83	57.7	3.26	155	45.6	1.87	12.1	3.49	0.002
IGF-1, *nmol/L*	239	83	21.2	1.04	156	19.5	0.66	1.7	1.18	0.157
Blood pressure, *mm Hg*										
Systolic	229	82	127.1	1.55	147	122.3	1.11	4.8	1.89	0.012
Diastolic	229	82	73.6	1.13	147	71.7	0.75	1.9	1.31	0.139
Lipids										
Total cholesterol, *mmol/L*	238	83	5.01	0.10	155	5.12	0.07	−0.11	0.12	0.352
LDL, *mmol/L*	238	83	3.09	0.09	155	3.13	0.06	−0.04	0.11	0.708
HDL, *mmol/L*	238	83	1.37	0.04	155	1.32	0.03	0.05	0.05	0.296
ln TGs, *ln(mmol/L)*	239	83	0.12	0.04	156	0.33	0.03	−0.21	0.05	0.001

[1] IGF-1, insulin-like growth factor-1; IPTH, immunoreactive parathyroid hormone; VO_2max, maximal aerobic capacity; 25(OH)D, 25-hydroxyvitamin D.
[2] Lean mass + bone mass.

TABLE 2 Body composition, fitness, and race as predictors of 25(OH)D[1]

			Predictor slope[2]			Race (black − white)[3]		
Predictor	*N*	R^2	β	SE	*P*	β	SE	*P*
BMI (kg/m^2)	239	0.27	−1.191	0.264	0.001	−12.62	2.77	0.0001
Waist (cm)	215	0.27	−0.306	0.133	0.023	−13.28	2.88	0.0001
Waist:hip ratio	214	0.25	−11.584	20.214	0.567	−13.47	2.94	0.0001
Fat-free mass (kg)	239	0.27	−0.504	0.235	0.033	−12.84	2.78	0.0001
Percentage fat (%)	239	0.25	−1.031	0.260	0.001	−13.93	2.75	0.0001
Visceral abdominal fat (cm^2)	198	0.33	−0.075	0.024	0.002	−18.04	2.90	0.0001
Subcutaneous abdominal fat (cm^2)	171	0.30	−0.007	0.017	0.670	−17.66	3.39	0.0001
Visceral fat:total abdominal fat ratio	171	0.32	−49.237	22.213	0.028	−20.42	3.40	0.0001
VO_2max [(mL/kg) · min^{-1}]	205	0.29	0.860	0.341	0.012	−16.58	2.95	0.0001

[1] Adjusted for age, race, month plasma was obtained for 25(OH)D assay, percentage fat and length of time plasma was stored before 25(OH)D, PTH, and IGF-1 assays were performed. (VO_2max, BMI, and percentage fat were not adjusted for percent fat). All models significant at $P < 0.001$. IGF-1, insulin-like growth factor-1; PTH, parathyroid hormone; VO_2max, maximal aerobic capacity; 25(OH)D, 25-hydroxyvitamin D.
[2] Difference in vitamin D concentration (nanomoles per liter) for each unit difference in predictor variable.
[3] Difference in vitamin D concentration (black − white) adjusted for all other terms in the model.

described (note that our scans, compared with those described previously, were obtained at the second and third rather than third and fourth vertebral body) (19,21,22). Three computed tomography scanners were used to image our participants [PQ 6000 (GE Healthcare); Hi-Light, High Speed Advantage 9800 (GE Healthcare); and Siemens Somatom Sensation 64 (Siemens)]. Once a day, the scanners were calibrated to a water phantom (standard) to ensure that readings from the scanners were equivalent. Maximal aerobic capacity [VO_2max; (mL/kg) · min^{-1}] was measured during a graded treadmill exercise stress test during which oxygen utilization and CO_2 production were continuously measured (23). Fasting plasma glucose and fasting plasma insulin (I_0) concentrations were measured at the beginning of a 2-h, 75-g oral glucose tolerance test conducted in the morning after a 10-h overnight fast. Two-hour plasma glucose and insulin concentrations were measured at the end of the test. Plasma glucose concentration was measured using a glucose oxidase assay; plasma insulin concentration was measured by RIA. Plasma lipid concentrations were measured using an automated colorimetric assay. HDL concentration was measured in the supernatant after precipitation of VLDL, IDL, and LDL with dextran sulfate (24). LDL concentration was computed from total cholesterol, HDL, and TG concentrations using the Friedewald equation (25). Reported lipid concentrations are the mean of 3 plasma samples taken while the participant was metabolically stable over a period of 2.6 (1.4–3.9) wk [median (25th–75th percentiles)]. 25(OH)D, parathyroid hormone (PTH), and insulin-like growth factor-1 (IGF-1) were measured specifically for this report, from a single plasma aliquot that had been stored at −80°C for 0–13.8 y [7.5 (2.9–9.6) y; median (25th–75th percentiles)]. We report the mean of 2 measurements from each aliquot. Samples that had evidence of evaporation or crystallization were not assayed. Plasma PTH was measured using immunoradiometric assay [Nichols Institute Diagnostics assay, sensitivity: 1.0 pg/mL (1 ng/L); intra-assay CV: 4.09%; interassay CV: 4.94%], plasma IGF-1 was measured using RIA [Nichols Institute Diagnostics assay, sensitivity: 0.1 μg/L (0.013 nmol/L); intra-assay CV: 7.15%; interassay CV: 9.63%]. Plasma 25(OH)D was measured using RIA [Diasorin assay, sensitivity: 3.0 μg/L (7.5 pmol/L); intra-assay CV: 5.19%; interassay CV: 7.90%].

Statistical methods. Analyses started with exploratory data analysis looking for extreme values. Extreme values were checked to ensure that they were not the result of transcription or other errors. This was followed by visual inspection of bivariate plots depicting the association of 25(OH)D with each of our study variables (**Tables 2** and **3**). Each plot included a smoothing spline fit to the data. Several of the plots suggested a critical 25(OH)D concentration of ~25 ng/dL (62.5 pmol/L) defining 2 regions—1 above and 1 below a critical value (knot)—such that, below the knot, the slope was steeper than that above the knot. For each non-obesity outcome (Table 3), we performed a piece-wise linear regression with a single knot (SAS proc nlin), adjusted for race, age, and time (days), plasma was stored prior to 25(OH)D assay. The regressions, performed with no a priori assumption about the location of the knot, were run to determine objectively if a knot existed, the location of the putative knot, and the slopes above and below the knot. [There were no statistically significant race × 25(OH)D interactions.] For 4 outcome measures—2-h glucose, I_0 and 2-h insulin, and HOMA-IR—evidence of a knot was found. For these outcomes, to determine whether the existence of a knot would survive additional adjustment, multiple variable piecewise continuous linear regressions were run (SAS proc GLM) in which the 25(OH)D concentration at which the knot was observed defined 2 straight lines: 1 below the knot and a second above the knot (**Table 4**). These analyses (and all other regressions unless otherwise noted) were adjusted for age, race, month plasma was obtained for 25(OH)D assay, time (number of days) plasma was stored before 25(OH)D, PTH, and IGF-1 assay, percentage fat (by DXA), and, depending on the outcome variable, the use or non-use of drug therapy for hyperlipidemia (outcome lipids), hypertension (outcome blood pressure), or postmenopausal hormonal replacement (outcome measures glucose, insulin, and HOMA-IR). To allow our results to be compared with studies that did not look for a critical 25(OH)D threshold, for each non-obesity outcome variable, we performed a multiple variable linear regression looking for a single slope relating 25(OH)D to the outcome variable (Table 3). To determine whether IGF-1 modulated the relation between 25(OH)D and outcome, we performed additional analyses in which IGF-1 and an IGF-1× 25(OH)D interaction were added to the multiple variable piecewise continuous linear regressions and the single-slope multiple-variable linear regressions. For the single-slope multiple-variable linear regressions, 2 terms were added to the model: *1)* IGF-1; and *2)* IGF-1 × 25(OH)D. The single-slope multiple-variable linear regressions were as follows:

$$\text{outcome} = \ldots + \beta_{\text{IGF-1}} \times \text{IGF-1} + \beta_{25(\text{OH})\text{D}} \times 25(\text{OH})\text{D} + \beta_{\text{IGF-1:25(OH)D}} \times \text{IGF-1} \times 25(\text{OH})\text{D},$$

where … represents terms for intercept, age, race, month plasma was obtained for 25(OH)D assay, time (number of days) plasma was stored before 25(OH)D, PTH, and IGF-1 assay, and other adjustments as described above; $\beta_{\text{IGF-1}}$ is the IGF-1 slope; $\beta_{25(\text{OH})\text{D}}$ is the 25(OH)D slope; and $\beta_{\text{IGF-1:25(OH)D}}$ is the IGF-1 × 25(OH)D interaction slope.

For the piecewise continuous linear regressions, IGF-1 and 2 interactions were added to the model: *1)* the product of vitamin D below the knot and IGF-1; and *2)* the product of IGF-1 and the difference between the IGF-1 slopes above and below the knot. The piecewise continuous linear regressions were as follows:

$$\begin{aligned}\text{outcome} = \ldots &+ \beta_{\text{IGF-1}} \times \text{IGF-1} + \beta_{25(\text{OH})\text{DBelowKnot}} \times 25(\text{OH})\text{D}_{\text{BelowKnot}} \\ &+ \beta_{\text{IGF-1:25(OH)DBelowKnot}} \times \text{IGF-1} \times 25(\text{OH})\text{D}_{\text{BelowKnot}} \\ &+ \beta_{25(\text{OH})\text{DDiff}} \times \max(25(\text{OH})\text{D} - \text{knot}, 0) \\ &+ \beta_{\text{IGF-1:25(OH)DDiff}} \times \text{IGF-1} \times \max(25(\text{OH})\text{D} - \text{knot}, 0),\end{aligned}$$

where $\beta_{25(\text{OH})\text{DBelowKnot}}$ and $\beta_{\text{IGF-1:25(OH)DBelowKnot}}$ are the 25(OH)D slope and interaction slope below the knot, $25(\text{OH})\text{D}_{\text{BelowKnot}}$ is the

TABLE 3 25(OH)D and race as predictors of metabolic outcome[1]

			25(OH)D slope [units predicted variable/(nmol/L)][2]			Race (black − white)[3]		
Predicted outcome	*N*	R^2	β[4]	SE	*P*	β	SE	*P*
Fitness								
VO_2max [(mL/kg) · min^{-1}]	205	0.27	0.0379	0.0150	0.012	−1.3616	0.6622	0.041
Body composition								
BMI (kg/m^2)	239	0.57	−0.0288	0.0119	0.017	0.9714	0.5183	0.062
Waist (cm)	215	0.32	−0.0848	0.0369	0.023	0.0609	1.5944	0.970
Fat-free mass (kg)	239	0.23	−0.0402	0.0188	0.033	1.6036	0.8149	0.050
Percentage fat (%)	239	0.13	−0.0638	0.0161	0.001	0.4831	0.7227	0.505
Visceral fat (cm^2)	198	0.22	−0.6667	0.2167	0.002	−26.3997	9.3598	0.005
Subcutaneous abdominal fat (cm^2)	171	0.63	−0.1655	0.3876	0.670	50.9069	17.2272	0.004
Visceral:total abdominal fat ratio	171	0.44	−0.0006	0.0003	0.028	−0.0595	0.0126	0.001
Glucose–insulin								
Fasting glucose (mmol/L)	217	0.14	−0.0043	0.0016	0.008	−0.1573	0.0722	0.030
2-h glucose (mmol/L)	216	0.13	−0.0114	0.0068	0.093	0.1999	0.3073	0.516
ln fasting insulin [ln(pmol/L)]	211	0.19	−0.0044	0.0016	0.007	0.1656	0.0721	0.023
ln 2-h insulin [ln(pmol/L)]	210	0.14	−0.0061	0.0026	0.022	0.2283	0.1178	0.054
ln HOMA-IR [ln(mg/dL) · (mmol/L)]	211	0.18	−0.0052	0.0018	0.004	0.1350	0.0785	0.087
Hormones								
IPTH (pg/mL)	238	0.19	−0.4129	0.0857	0.001	5.1038	3.7277	0.172
ln IGF-1 (nmol/L)	239	0.10	−0.0025	0.0014	0.071	0.0275	0.0609	0.652
Blood pressure (mm Hg)								
Systolic	220	0.27	−0.0489	0.0466	0.295	4.7731	2.0964	0.024
Diastolic	220	0.38	−0.0142	0.0300	0.637	3.2609	1.3505	0.017
Lipids								
Total cholesterol (mmol/L)	225	0.12	−0.0004	0.0032	0.892	−0.1406	0.1389	0.313
LDL (mmol/L)	225	0.10	0.0015	0.0029	0.592	−0.0041	0.1253	0.974
HDL (mmol/L)	225	0.08	0.0005	0.0013	0.721	0.0129	0.0553	0.816
ln TGs [ln(mmol/L)]	226	0.20	−0.0043	0.0013	0.001	−0.2888	0.0552	0.001

[1] IGF-1, insulin-like growth factor-1; IPTH, immunoreactive parathyroid hormone; VO_2max, maximal aerobic capacity; 25(OH)D, 25-hydroxyvitamin D.
[2] Change in outcome variable for each unit increase in vitamin D expressed in units predicted variable/(nmol/L). Adjusted for age, race, month plasma was obtained for 25(OH)D assay, percentage fat, and length of time plasma was stored before 25(OH)D, PTH, and IGF-1 assays were performed.) VO_2max and percentage fat were not adjusted for percentage fat. Additional adjustments were made for hormone replacement therapy (glucose, insulin, HOMA-IR), antihypertensive therapy (blood pressure), and antihyperlipidemia therapy (lipids).
[3] Difference in vitamin D concentration in blacks − whites adjusted for all other terms in the model.
[4] Difference in outcome variable for each unit difference in vitamin D [units predicted variable /(nmol/L)].

25(OH)D concentration below the knot, and $\beta_{25(OH)DDiff}$ and $\beta_{IGF\text{-}1:25(OH)DDiff}$ are the differences between the 25(OH)D and interaction slopes above and below the knot. Max(25(OH)D − knot, 0) is the maximum value of 25(OH)D − knot and zero. For a 25(OH)D concentration below the knot, the value is zero. For a concentration above the knot, the value is the 25(OH)D concentration centered at the value of the knot. This parameterization is a convenient way to implement piecewise continuous regression analysis (26).

For both the single-slope multiple-variable linear regressions and the multiple-variable piecewise continuous linear regressions, we quantified the effect the IGF-1 × 25(OH)D interaction had on the vitamin D slope by calculating the fractional change in the value of each outcome variable associated with a 10 μg/L (25 pmol/L) increase in 25(OH)D concentration at 2 different IGF-1 concentrations. The IGF-1 concentrations used in the calculations, 86 and 234 μg/L (11.3 and 30.7 nmol/L), were the 10th and 90th percentiles of the distribution of IGF-1 values. For the multiple-variable piecewise continuous linear regressions, these computations were performed separately for the line below and above the knot. Because not all participants had every outcome measured, the number of participants reported varies by outcome. A 2-tailed $P < 0.05$ was considered significant.

Results

Clinical characteristics of study participants. The 83 black women on average were younger and more obese (higher BMI and percentage fat), had a greater waist circumference, had more subcutaneous abdominal fat [and a higher proportion of their total abdominal fat (subcutaneous + visceral) was subcutaneous], had lower fitness (lower VO_2max), and had lower 25(OH)D concentration than the 156 white women (Table 1). These differences were unchanged after adjustment for age (data not shown). According to the 25(OH)D cut points defined by the IOM (4), 62% of the black participants had either an insufficient (40%) or deficient (22%) 25(OH)D concentration compared with 27% of the whites (23% insufficient, 4% deficient) (**Fig. 1**). If the ≥30 μg/L concentration (75 nmol/L) defined by the Endocrine Society (3) is accepted as normal, 92% of the black and 76% of the whites would be classified as having an abnormal 25(OH)D concentration. Plasma PTH concentration was higher in blacks than whites ($P < 0.002$). There was no evidence of a significant difference in IGF-1 concentration in blacks vs. whites (Table 1). There was no difference by race in the percentage of participants who had impaired fasting glucose (blacks: 30%, 24 of 81 vs. whites: 28%, 43 of 155; $P < 0.76$) or impaired glucose tolerance (blacks: 43%, 34 of 80 vs. whites: 36%, 55 of 155; $P < 0.29$). Despite this, blacks had higher I_0 and 2-h insulin concentrations and greater insulin resistance (higher HOMA-IR) than whites (Table 1). Women with insufficient [IOM criteria (4), 12–19.9 μg/L (30.0–49.7 pmol/L)] or deficient [<12 μg/L (<30.0 pmol/L)] 25(OH)D had significantly higher

TABLE 4 25(OH)D and race as predictors of metabolic outcome[1]

Predicted outcome	N	R^2	P Model	25(OH)D threshold[6]	25(OH)D slope [units predicted variable/(nmol/L)][2]								Race (black − white)[5]		
					Below threshold			Above threshold[3]			Difference (above − below)[4]				
					β	SE	P	β	SE	P	β	SE	P	β	SE	P
				nmol/L												
Fasting glucose (mmol/L)	217	0.15	0.017	58.7	−0.0068	0.0031	0.030	−0.0023	0.0027	0.395	0.0045	0.0048	0.346	−0.1654	0.0727	0.024
2-h glucose (mmol/L)	216	0.14	0.022	53.3	−0.0410	0.0155	0.009	0.0040	0.0099	0.684	0.0450	0.0213	0.035	0.1234	0.3068	0.688
ln fasting insulin [ln(pmol/L)]	211	0.21	0.000	64.6	−0.0091	0.0026	0.001	0.0018	0.0031	0.575	0.0109	0.0048	0.024	0.1496	0.0717	0.038
ln 2-h insulin [ln(pmol/L)]	210	0.19	0.002	58.1	−0.0193	0.0050	0.000	0.0045	0.0043	0.300	0.0237	0.0077	0.002	0.1930	0.1158	0.097
ln HOMA-IR [ln[(mg/dL) · (mmol/L)]	211	0.20	0.001	62.6	−0.0105	0.0030	0.001	0.0008	0.0032	0.799	0.0113	0.0052	0.030	0.1182	0.0782	0.132

[1] Evidence for different slopes above and below a critical 25(OH)D concentration. IGF-1, insulin-like growth factor-1; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
[2] Change in outcome variable for each unit increase in vitamin D expressed as units predicted variable/[nmol/L · (25(OH)D)$^{-1}$]. Adjusted for age, month plasma was obtained for 25(OH)D assay, length of time plasma was stored before 25(OH)D, PTH, and IGF-1 assays were performed percentage fat, race, and postmenopausal hormone replacement therapy.
[3] Computed from the coefficients returned by the piecewise continuous linear regression and the variance–covariance estimates of the parameters.
[4] Slope above knot minus slope below knot.
[5] Difference in 25(OH)D concentration in blacks − whites adjusted for all other terms in the model.
[6] Knot, critical 25(OH)D concentration separating regions with different slopes.

FIGURE 1 25(OH)D, by age and race, within 3 diagnostic categories defined by the IOM. The values are the number and percentage of participants within each IOM category. IOM, Institute of Medicine; 25(OH)D, 25-hydroxyvitamin D.

measures of adiposity (percentage fat, visceral fat, subcutaneous abdominal fat, and BMI) than participants with sufficient values [>20 μg/L (49.4 pmol/L)] regardless of race (data not shown).

Body composition and aerobic fitness as predictors of plasma 25(OH)D concentration. BMI, waist circumference, fat-free mass, percentage fat, visceral fat area, and the ratio of visceral fat to total abdominal fat [visceral/(visceral + subcutaneous)] were significant inverse predictors of 25(OH)D concentration; aerobic fitness (VO_2max) was directly related to 25(OH)D (Table 2). The waist-to-hip ratio and subcutaneous fat area were not significantly related to 25(OH)D concentration. Blacks had lower 25(OH)D concentrations than whites independent of age, percentage fat, the various measures of body composition, or VO_2max (race term; all $P < 0.01$) (Table 2). PTH did not affect the association of the measures of obesity or VO_2max with 25(OH)D, and there were no significant interactions with PTH (data not shown).

Plasma 25(OH)D as a predictor of metabolic and physiologic measures: single-slope linear regression. Plasma 25(OH)D concentration was a significant predictor of glucose concentration and insulin resistance (all slopes $P \leq 0.04$ except 2-h glucose, $P < 0.093$) and a significant predictor of PTH concentration ($P < 0.01$) (Table 3). The slopes were all negative, indicating that measures of glucose–insulin physiology improved (i.e., decreased) and that PTH decreased with increasing 25(OH)D concentration. 25(OH)D concentration was unrelated to blood pressure, IGF-1 ($P < 0.071$), or lipids other than TGs. There was no evidence of different slopes relating 25(OH)D to any of the physiologic measures studied in blacks and whites [all race × 25(OH)D interaction terms were nonsignificant and were dropped from the models]. At any 25(OH)D concentration, fasting plasma glucose concentration and TG concentration were significantly lower and I_0 was significantly higher in blacks than whites (race term) (Table 3).

IGF-1 modulated the relation between 25(OH)D and both I_0 and insulin resistance (HOMA-IR). When IGF-1 and an IGF-1 × 25(OH)D interaction were added to the equation predicting logI_0, the interaction was almost significant ($P < 0.052$). When the interaction was added to the equation predicting logHOMA-IR, the interaction was of borderline significance ($P < 0.073$).

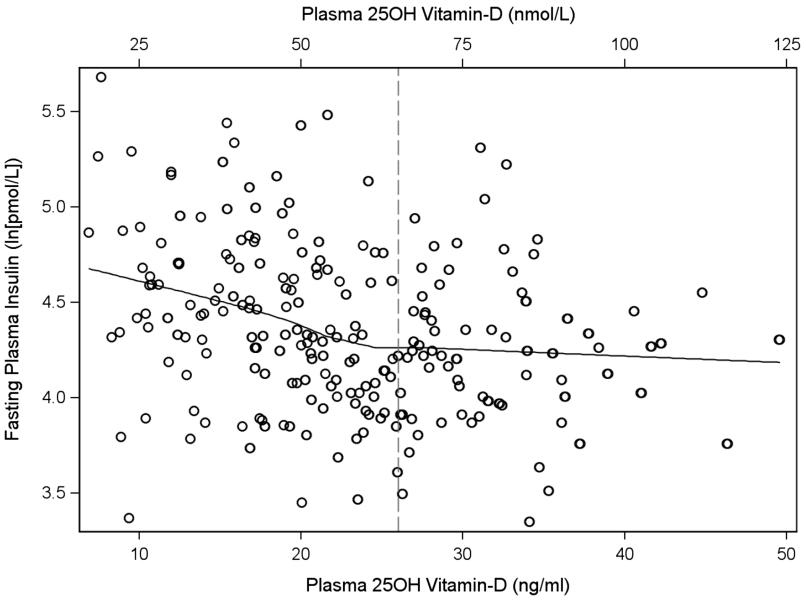

FIGURE 2 Scatter plot of the ln of fasting insulin and 25OH Vitamin-D and concentrations along with a smoothing cubic spline (43) fit to the data suggesting regions with 2 distinct slopes. The vertical line corresponds to the location of the knot (26 ng/m) separating the 2 regions. The location of the knot was determined by a piecewise linear regression. For details, see Statistical methods and Results. 25OH Vitamin-D, 25-hydroxyvitamin D.

In a woman with an IGF-1 of 86 μg/L (11.3 nmol/L), a 10 μg/L (25 pmol/L) increase in 25(OH)D concentration resulted in a 16% ($P < 0.001$) decrease in I_0 concentration and an 18% ($P < 0.001$) decrease in HOMA-IR. For a woman with an IGF-1 of 234 μg/L (30.7 nmol/L), a 10 μg/L (25 pmol/L) increase in 25(OH)D produced a minimal, nonsignificant 3% ($P = 0.40$) decrease in I_0 and a minimal, nonsignificant 5% decrease in HOMA-IR ($P = 0.55$).

Evidence for a critical 25(OH)D concentration: piecewise continuous regression. Bivariate scatter grams of 2-h plasma glucose concentration, 2-h and fasting insulin concentrations, and HOMA-IR plotted against 25(OH)D (**Fig. 2**, only fasting insulin shown) suggested the presence of a critical 25(OH)D concentration (knot) defining regions with 2 different slopes. 25(OH)D concentrations below the knot were inversely related to the metabolic variable included in the plots. 25(OH)D concentrations above the knot were unrelated to the metabolic variable. The suggestion by the plot of 2 regions, each with a distinct slope, was confirmed by multivariable piecewise linear regression with a single knot (Table 4). The slopes below the knots (threshold) were uniformly negative and statistically significant [indicating that, below the knot, decreasing 25(OH)D concentration was associated with progressively higher glucose concentration and greater insulin resistance]. The slopes above the knot were uniformly nonsignificant and significantly different from the slopes below the knot. The knots for the 4 measures of plasma glucose and insulin physiology occurred between 21.8 and 25.9 μg/L (53.3 and 64.6 pmol/L) 25(OH)D. There was no evidence of different slopes in blacks vs. whites. After adding interaction terms, neither of the 2 race $\times$ 25(OH)D interactions (1 for the slope below the knot and a second for the slope above the knot) were statistically significant in any of the analyses (data not shown). There was no evidence of a knot in the association between 25(OH)D and lipids, blood pressure, or VO_2max.

IGF-1 modulates the actions of 25(OH)D below but not above 25(OH)D threshold concentrations: piecewise continuous regression. IGF-1 modulated the relation between 25(OH)D and both I_0 and insulin resistance (HOMA-IR). In the equation predicting I_0, there was a significant IGF-1 $\times$ 25(OH)D interaction below the knot [25.9 g/L (64.6 pmol/L), $P < 0.004$] but not above ($P = 0.10$), and in the equation predicting HOMA-IR, there was a significant interaction below the knot [25.1 g/L (62.6 pmol/L), $P < 0.007$] but not above ($P = 0.13$).

Women with a low 25(OH)D concentration (25 nmol/L) and a low IGF-1 concentration (11.3 nmol/L) had the highest I_0 concentration and HOMA-IR (**Table 5**). Increasing IGF-1 to 30.0 nmol/L resulted in a statistically significant decrease in both I_0 (122–84 pmol/L, $P < 0.004$) and HOMA-IR [4.9–3.4 (mg/dL) · (mmol/L), $P < 0.008$]. Increasing IGF-1 in a woman with a higher 25(OH)D, 50 nmol/L, made no statistically significant change in either I_0 (80–78 pmol/L, $P < 0.74$) or HOMA-IR (3.1–3.1, $P < 0.91$). Increasing IGF-1 in a woman with a 25(OH)D of 70 nmol/L, and an IGF-1 of 11.3 resulted in a borderline statistically significant increase in I_0 (64–75 pmol/L, $P = 0.11$) and HOMA-IR (2.5–2.9, $P = 0.13$). Increasing IGF-1 in a woman with a 25(OH)D of 95 nmol/L and an IGF-1 of 11.3 resulted in no statistically significant increase in I_0 (70–78 pmol/L, $P < 0.57$) or HOMA-IR (2.6–3.0, $P < 0.58$).

PTH. PTH had a moderate, statistically significant correlation with 25(OH)D ($r = -0.35$, $P < 0.001$). When PTH alone or a PTH $\times$ 25(OH)D interaction was added to the models (multiple-variable linear regression or piecewise continuous regression), there was no evidence of PTH, either as a main effect or an interaction, having a significant effect on the outcome measures studied (data not shown).

Discussion

We found in postmenopausal, overweight and obese women without diabetes that low 25(OH)D was associated with elevated glucose concentration and increased insulin resistance. To the best of our knowledge, this is the first study to report evidence of a threshold effect of 25(OH)D with these measures. As discussed below, our findings suggest that there is no need for race-specific vitamin D cut points defining normal vs. abnormal 25(OH)D concentrations and that IGF-1 modulates the relation between 25(OH)D and outcome but only below a critical 25(OH)D concentration. We found no relation between 25(OH)D and blood pressure or lipids (other than TGs) or fitness.

No need for race-specific vitamin D cut points. Our finding that the slopes relating 25(OH)D to outcome did not differ in blacks and whites suggests that response to vitamin D supplementation [the change in outcome for a given change in plasma 25(OH)D concentration] would be the same in blacks and

TABLE 5 IGF-1 modulates the relation between 25(OH)D and outcome (insulin and HOMA-IR) below, but not above, the knot[1]

	Predicted Values			
	Fasting insulin		HOMA-IR	
	IGF-I used in computation			
25(OH)D Location	11.3 nmol/L	30.0 nmol/L	11.3 nmol/L	30.0 nmol/L
	pmol/L		*(mg/dL) · (mmol/L)*	
Below the knot				
25 nmol/L	122 (96, 155)	84 (67, 106)	4.9 (3.8, 6.4)	3.4 (2.6, 4.4)
50 nmol/L	80 (68, 95)	78 (66, 93)	3.1 (2.6, 3.7)	3.1 (2.5, 3.7)
Above the knot				
70 nmol/L	64 (53, 77)	75 (63, 90)	2.5 (2.0, 3.1)	2.9 (2.4, 3.5)
95 nmol/L	70 (55, 89)	78 (58, 104)	2.6 (2.0, 3.4)	3.0 (2.1, 4.1)

[1] Data are presented as the predicted value (95% CI). 25(OH)D knots: fasting insulin, 58.7 nmol/L; HOMA-IR, 62.6 nmol/L. Values were computed for a 50-y-old black woman taking postmenopausal replacement hormones, whose body composition was 40% fat and whose blood was obtained in December and stored 6.3 y before 25(OH)D, IGF-1, and PTH-1 assays. IGF-1, insulin-like growth factor-1; PTH-1, parathyroid hormone-1; 25(OH)D, 25-hydroxyvitamin D.

whites. Although we found that, for a given 25(OH)D concentration, fasting glucose and TG concentrations were lower and I_0 and HOMA-IR were higher in blacks than whites (race term) (Table 3), the race-related differences were small and of minimal biologic importance. These findings suggest that, at least for non-bone–related outcome measures, there is no need for race-specific definitions of normal vs. abnormal 25(OH)D concentration; the same cut points can be used in blacks and whites. It is possible that our inability to find any race × 25(OH)D interactions was a Type II error (failure to identify a true effect). To the best of our knowledge, our study is the first to look for race-related differences in the relation between 25(OH)D and the non-bone–related outcome measures. Larger studies will be needed to confirm our findings.

Support for a cut point separating normal from insufficient 25(OH)D between 20 and 26 μg/L. Other investigators found associations between 25(OH)D and measures of glucose–insulin physiology (27), but we believe we are the first to report evidence of a threshold effect for 25(OH)D concentration on these associations. We found that, for 25(OH)D concentrations <21–26 μg/L (52.5–65.0 pmol/L), glucose–insulin metabolism worsened with progressively lower 25(OH)D, whereas >26 μg/L (65.0 pmol/L), there was no relation. These results suggest that, at least for glucose homeostasis and insulin resistance, there is 25(OH)D insufficiency >20 but <30 μg/L (50 and 75 pmol/L) and that a more appropriate cut point separating normal from insufficient would be in the region of 20–26 μg/L (50.0–65.0 pmol/L). A recent meta-analysis (8) found that diabetes was less likely when 25(OH)D was ≥25 μg/L (62.5 pmol/L), supporting the idea that the lower normal range of 25(OH)D should be higher than 20 μg/L (50.0 pmol/L). Our study was of modest size; larger studies are needed to precisely define the 25(OH)D concentration threshold for measures of glucose–insulin metabolism.

Glucose metabolism and insulin resistance in blacks and whites. Consistent with our previous reports from 3 (17,18,21) of the 7 studies that contributed participants to the present study and as reported by others (28), we demonstrate that middle-aged black women have lower fasting glucose concentration, higher I_0 concentrations, and higher HOMA-IR than whites of the same age. This study extends these findings by showing that the lower fasting glucose concentration, higher I_0 concentrations, and higher HOMA-IR in blacks than whites are not caused by the lower 25(OH)D concentrations in blacks than whites [the findings remained after controlling for 25(OH)D concentration (Table 3)]. The lower fasting glucose, despite greater insulin resistance in blacks than whites, may indicate the presence in blacks of metabolic factors that compensate for the deleterious effects of lower 25(OH)D concentration on glucose homeostasis.

IGF-1 modulates metabolic effects of 25(OH)D. In our study, IGF-1 modulated the relation between 25(OH)D concentration and outcome: I_0 concentration and HOMA-IR. We found that higher IGF-1 concentrations were associated with lower I_0 concentration and less insulin resistance. Other researchers found similar results (29–31). We extend the previous studies by demonstrating that IGF-1 affects the slope of the relation between 25(OH)D and outcome at low, but not high, 25(OH)D concentrations [i.e., below, but not above, the threshold 25(OH)D concentrations]. The interplay between vitamin D, IGF-1 I_0, and HOMA-IR may have important health implications. Low 25(OH)D concentration increases the risk of adverse cardiometabolic outcome (32). Vitamin D reduces inflammatory processes (33), resulting in improved insulin sensitivity (34), and has been shown to reduce inflammation after coronary events (35). Conversely, low 25(OH)D raises PTH concentration, which is associated with insulin resistance in healthy adults (36).

Body composition predicting 25(OH)D. In this study and in our previous work (17,18,21), we found that black women compared with white women of the same age had greater adiposity (BMI, percentage fat) and larger waist circumference and that a higher proportion of their abdominal fat was stored in the subcutaneous, compared with the visceral, compartment. We found that BMI, waist circumference, percentage fat, and visceral fat, but not subcutaneous abdominal fat or waist-to-hip ratio (Table 2), predicted 25(OH)D.

Although others report inverse associations between 25(OH)D and subcutaneous fat (37), the physiology underlying the association is not fully understood. It has been suggested that 25(OH)D is sequestered in adipose tissue (15), resulting in lower circulating 25(OH)D with increasing body fat. Drincic et al. (38) propose another explanation for the inverse ratio: volumetric dilution. They propose that 25(OH)D is not sequestered within fat but passively diffuses into and out of adipose tissue based on an equilibrium between blood 25(OH)D (bound to D-binding protein) and 25(OH)D within body fat depots.

Our finding that the slopes relating measures of obesity and regional adiposity to 25(OH)D were similar in blacks and whites (Table 2) suggests that changes in body composition have similar effects on 25(OH)D in black and white women. The statistically significant and negative race term indicating that 25(OH)D concentration is lower in blacks than whites for a given amount of visceral or subcutaneous abdominal fat suggests that vitamin D is stored differentially in different fat depots in blacks and whites.

Storage before assay. The plasma used in our 25(OH)D, IGF-1, and PTH assays was stored at −80°C for up to 13.8 y [7.5 (2.9–9.6) y; median (25th–75th percentiles)] before assay. All other biochemical assays were run shortly after plasma was obtained. We have 2 reasons to believe that the storage time did not affect our results. First, to see whether storage time affected our results, we compared results from analyses in which we controlled for time of storage vs. analyses in which we did not control for time of storage. The results of the 2 sets of analyses were remarkably similar; inferences derived from the 2 sets of analyses were the same. Second, 25(OH)D has been shown to be stable in long-term frozen storage. Serum 25(OH)D concentrations measured from frozen samples stored from 6–24 y showed practically no correlation with storage time (39). IGF-1 is also stable in storage. Serum values of IGF-1 obtained from serum stored for 9 years at −80°C were similar to concentrations found in freshly collected sera (40). The long-term stability of PTH is more controversial. A comparison of PTH concentrations obtained from samples stored in EDTA tubes for 5 y with matched controls found no significant difference between the concentrations (41). In contrast, a 27% decrease in PTH was found in serum samples stored for 10 mo and subjected to 1 freeze–thaw cycle before analysis (42).

In summary, we report an inverse relation between 25(OH)D and fasting plasma glucose, I_0 and 2-h insulin, HOMA-IR, and TGs and found evidence for a threshold effect of 25(OH)D on 2-h glucose [21.4 μg/L (53.5 pmol/L)], 2-h insulin [23.3 μg/L (58.1 pmol/L)], I_0 [25.9 μg/L (64.6 pmol/L)], and HOMA-IR [25.1 μg/L (62.6 pmol/L)]. Although the slopes of the relations between 25(OH)D and fasting glucose, I_0, and TGs were similar in blacks and whites, glucose and TG concentrations were lower and insulin concentrations were higher for a given 25(OH)D in blacks than whites. Our findings suggest a threshold effect of 25(OH)D on glucose–insulin metabolism such that 25(OH)D concentrations <26 μg/L (65.0 pmol/L) are inversely associated with outcome and concentrations ≥26 μg/L (65.0 pmol/L) are unrelated to outcome in overweight and obese black and white postmenopausal women. Additional studies are needed to confirm that our results hold in other populations, including other racial and ethnic groups (e.g., Hispanics and Asians), studies in men, and studies in younger and older participants. A large, prospective interventional study in black and white women will be needed to confirm that increasing 25(OH)D concentration above ~26 μg/L (65.0 pmol/L) improves glucose homeostasis and insulin sensitivity with little improvement above this value.

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