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Regional Adipose Tissue Associations With Calcified Atherosclerotic Plaque: African American–Diabetes Heart Study

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Abstract

Coronary artery calcified atherosclerotic plaque (CP) is strongly associated with nonsubcutaneous adipose tissue, particularly pericardial adipose tissue (PAT), in community-based studies. We tested for relationships between regional adipose tissue depots and CP in African Americans with longstanding type 2 diabetes. Infrarenal aorta, coronary, and carotid artery CP and pericardial, visceral, intermuscular, and subcutaneous organ-specific adipose tissue volumes were measured using single and multidetector computed tomography (CT) in 422 African Americans with type 2 diabetes. Generalized estimating equations using exchangeable correlation and the sandwich estimator of the variance were used to test for associations between CP and adipose tissue depots. Mean (s.d.) age was 56.5 (7.6) years, diabetes duration 10.3 (7.6) years, PAT 85.3 (36.1) cm^3/45 mm and visceral adipose tissue (VAT) 174.9 (70.1) cm^3/15 mm. Adjusting for age, gender, BMI, blood pressure, medications, proteinuria, smoking, lipids, and 25-hydroxyvitamin D, PAT was positively associated with the presence ($P = 0.009$) and quantity of coronary artery CP in African Americans ($P = 0.004$), as well as the quantity of infrarenal aorta CP ($P = 0.004$). As in European Americans, PAT is associated with CP in African Americans with type 2 diabetes. Ethnic differences in the relationships between organ-specific adipose tissue depots and atherosclerosis require further study.

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DISCLOSURE

The authors declared no conflict of interest.

INTRODUCTION

Markedly lower levels of coronary artery calcified atherosclerotic plaque (CP) are present in African Americans with and without type 2 diabetes mellitus, relative to European Americans (1–5). These consistent ethnic differences occur despite exposure to similar or more severe conventional cardiovascular disease risk factors in African Americans, suggesting that risk factors may have differential impacts on atherosclerosis between races (6). However, both presence and amount of coronary artery CP are associated with increased risk of cardiovascular events in individuals from all ethnic groups (7).

Pericardial adipose tissue (PAT) volumes were associated with presence of coronary artery CP in 227 European American and 171 African American Multi-Ethnic Study of Atherosclerosis (MESA) participants, although only 84 MESA subjects had detectable CP (8). PAT was also associated with incident coronary disease in 998 MESA participants (220 were African American) without baseline cardiovascular disease (9). These findings support the theory that local effects of adipose tissue directly impact atherosclerotic risk in adjacent arteries. Pericardial and visceral adipose associations with CP have not been performed in large numbers of African Americans or in those with diabetes. Furthermore, few studies have explored the relationship of regional or total adipose tissue measures with CP in the carotid artery or abdominal aorta.

The African American–Diabetes Heart Study (DHS) is exploring the inherited and environmental causes of CP in African Americans with type 2 diabetes. PAT, visceral adipose tissue (VAT), intermuscular adipose tissue (IMAT) and subcutaneous adipose tissue (SAT) volumes were measured with computed tomography (CT) in African Americans with longstanding type 2 diabetes and relatively preserved kidney function. Relationships between organ-specific adipose tissue depots and coronary artery, carotid artery and aorta CP were computed.

METHODS AND PROCEDURES

Study populations

African Americans with type 2 diabetes recruited in the initial DHS and in the follow-up African American–DHS formed the study population (3). The original DHS recruited sibling pairs and used age-at-diabetes onset of 35 years, because most of the participants were European American. The African American–DHS was limited to unrelated African Americans and age-at-diabetes onset was 30 years, because type 2 diabetes is reported at younger ages in African Americans, relative to European Americans. Subjects who underwent prior coronary artery bypass surgery or carotid endarterectomy were excluded from these analyses as it was felt that the CP mass score in relevant arteries would be impacted by these procedures; those with prior myocardial infarction or stroke were included. The study was approved by the institutional review board at the Wake Forest University School of Medicine and all participants provided written informed consent.

Vascular imaging

Calcified atherosclerotic plaque was measured in the coronary, carotid, and infrarenal abdominal aorta arteries with single and multidetector CT systems using a standard electrocardiogram-gated CT scanning protocol based on those currently implemented in the National Heart Lung and Blood Institute's MESA (10). The calcium mass score (SmartScore, General Electric (GE) Healthcare, Waukesha, WI), which accounts for the volume and density of CP on a pixel by pixel basis and is highly correlated with the standard Agatston score using a 90 Hounsefield Unit threshold, 0.5 mm^2 minimum lesion size (two adjacent pixels), was

employed. As noted by multiple authors, the Agatston scoring system has established limitations as relates to measurement error (11). Furthermore, the weighting factors used in the coronary arteries which are <4 mm in diameter, are not appropriate for the carotid arteries and abdominal aorta which are more than ten times greater in size. By using SI units of calcium mass we have a more stable measure which increases statistical validity, is superior for comparing between the vascular beds, and has the benefits of assessing the volume of CP while also taking into account the density or mass of calcification.

Adipose tissue imaging

PAT and VAT were measured from volumetric CT acquisitions to reduce variability related to slice location using the Volume Analysis software (Advantage Windows Workstation, GE Healthcare, Waukesha, WI) and a threshold of −190 to −30 as the definitions of adipose tissue containing tissue. PAT is the combined adipose tissue superficial (paracardial) and deep (epicardial) to the pericardium (12); however, the pericardium extends superiorly to encase the great vessels and inferiorly borders the diaphragm. Our methods for measuring PAT segments a volume for measurement that covers 45 mm in length along the z axis (cephalo-caudad) of the individual based on origin of the left main coronary such that it extends 15 mm above and 30 mm below. This PAT volume includes the majority of the coronary arteries and myocardium and excludes PAT located superiorly around the aorta and pulmonary arteries and adjacent to the abdomen, as reported (13).

In the abdomen, VAT, SAT, and IMAT were measured on abdominal CT scans with technical factors: helical mode, 120 kVp, 250 mA, 4 × 2.5 mm collimation, standard reconstruction kernel, and a display field of view of 500 mm. The landmark for analysis was the first lumbar disk above the lumbar-sacrum junction, most commonly designated as L4–L5. A volume 15 mm in z axis length of the abdomen was segmented for the subcutaneous, abdominal wall and intra-abdominal compartments. VAT was defined as the adipose tissue containing pixels located within the abdominal cavity, SAT was defined as the adipose tissue containing pixels between the skin surface and lean tissue of the abdominal wall and IMAT was measured within the abdominal wall and paraspinal muscles. Studies in human cadavers revealed that the area measured by CT is an accurate estimate of VAT, SAT, and IMAT volume.

Statistical methods

A series of generalized estimating equations assuming exchangeable correlation for the sibling pairs and using the sandwich estimator to estimate the variance of parameter estimates while adjusting for familial correlation was computed to test for associations between each adipose tissue depot and CP (14). The exchangeability assumption is expected to hold here since the clusters are made of only first degree relatives. The modified Poisson approach (15) was used for analyses that treat the CP variables as binary (presence vs. absence) traits, whereas an identity link was used for quantitative traits following the appropriate transformations. The choice of the modified Poisson approach is justified by the fact that the fraction of participants with detectable CP was high, making the rare disease assumption untenable. The Box-Cox method was applied to identify the appropriate transformation of each outcome variable that would best approximate the distributional assumptions of conditional normality and homogeneity of variance of the residuals (16). The natural log of (CorCP + 1), (CarCP + 1), and (AorCP + 1) were analyzed. Prior to these transformations, observed values of aorta, carotid and coronary CP exceeding the 95th percentile were winsorized at their 95th percentile.

All analyses were run adjusting for (i) age and gender, (ii) age, gender, and BMI, (iii) age, gender, and 25-hydroxyvitamin D, and (iv) age, gender, C-reactive protein, BMI, high-density lipoprotein cholesterol, systolic blood pressure, triglycerides, blood pressure, smoking history, lipid-lowering medications, and 25-hydroxyvitamin D. Standard regression diagnostics for

collinearity and influence were computed for each model reported. All analyses satisfied the normality assumption made about the distribution of the residuals.

RESULTS

The study population consisted of 422 African Americans with type 2 diabetes mellitus from 323 families. Table 1 contains demographic characteristics of the study group and Table 2 lists laboratory results. Participants had mean (s.d.) PAT volume 85.3 (36.1) cm^3/45 mm, VAT 174.9 (70.1) cm^3/15 mm, IMAT 11.1 (7.4) cm^3/15 mm, and SAT 437.1 (184.7) cm^3/15 mm. The correlation between the adipose tissue depots was highly significant (spearman correlation = 0.63, *P* value < 0.001). However, this correlation translates to only a 40% (0.63^2) reliability estimate between the variables; therefore, it was possible to detect statistically significant effect with one variable, but not another. We next computed the partial correlation between log (coronary CP + 1) with intermuscular and PATs, accounting for age. The correlations were (0.16, *P* value = 0.001) for log (coronary CP + 1) with PAT and (0.05, *P* value = 0.29) for log (coronary CP + 1) with IMAT. This suggests that the initial correlation between log (coronary CP + 1) with intermuscular adipose was due to the strong correlation that exists between these variables and age (age appears to act as a confounding variable). The fact that the partial correlation adjusted for age is not significant explains why, despite the strong correlation between intermuscular and PAT, any model that included age as a covariate shows no statistically significant association between log (coronary CP + 1) and IMAT.

Table 3 contains results of analyses comparing presence or absence of CP in the coronary arteries, carotid arteries and aorta with regional adipose tissue. In the fully adjusted model (age, gender, BMI, C-reactive protein, BMI, high-density lipoprotein cholesterol, systolic blood pressure, triglycerides, blood pressure, smoking history, lipid-lowering medications, and 25-hydroxyvitamin D) a significant positive association between presence of coronary artery CP and PAT (parameter estimate 0.011; *P* = 0.009) was observed.

Relationships between quantitative measures of CP and regional adipose tissue depots are presented in Table 4. PAT was strongly and positively associated with quantitative coronary CP (parameter estimate = 0.015, *P* = 0.004). PAT was also positively associated with aorta CP (parameter estimate = 0.014, *P* = 0.004). Significant relationships were not observed between other regional adipose tissue depots and CP using fully adjusted models.

DISCUSSION

Adipose tissue has established autocrine, paracrine, and endocrine effects (17) and may directly influence the process of atherosclerosis through mechanisms related to release of adipocytokines and fatty acids (18–20). This study extends previous reports by evaluating larger numbers of African Americans with type 2 diabetes, by including quantitative measures of CP from three vascular beds, and by including CT-derived adipose tissue volumes from four adipose tissue depots. A strong positive association was detected between PAT and both the presence and amount of coronary artery CP. In addition, PAT was significantly associated with the quantitative measure of infrarenal aorta CP.

This finding extends the initial reports of association between PAT and coronary artery CP (8,9,21). MESA participants lacked symptomatic coronary artery disease and generally had lower amounts of CP than the diabetes-affected subjects in this report. The positive relationship between PAT and coronary artery CP herein is similar to that observed in MESA subjects, where after adjustment for demographics, lifestyle factors and height, coronary artery CP was associated with a 1 s.d. increment in the PAT index (odds ratio 1.38, 95% confidence interval 1.04–1.84) (8). Furthermore, a preliminary MESA report limited to 398 participants with

abdominal CT scans (171 African American; 227 European American) revealed VAT associations with coronary artery CP in European Americans (odds ratio 1.41; 95% CI 1.01–1.97), but not African Americans (odds ratio 1.17; 95% CI 0.75–1.83) suggesting that ethnic differences in development of CP based upon neighboring adipose tissue depots may exist (J. Ding, unpublished data). Interpretation of this preliminary finding will require analysis in the full MESA sample.

Despite higher mean BMI in African Americans compared to a random sample of 180 European Americans from the DHS, pericardial, visceral, intermuscular and SAT volumes were lower (data not shown) (22). It is tempting to speculate that deposition of adipose tissue in the pericardium, adipose tissue with a relatively high metabolic activity and cytokine secretion in close proximity to the coronary arteries, impacts the local development of CP. This report also reveals that PAT is associated with atherosclerosis in more distant vascular beds, such as the infrarenal aorta. VAT, nearer the aorta, was not associated with infrarenal aorta CP. Inflammatory mediators, leptin, adipo-nectin, and other as yet undetermined hormones/cytokines may prove to be associated with development of CP. The African American–DHS has a biorepository containing frozen serum, plasma and urine samples. Analyses of circulating factors and polymorphisms in the genes that regulate their synthesis will be performed once local recruitment is complete. These analyses may reveal the factor(s) underlying the strong association between PAT and systemic atherosclerosis.

As discussed, ethnic differences in CP have widely been observed, with African Americans at lower risk than European Americans despite presence of similar or more severe cardiovascular disease risk factors (1–5). It remains possible that VAT and PAT manifest ethnic differences in their effects on susceptibility to development of CP; this question will require additional analyses. An important limitation of this and the MESA reports are their cross-sectional nature. It will be important to quantify PAT and other regional adipose volumes using CT scans performed to measure CP in studies with longitudinal follow-up.

In conclusion, PAT and neighboring coronary artery CP were significantly and positively associated in African Americans with diabetes. In addition, significant positive associations were observed between PAT and infrarenal aorta CP. It will be important to determine whether ethnic differences in the relationships between adipose tissue depots and regional CP contribute to observed ethnic differences in CP mass, with African Americans having less CP relative to European Americans. The factors that mediate the relationship between PAT and atherosclerosis remain to be identified.

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Table 1

Demographic characteristics of African American–Diabetes Heart Study participants

Variable		Female (*n* = 270)	Male (*n* = 152)	All (*n* = 422)
Age (years)	Mean (s.d.)	56.2 (9.5)	57 (9.9)	56.5 (9.6)
	Median	56	57	56
Diabetes duration (years)	Mean (s.d.)	10.3 (7.4)	10.3 (8.1)	10.3 (7.6)
	Median	8	7.5	8
BMI (kg/m^2)	Mean (s.d.)	36.8 (8.2)	32 (7)	35 (8.1)
	Median	35.8	31	33.7
ACEI or ARB use	*N* (%)	136 (50.37)	75 (49.34)	211 (50)
Systolic BP (mm Hg)	Mean (s.d.)	137.5 (21.5)	135.7 (17.9)	136.8 (20.3)
	Median	135	135	135
Diastolic BP (mm Hg)	Mean (s.d.)	76 (12.4)	77.5 (11.1)	76.6 (11.9)
	Median	75.5	77.5	76
Lipid meds	*N* (%)	132 (49.07)	69 (46)	201 (47.97)
Insulin	*N* (%)	117 (43.49)	61 (40.13)	178 (42.28)
Smoking	Never	127 (51.84)	36 (25.53)	163 (42.23)
	Former	70 (28.57)	59 (41.84)	129 (33.42)
	Current	48 (19.59)	46 (32.62)	94 (24.35)

Excludes 26 subjects with coronary artery bypass surgery and 9 with carotid endarterectomy.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BP, blood pressure.

Table 2

Laboratory characteristics of African American–DHS study participants (mean (s.d.) median)

	Female (*n* = 270)	Male (*n* = 152)	All (*n* = 422)
GFR, ml/s	1.38 (0.5) 1.31	1.46 (0.5) 1.39	1.41 (0.5) 1.35
Urine albumin: creatinine, mg/g	241.8 (828) 16.3	209.6 (618.4) 19	230.1 (758.4) 17.5
Serum creatinine, μmol/l	85.8 (35.4) 79.6	106.1 (35.4) 97.2	92.8 (35.4) 88.4
Fasting blood sugar, mmol/l	8.6 (3.7) 7.8	8.9 (4.3) 8.0	8.7 (3.9) 7.9
HbA_{1c}, proportion of total	0.085 (0.03) 0.078	0.084 (0.02) 0.080	0.085 (0.02) 0.079
Serum calcium, mmol/l	2.38 (0.1) 2.38	2.35 (0.1) 2.35	2.35 (0.1) 2.38
Serum phosphorus, mmol/l	1.13 (0.2) 1.13	1.07 (0.2) 1.03	1.10 (0.2) 1.10
25-hydroxyvitamin D, nmol/l	49.5 (30.4) 39.9	44.4 (21.6) 42.4	47.5 (27.2) 41.2
Coronary CP	548 (1452) 23	882 (1824) 111	669 (1602) 52
Coronary CP >0%	215 (80.83)	125 (83.33)	340 (81.73)
Carotid CP	164 (531) 2	194 (651) 6	175 (576) 3
Carotid CP >0%	143 (54.17)	88 (58.67)	231 (55.80)
Aorta CP	4697 (8991) 651	5192 (11225) 1014	4883 (9879) 724
Aorta CP >0%	198 (82.85)	120 (88.33)	318 (83.03)
HDL-cholesterol, mmol/l	1.31 (0.4) 1.30	1.15 (0.3) 1.14	1.25 (0.4) 1.19
LDL cholesterol, mmol/l	2.91 (1.0) 2.85	2.78 (1.0) 2.67	2.87 (1.0) 2.77
Triglycerides, mmol/l	1.39 (1.3) 1.08	1.53 (2.0) 1.18	1.45 (1.6) 1.13
PAT, cm^3/45 mm	82.4 (31.3) 77.3	90.3 (43) 79.8	85.3 (36.1) 78.7
Visceral adipose tissue, cm^3/15 mm	174.4 (63.8) 167.8	175.9 (79.9) 166.2	174.9 (70.1) 167.8
Intermuscular adipose tissue, cm^3/15 mm	11.9 (7.5) 10.3	9.6 (7.2) 7.8	11.1 (7.4) 9.3
Subcutaneous adipose tissue, cm^3/15 mm	498.9 (172.8) 485.7	332.4 (154.9) 313.4	437.1 (184.7) 415.2

CP, calcified atherosclerotic plaque; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAT, pericardial adipose tissue.

Table 3

Associations between presence/absence of CP and organ-specific adipose tissue volumes

		Coronary CP			Carotid CP			Aorta CP		
Variable	**Adjustment**	**Parameter**	**Prevalence ratio**	***P* value**	**Parameter**	**Prevalence ratio**	***P* value**	**Parameter**	**Prevalence ratio**	***P* value**
Intermuscular	A & G	0.020	1.163	0.291	0.016	1.069	0.300	−0.009	0.9328	0.551
1st PC of adipose tissue measures	A & G	0.127	1.202	0.078	0.115	1.091	0.111	−0.087	0.8818	0.209
Pericardial	A & G	0.010	1.435	**0.003**	0.000	1.011	0.912	0.001	1.0350	0.728
Subcutaneous	A & G	0.000	0.970	0.787	0.001	1.110	0.060	−0.001	0.7687	0.014
Visceral	A & G	0.001	1.078	0.448	0.003	1.098	0.045	−0.001	0.9205	0.361
Intermuscular	A, G & BMI	0.015	1.117	0.470	0.007	1.037	0.673	0.003	1.0209	0.873
1st PC of adipose tissue measures	A, G & BMI	0.154	1.251	0.166	0.047	1.048	0.651	0.035	1.0516	0.722
Pericardial	A, G & BMI	0.011	1.480	**0.005**	−0.003	0.962	0.423	0.005	1.2035	0.121
Subcutaneous	A, G & BMI	−0.002	0.680	0.015	0.001	1.088	0.382	−0.001	0.7875	0.168
Visceral	A, G & BMI	0.000	1.014	0.911	0.002	1.075	0.204	0.000	1.0134	0.904
Intermuscular	A, G & Vit D	0.030	1.245	0.286	0.011	1.052	0.619	−0.004	0.9711	0.851
1st PC of adipose tissue measures	A, G & Vit D	0.183	1.305	0.024	0.106	1.087	0.279	0.010	1.0148	0.901
Pericardial	A, G & Vit D	0.012	1.520	**0.001**	0.004	1.077	0.334	0.003	1.1319	0.293
Subcutaneous	A, G & Vit D	0.000	1.061	0.663	0.000	1.019	0.766	−0.001	0.8075	0.101
Visceral	A, G & Vit D	0.002	1.145	0.243	0.002	1.086	0.230	0.001	1.0433	0.719
Intermuscular	Full	0.020	1.158	0.425	0.016	1.064	0.562	−0.004	0.9718	0.872
1st PC of adipose tissue measures	Full	0.226	1.387	0.097	0.091	1.088	0.549	0.092	1.1430	0.497
Pericardial	Full	0.011	1.504	**0.009**	0.002	1.058	0.633	0.006	1.2386	0.155
Subcutaneous	Full	−0.001	0.793	0.275	0.001	1.019	0.724	0.000	0.9618	0.842
Visceral	Full	0.001	1.090	0.580	0.000	1.011	0.984	0.000	1.0287	0.873

A & G: adjusting for age and gender; A, G & BMI: adjusting for age, gender, and BMI; A, G & Vit D: adjusting for age, gender, and 25-hydroxyvitamin D; Full: fully adjusted model accounting for age, gender, C-reactive protein, cholesterol medication, albumin creatinine ratio, ACE-inhibitor (ACE_ARB), height, smoking history, HDL-cholesterol, systolic blood pressure, triglycerides level, BMI, and 25-hydroxyvitamin D; 1st PC: first principal component computed using the correlation matrix between all the adipose tissue measures. Prevalence ratios are given for an effect size of one standard deviation for each variable. The standard deviations are the values given in parentheses in Table 2 under the column labeled “All”. Significant *P* values are shown in boldface.

Table 4

Associations between quantity of calcified plaque and organ-specific adipose tissue volumes

		Coronary CP		Carotid CP		Aorta CP	
Variable	**Adjustment**	**Estimate**	***P* value**	**Estimate**	***P* value**	**Estimate**	***P* value**
Intermuscular	A & G	0.026	0.123	−0.007	0.651	−0.020	0.252
1st PC of adipose tissue measures	A & G	0.195	0.020	−0.088	0.252	−0.116	0.225
Pericardial	A & G	0.012	$\mathbf{1.17 \times 10^{-4}}$	0.000	0.927	0.005	0.128
Subcutaneous	A & G	0.000	0.638	−0.001	0.260	−0.002	$\mathbf{5.12 \times 10^{-3}}$
Visceral	A & G	0.002	0.295	−0.003	0.023	−0.004	0.054
Intermuscular	A, G & BMI	0.019	0.289	0.001	0.968	0.004	0.823
1st PC of adipose tissue measures	A, G & BMI	0.239	0.040	−0.034	0.760	0.182	0.167
Pericardial	A, G & BMI	0.013	$\mathbf{2.29 \times 10^{-4}}$	0.003	0.418	0.014	$\mathbf{2.80 \times 10^{-4}}$
Subcutaneous	A, G & BMI	−0.001	0.386	0.000	0.762	−0.001	0.364
Visceral	A, G & BMI	0.001	0.687	−0.003	0.053	−0.001	0.710
Intermuscular	A, G & Vit D	0.040	0.121	−0.012	0.610	−0.006	0.838
1st PC of adipose tissue measures	A, G & Vit D	0.331	0.003	−0.098	0.354	0.043	0.747
Pericardial	A, G & Vit D	0.017	$\mathbf{2.57 \times 10^{-5}}$	−0.001	0.904	0.011	$\mathbf{1.54 \times 10^{-2}}$
Subcutaneous	A, G & Vit D	0.001	0.423	−0.001	0.578	−0.002	0.102
Visceral	A, G & Vit D	0.004	0.096	−0.003	0.119	−0.001	0.850
Intermuscular	Full	0.029	0.308	−0.006	0.829	−0.009	0.761
1st PC of adipose tissue measures	Full	0.419	0.014	−0.001	0.993	0.212	0.227
Pericardial	Full	0.015	$\mathbf{1.04 \times 10^{-3}}$	0.002	0.692	0.014	$\mathbf{4.20 \times 10^{-3}}$
Subcutaneous	Full	−0.001	0.675	0.000	0.891	−0.001	0.452
Visceral	Full	0.004	0.163	−0.001	0.747	0.001	0.772

A & G: adjusting for age and gender; A, G & BMI: adjusting for age, gender, and BMI; A, G & Vit D: adjusting for age, gender, and 25-hydroxyvitamin D; Full: fully adjusted model accounting for age, gender, C-reactive protein, cholesterol medication, albumin creatinine ratio, ACE-inhibitor (ACE_ARB), height, smoking history, HDL cholesterol, systolic blood pressure, triglycerides level, BMI, and 25-hydroxyvitamin D; 1st PC: first principal component computed using the correlation matrix between all the adipose tissue measures. Significant *P* values are shown in boldface.

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