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Association of the *CPT1B* Gene with Skeletal Muscle Fat Infiltration in Afro-Caribbean Men

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Abstract

Skeletal muscle fat is greater in African ancestry individuals compared with whites, is associated with diabetes, and is a heritable polygenic trait. However, specific genetic factors contributing to skeletal muscle fat in humans remain to be defined. Muscle carnitine palmitoyltransferase-1B (CPT1B) is a key enzyme in the regulation of skeletal muscle mitochondrial β-oxidation of long-chain fatty acids, and as such is a reasonable biological candidate gene for skeletal muscle fat accumulation. Therefore, we examined the association of three nonsynonymous coding variants in *CPT1B* (G531L, I66V, and S427C; a fourth, A320G, could not be genotyped) and quantitative computed tomography measured tibia skeletal muscle composition and BMI among 1,774 Afro-Caribbean men aged ≥40, participants of the population-based Tobago Health Study. For all variants, no significant differences were observed for BMI or total adipose tissue. Among individuals who were homozygous for the minor allele at G531L or I66V, intermuscular adipose tissue (IMAT) was 87% ($P = 0.03$) and 54% lower ($P = 0.03$), respectively. In contrast, subcutaneous adipose tissue (SAT) was 11% ($P = 0.017$) and 7% ($P = 0.049$) higher, respectively, than among individuals without these genotypes. These associations were independent of age, body size, and muscle area. Finally, no individuals with type 2 diabetes were found among those who were homozygous for the minor allele of either at G531L and I66V whereas 14–18% of men with the major alleles had type 2 diabetes ($P = 0.03$ and 0.007, respectively). Our results suggest a novel association between common nonsynonymous coding variants in *CPT1B* and ectopic skeletal muscle fat among middle-aged and older African ancestry men.

INTRODUCTION

Skeletal muscle accounts for about 80% of glucose disposal in humans, and therefore, is crucial for maintaining glucose homeostasis (1). Emerging evidence suggests that ectopic adipose tissue accumulation within the fascia surrounding skeletal muscle (i.e., intermuscular adipose tissue, IMAT) may be an independent risk factor for insulin resistance and type 2 diabetes

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(2–4). The physiological and molecular mechanisms responsible for the accumulation of this metabolically important fat depot are yet to be defined.

Some have proposed that defects in mitochondrial β-oxidation may contribute to increased fat accumulation in skeletal muscle in addition to increased fatty acid delivery (5). Carnitine palmitoyltransferase-1 (CPT1) is the rate- controlling enzyme in the long-chain fatty acid β-oxidation pathway in muscle mitochondria. This enzyme is required for the net transport of long-chain fatty acyl-CoAs from the cytoplasm into the mitochondria (6,7). In mammals, three CPT1 isoforms have been identified: liver (CPT1A), muscle (CPT1B), and brain (CPT1C). CPT1 inhibition results in the accumulation of long-chain fatty acyl-CoAs derivatives such as triglycerides, which is an early occurrence in the development of insulin resistance (8). Pharmacological inhibition of muscle CPT1 leads to fat accumulation in skeletal muscle and insulin resistance in rats (9). Therefore, skeletal muscle CPT1 may have an important role in accumulation of skeletal muscle fat, insulin resistance, and development of diabetes.

We have recently shown that skeletal muscle fat infiltration is a heritable trait (heritability 35.0%) (10). However, the specific genetic variants contributing to adipose tissue accumulation in skeletal muscle in humans remain to be defined. The identification of such factors may provide fundamental insight on the biological pathways contributing to ectopic fat accumulation in skeletal muscle and the etiology of diabetes. In this study, we tested the hypothesis that common nonsynonymous coding variants in *CPT1B* are associated with skeletal muscle fat accumulation and total adiposity in a large homogeneous population-based cohort of middle-aged and older African ancestry men, who have a high prevalence of type 2 diabetes (11).

METHODS AND PROCEDURES

Study population

Between 1998 and 2003 >3,000 men were recruited for a population-based prostate cancer screening study on the Island of Tobago, Trinidad & Tobago. A detailed summary of the Tobago Prostate Cancer Survey has been published elsewhere (12). Briefly, the aim of the observational cohort study was to determine the prevalence and incidence of prostate cancer in otherwise healthy men aged ≥40 years on the island. To be eligible, men had to be ambulatory, noninstitutionalized, and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island Parishes. The recruited cohort was 97% African, 2% East Indian, <1% white, and <1% “other” as defined by participant-report of paternal and maternal grandparents’ ethnicity. Ancestry informative genetic markers have confirmed a low admixture (6% non-African) in this population (13) compared to the more genetically heterogeneous African-American population which has much higher degree of non-African admixture (17–23.9%) (14–16).

Men who participated in the study were re-contacted for a follow-up clinic examination beginning in 2004. A total of 2,031 men in the cohort (70% of survivors) returned at the time of the present analysis. At the follow-up visit, we also recruited 451 new participants aged ≥40 years. The present analysis is limited to men at the follow-up visit who were Afro-Caribbean (self-report of four grandparents of African ancestry) and who had complete data on anthropometric measures, demographic information, medical history, and peripheral quantitative computed tomography (pQCT) measures of skeletal muscle composition at the time of this analysis ($N = 1,774$). Written informed consent was obtained from every participant using forms approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health.

Other measures

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from height and weight (kg/m^2). Information on lifestyle habits, demographic information, medical conditions, family history, and medication use, were also assessed using standardized interviewer administered questionnaires that were reviewed with the participant in the clinic. Alcohol drinking frequency was self-reported in predefined categories. We arbitrarily created two categories of alcoholic use (<1 and ≥1 drink per week). We also recorded information on self-reported walking, because walking is the predominant form of physical activity on the island. Information on family history of diabetes was collected for first-degree relatives (parents, siblings, and offspring).

Participants were classified as having type 2 diabetes if they were told by a doctor that they had diabetes and/or if they were currently taking antidiabetic therapy (insulin or oral antidiabetic medications); whereas they were classified as overweight if their BMI was 25–29.9 kg/m^2 or as obese if their BMI was ≥30 kg/m^2.

pQct

A pQCT scan of the tibia was performed using a Stratec XCT-2000 scanner (Orthometrix, White Plains, NY) in order to evaluate the total, muscle, and adipose tissue cross-sectional areas. Scans were obtained at 66% of the tibial length, proximal to the terminal end of the tibia. This site was chosen as it is the region of the lower leg with the largest circumference of the calf with little variability across individuals (17). Images of the cross-sectional area of skeletal muscle and adipose tissue were analyzed using the Stratec analysis software version 5.5D (Orthometrix). To maintain consistency, all images were analyzed by a single investigator (C.L.G.). We obtained measures of the cross-sectional total adipose tissue (TAT) area (mm^2), the cross-sectional subcutaneous adipose tissue (SAT) area, and cross-sectional IMAT area and expressed these measures as a percentage of the TAT. SAT percentage (%) was determined as: (SAT cross-sectional area/calf total adipose tissue (TAT) cross-sectional area) × 100. IMAT percentage (%) was determined as: (IMAT cross-sectional area/TAT cross-sectional area) × 100.

Selection and genotyping of nonsynonymous coding single-nucleotide polymorphisms

We identified and successfully genotyped three of four validated (two-hit, frequency available) nonsynonymous coding single-nucleotide polymorphisms (nsSNPs) in the *CPT1B* gene from the National Center for Biotechnology Information SNP database (Build 129): rs470117 (Glu531Lys), rs3213445 (Ile66Val) and rs8142477 (Ser427Cys). One validated nonsynonymous coding SNP in *CPT1B* could not be genotyped by TaqMan (rs2269383, Asp320Gly).

Genotyping was completed using genomic DNA prepared from blood clots or whole blood. All SNPs were genotyped using the fluorogenic 5′-nuclease TaqMan allelic discrimination assay system (Applied Biosystems, Foster City, CA). The assays were performed under standard conditions on a 7900HT real-time polymerase chain reaction instrument with probes and reagents purchased from Applied Biosystems. All genotype calls were determined by two independent investigators, and only concordant calls were used. The average genotyping completeness rate for successful assays was 96.5% and the lowest genotyping completeness rate was 95.5%. The average genotyping consensus rate for 210 replicate samples across the three genotyping assays was 99.7% (range, 99.52–100%). All three variants were in Hardy–Weinberg equilibrium in the Afro-Caribbean population.

Statistical analysis

We calculated site-specific allele frequencies by gene counting and tested for departures from Hardy–Weinberg equilibrium using a goodness of fit statistic. Pairwise estimates of linkage disequilibrium were measured as D' and R^2 from the diploid data, and were calculated with HAPLOVIEW (18). *CPT1B* polymorphisms were tested for their association with BMI and pQCT measured fat traits (TAT, IMAT%, and SAT%). Linear regression was used to test for association between the number of minor alleles and BMI or pQCT skeletal muscle fat traits. ANOVA was performed using SAS GLM to assess the dominant and recessive models to obtain adjusted means. For pQCT measured fat traits (TAT, IMAT%, and SAT%) all analyses appeared to follow a recessive model and only these results are presented in tables. The association between *CPT1B* genotypes and type 2 diabetes was assessed using logistic regression analysis adjusting for age and BMI. Furthermore, we determined an allelic score by counting the number of minor alleles for G531L and I66V. For each subject having genotypes for both G531L and I66V, we assigned an allele score of 0–4 as follows: 0 for subjects with no minor alleles, 1 for subjects with one minor allele, 2 for subjects with two or more minor alleles. No participants had four protective alleles. Prevalence of type 2 diabetes was then compared between genotypes using Fisher's exact test. Our study had 85% power to detect an association that explained 0.5% of the total phenotypic variance in IMAT% and SAT% at $\alpha < 0.05$ and at minor allele frequency (MAF) of ≥9.6%. Analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Characteristics of the 1,774 men are presented in Table 1. All men were of Afro-Caribbean ancestry with mean age of 59 years (range 40–92 years). Almost 45% of men were overweight, 25% were obese, and 17% self-reported type 2 diabetes. Nearly half of all participants reported a family history of diabetes.

The MAF of each variant is reported in Table 2. The MAFs for all three variants were comparable to those reported in African ancestry subjects from the Yoruban population of Ibadan, Nigeria (www.hapmap.org).

Table 3 presents the adjusted means of IMAT% and SAT% by genotype. For all three SNPs, mean IMAT% and SAT% of heterozygotes were similar to those of homozygotes with the major allele, suggesting a recessive effect on these traits. Consistent with this, formal analysis under additive and dominant models did not reveal a significant association between any of the three SNPs and IMAT% or SAT% ($P < 0.05$), with the exception of a borderline significant association between G531L and SAT% (additive P value = 0.051). Under a recessive model, individuals who were homozygous for the minor allele at G531L and I66V had significantly lower IMAT% compared to those with the major allele ($P = 0.033$ and 0.029, respectively). In contrast, the mean SAT% was higher among men who were homozygous for the minor alleles than for individuals who were not homozygous for the minor allele ($P = 0.017$ and 0.049, respectively). The magnitude of difference in IMAT% between homozygous genotype groups was 87 and 54% for G531L and I66V, respectively. The magnitude of difference in SAT% between homozygous genotype groups was 12 and 7%, respectively. Individuals homozygous for the minor allele at S427C had slightly higher SAT% ($P = 0.04$) than carriers of the major allele, but differences in IMAT% were not statistically significant. For all three variants, no significant differences were observed for BMI or total adipose tissue measured by pQCT under additive, dominant, or recessive models. There was also a significant difference in the prevalence of type 2 diabetes between genotypes at G531L and I66V. Results from the logistic regression analysis showed an association between the number of minor alleles at G531L (OR = 0.70, 95%CI = 0.49–0.98, $P = 0.044$) and I66V (OR = 0.63, 95%CI = 0.46– 0.88, $P = 0.006$) with type 2 diabetes adjusted for age and BMI. However, we found no significant association

of S427C (OR = 0.95, 95%CI = 0.78–1.15, $P = 0.61$) with type 2 diabetes. We repeated the analyses without individuals who had type 2 diabetes ($N = 1,440$) and found similar results (data not shown).

We next created an "allele score" combining genotypes from both G531L and I66V. We found decreased IMAT% and increased SAT% among men with ≥2 minor alleles for either variant compared to men who had no minor alleles ($P < 0.05$) (Table 4). The association with type 2 diabetes prevalence was particularly striking where only 2.5% of men with ≥2 minor alleles for G531L and I66V had diabetes compared to 19% of men without any minor alleles (Table 4).

DISCUSSION

In this study, we examined the association between ectopic fat accumulation in skeletal muscle and coding variants in the gene encoding carnitine palmitoyltransferase-1B (*CPT1B*), the rate limiting enzyme in mitochondrial β-oxidation of long-chain fatty acids, the most abundant fatty acids in energy metabolism. To our knowledge, there have been very few genetic variants reported to date that might affect skeletal muscle ectopic fat accumulation in humans (19) despite recent evidence that this fat depot is a heritable trait (10). Our analysis suggests that men with minor alleles for G531L and I66V in *CPT1B* have lower intermuscular and higher subcutaneous fat independent of overall adiposity. This association implies that mutations in the *CPT1B* gene might influence susceptibility to the development of ectopic skeletal muscle fat.

Our results suggest that inherited differences in the function of CPT1B, a key enzyme in the regulation of the mitochondrial β-oxidation, may impair long-chain fatty acid metabolism and predispose to increased skeletal muscle fat infiltration (5). Magnetic resonance spectroscopy studies have revealed that inherited defects in mitochondrial function are indeed associated with increased ectopic skeletal muscle fat (20,21). Fatty acid oxidation is compromised in lean, insulin-resistant offspring of diabetic individuals compared to insulin-sensitive nondiabetic controls suggesting that alterations in lipid metabolism may be an early defect in the pathogenesis of diabetes (22). Our findings support the possibility that inherited defects in CPT1B may contribute to impaired energy metabolism, insulin resistance and diabetes.

Although the biological significance of the rs470117 (G531L) and rs3213445 (I66V) variants is unclear, these amino acid substitutions may change the activity of the encoded CPT1B protein. rs470117 changes the amino acid at residue 531 from medium size and acidic (Glu) to large size and basic (Lys). A sequence alignment analysis of 17 vertebrates, including mammalian, amphibian, bird, and fish species (http://genome.ucsc.edu) revealed that Glu at position 531 is highly conserved. rs3213445 changes Ile to Val at residue 66 within a potential transmembrane domain but both residues are medium size and hydrophobic. The human and chimp genome reference sequences contain Ile at residue 66 whereas this residue is Val in the macaque and other distantly related species (www.ucsc.org). PolyPhen analysis (23) predicts that both G531L and I66V are probably benign variants. However, *in silico* analysis using FASTSNP (24) suggests that both variants may change putative exonic splice enhancer sequences. Exonic splice enhancer sequences play an important role in constitutive and alternative pre-mRNA splicing and variants within these elements may alter splicing regulation. These hypotheses will, of course, need to be verified in future functional experiments of each amino acid substitution.

Genome-wide association studies have not reported significant evidence of association between rs470117, rs3213445, and rs8142477 and type 2 diabetes (25–29). However, rs3213445 and rs470117 were nominally associated with triglycerides and low-density

lipoprotein-cholesterol levels in three genome-wide association studies, although these associations did not achieve genome-wide significance ($P = 0.03$ for both) (30). All of these genome-wide association studies were conducted in populations of non-African ancestry.

CPT1B overexpression may protect against the accumulation of lipid metabolites, such as diacylglycerol, long-chain acyl-CoA esters (acyl-CoAs) and ceramide (31). These metabolites have been implicated as a potential cause of ectopic fat induced insulin resistance as they activate signal transduction pathways that lead to impaired insulin signaling (32,33). Interestingly, in our study, none of the homozygous carriers of the G531L or I66V minor alleles had diabetes. Similarly, only 2.5% of the men who had at least two copies of the minor G531L and I66V allele had type two diabetes compared to 19% among of men without any minor alleles. Although statistically significant, these results should be interpreted with caution as the number of men in the homozygous genotype groups was small. Associations between diabetes risk and genetic variation at the *CPT1B* locus should be explored in a much larger sample. Further studies are also warranted to examine whether other variants in the *CPT1B* gene contribute to skeletal muscle fat accumulation and risk for type 2 diabetes.

We found that the allele frequencies of the three CPT1B variants are very similar to those observed in the West African Yoruba population from Ibadan, Nigeria (www.hapmap.org). However, the allelic and genotypic distributions of the three CPT1B variants were considerably different between African, white and Asian ancestry populations from HapMap. The minor alleles for both rs470117 (G531L) and rs8142477 (S427C) were substantially lower in African compared with white, Japanese, and Chinese ancestry individuals. In contrast, among African ancestry individuals the MAF for rs3213445 (I66V) was greater than in whites, but lower than in Japanese and Chinese HapMap populations.

African Americans have more intermuscular fat compared to whites, even after adjustment for differences in total adiposity and other potential covariates (3,4), but the factors underlying black–white differences in skeletal muscle fat accumulation are unknown. Interestingly, the minor allele at G531L is substantially more common in populations of European origin (MAF = 50.8%). These population differences raise the possibility that ethnic differences in *CPT1B* allele frequencies may contribute, in part, to ethnic variation in skeletal muscle fat accumulation. This hypothesis will need to be tested by a more comprehensive evaluation of *CPT1B* gene variation in a population of European ancestry.

There is evidence that abnormal CPT1 activity might contribute to overall obesity in human and rodent models. For example, muscle CPT1 mRNA enzyme activity was reduced in obese individuals in one study (34). Despite the accumulating evidence for a role of CPT1B in diabetes and obesity, there has been only one study of *CPT1B* gene variants and obesity in humans (35). Interestingly, G531L, common in this study of 351 French Canadians (MAF = 40%), was found to be modestly associated with BMI and waist circumference, whereas I66V and S427C showed no associations with these traits. In our study, we also did not find associations between BMI, waist circumference (data not shown), or total adipose tissue measured by pQCT with the I66V and S427C variants. However, in contrast to this earlier study, we did not observe an association between the G531L variant and measures of overall adiposity despite having a larger sample size. Additionally, it is interesting that the *CPT1B* minor alleles were associated with decreased skeletal muscle fat and increased subcutaneous fat. A possible explanation may be that *CPT1B* mediated differences in skeletal muscle fatty acid oxidation lead to an overflow of fatty acids, and therefore, spillover of fat that is stored in subcutaneous depots. It is also possible that these differences are related to skeletal muscle fat depot differences in the expression of the *CPT1B* gene, which is expressed in both adipocytes and myocytes (36). These expression patterns should be investigated in future experimental studies.

This study has several potential limitations in addition to strengths. We only studied middle-aged and older men of Afro-Caribbean ancestry and our findings may not be generalizable to other populations including younger men, men of other ethnicities or to women. Next, as we did not obtain fasting serum glucose measurements for the whole data set, participants were classified as having type 2 diabetes if they were told by their doctor that they had diabetes and if they were currently taking antidiabetic therapy (insulin or oral antidiabetic medications). In a subset of 1,000 men with available fasting serum glucose, we found that 5% of those not reporting diabetes or current antidiabetic treatment had fasting glucose ≥126 mg/dl. Of those who were told by a doctor that they had diabetes 89% subsequently reported current treatment of diabetes. We can thus conclude that in the Tobago Health Study, self-reported doctor's diagnosis of type 2 diabetes was highly consistent with treatment claims data, and that patient self-report of diabetes is a solid surrogate of diabetes classification based on fasting glucose measurements. Further, although we prioritized genotyping of nonsynonymous coding variants, intronic tag SNPs and additional variants within or flanking *CPT1B* may also influence CPT1B expression and/or function and ectopic fat in skeletal muscle and should be considered in future studies. Indeed, some investigators hypothesize that individual differences in medically relevant traits are due, in part, to the summation of the effects of polymorphisms that change the amino acid sequence of the encoded protein (37). Detailed knowledge of the genetic causes of Mendelian diseases and more recently quantitative traits (38) have illustrated the central role of nonsynonymous mutations (39). We focused on validated nonsynonymous polymorphisms in CPT1B based on the hypothesis that these protein-altering variants might be enriched for alleles of higher-impact on skeletal muscle composition. We were unable to develop a successful assay for an additional validated coding variant in CPT1B (rs2269383, G320D) and thus cannot exclude its potential influence on ectopic skeletal muscle fat or diabetes. This variant is predicted to be probably damaging by PolyPhen analysis (23) and damaging by Sorting Intolerant From Tolerant analysis (40,41) and thus should be considered for inclusion in future studies. Finally, we did not test for associations between measures of mitochondrial function or lipid metabolism and CPT1B genotype nor did we perform functional experiments of the coding variants.

In conclusion, our results suggest a possible role of genetic variation in CPT1B in the distribution of ectopic fat in skeletal muscle. A better understanding of such genetic factors may create opportunities for novel therapies that influence the development of insulin resistance and type 2 diabetes.

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Table 1

Characteristics of the Tobago Health Study population

N	1,774
Age	59.3 ± 10.4
Anthropometric characteristics	
Weight (kg)	84.5 ± 16.3
Height (cm)	175.0 ± 6.9
Waist circumference (cm)	93.2 ± 11.8
BMI (kg/m^2)	27.5 ± 4.9
pQCT lower leg skeletal muscle composition	
TAT (mm^2)	1,808 ± 817
IMAT (%)	16.0 ± 13.5
SAT (%)	73.5 ± 15.4
Total muscle area (mm^2)	7,433 ± 1,325
Lifestyle characteristics	
Ever smoked (%)	31.6
Currently smoke (%)	9.9
Walking per week (h)	3.0 ± 4.4
Alcohol intake ≥1 drink per week (%)	18.2
Medical conditions	
Overweight (%)	44.9
Obesity (%)	24.9
Type 2 diabetes (%)	16.6
Family history of diabetes (%)	49.1

Values are means ± s.d.

IMAT, intermuscular adipose tissue cross-sectional area; pQCT, peripheral quantitative computed tomography; SAT, subcutaneous adipose tissue cross-sectional area; TAT, total adipose tissue cross-sectional area.

Table 2

MAF distributions for three nonsynonymous variants in CPT1B

	MAF (%)				
CPT1 SNPs	**Afro-Caribbeans**	**YRI-HapMap**	**CEU-HapMap**	**JPT-HapMap**	**CHB-HapMap**
rs470117 (G531L)	9.6	9.2	50.8	51.2	46.4
rs3213445 (I66V)	11.6	11.7	4.2	28.9	40.0
rs8142477 (S427C)	32.9	25.8	93.3	58.0	47.8

CEU, U.S. Utah residents with ancestry from northern and western Europe; CHB, Han Chinese in Beijing, China; CPT1B, carnitine palmitoyltransferase-1B; JPT, Japanese in Tokyo, Japan; MAF, minor allele frequencies; SNP, single-nucleotide polymorphism; YRI, Yoruba of African ancestry in Ibadan, Nigeria.

Table 3

Adjusted mean (±s.e.) for adiposity traits and proportion of individuals with type 2 diabetes by CPT1B genotype

	G531L genotype				I66V genotype				S427C genotype			
	1/1 (*N* = 1,451)	**1/2 (*N* = 304)**	**2/2 (*N* = 19)**	***P* value**	**1/1 (*N* = 1,373)**	**1/2 (*N* = 351)**	**2/2 (*N* = 28)**	***P* value**	**1/1 (*N* = 787)**	**1/2 (*N* = 730)**	**2/2 (*N* = 178)**	***P* value**
IMAT (%)	16.1 ± 0.35	15.7 ± 0.8	8.6 ± 3.0	0.033[a]	16.0 ± 0.4	16.0 ± 0.7	10.4 ± 2.4	0.029[a]	16.0 ± 0.5	16.1 ± 0.5	14.7 ± 1.0	0.11[a]
SAT (%)	73.3 ± 0.4	74.6 ± 0.9	81.4 ± 3.2	0.017[a]	73.6 ± 0.4	72.8 ± 0.8	78.8 ± 2.7	0.049[a]	73.4 ± 0.5	73.3 ± 0.5	75.4 ± 1.0	0.03[a]
TAT (mm^2)	1,813 ± 22.4	1,822 ± 50.1	2,023 ± 187	0.27[b]	1,812 ± 23.0	1,811 ± 45.2	1,780 ± 155	0.85[b]	1,798 ± 30.3	1,816 ± 31.5	1,816 ± 60.1	0.81[b]
BMI (kg/m^2)	27.5 ± 0.13	27.8 ± 0.29	28.0 ± 1.0	0.71[c]	27.5 ± 0.13	27.8 ± 0.26	27.0 ± 0.94	0.68[c]	27.7 ± 0.18	27.5 ± 0.18	27.4 ± 0.35	0.65[c]
Diabetes (%)	17.5	13.9	0	0.044[d]	18.0	14.5	0	0.006[d]	17.9	16.3	16.6	0.61[d]

CPT1B, carnitine palmitoyltransferase-1B; IMAT, intermuscular adipose tissue cross-sectional area; SAT, subcutaneous adipose tissue cross-sectional area; TAT, total adipose tissue cross-sectional area.

[a] Recessive model, adjusted for age, height and pQCT total muscle area.

[b] Recessive model, adjusted for age and height.

[c] Recessive model, adjusted for age.

[d] *P* value based on logistic regression, adjusted for age and BMI.

Table 4

Adjusted mean (±s.e.) for intermuscular and subcutaneous fat percent and proportion of individuals with type 2 diabetes according to allelic score for G531L and I66V CPT1B variants

	0 Protective alleles (*N* = 1,084)	1 Protective allele (*N* = 574)	2 or 3 Protective alleles (*N* = 79)
IMAT (%)	16.1 ± 0.4	16.2 ± 0.6	11.9 ± 1.5*
SAT (%)	73.3 ± 0.5	73.3 ± 0.6	77.6 ± 1.7*
Diabetes (%)	18.9	15.2	2.5**

IMAT, intermuscular adipose tissue cross-sectional area; SAT, subcutaneous adipose tissue.

* $P < 0.05$, adjusted for age, height and pQCT total skeletal muscle area.

** $P < 0.0001$.