Genetic polymorphisms in *carnitine palmitoyltransferase 1A* gene are associated with variation in body composition and fasting lipid traits in Yup'ik Eskimos[S]

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Abstract **Variants of carnitine palmitoyltransferase 1A (*CPT1A*), a key hepatic lipid oxidation enzyme, may influence how fatty acid oxidation contributes to obesity and metabolic outcomes. *CPT1A* is regulated by diet, suggesting interactions between gene variants and diet may influence outcomes. The objective of this study was to test the association of *CPT1A* variants with body composition and lipids, mediated by consumption of polyunsaturated fatty acids (PUFA). Obesity phenotypes and fasting lipids were measured in a cross-sectional sample of Yup'ik Eskimo individuals (n = 1141) from the Center of Alaska Native Health Research (CANHR) study. Twenty-eight tagging *CPT1A* SNPs were evaluated with outcomes of interest in regression models accounting for family structure. Several *CPT1A* polymorphisms were associated with HDL-cholesterol and obesity phenotypes. The P479L (rs80356779) variant was associated with all obesity-related traits and fasting HDL-cholesterol. Interestingly, the association of P479L with HDL-cholesterol was still significant after correcting for body mass index (BMI), percentage body fat (PBF), or waist circumference (WC). Our findings are consistent with the hypothesis that the L479 allele of the *CPT1A* P479L variant confers a selective advantage that is both cardioprotective (through increased HDL-cholesterol) and associated with reduced adiposity.**—Lemas, D. J., H. W. Wiener, D. M. O'Brien, S. Hopkins, K. L. Stanhope, P. J. Havel, D. B. Allison, J. R. Fernandez, H. K. Tiwari, and B. B. Boyer. **Genetic polymorphisms in *carnitine palmitoyltransferase 1A* gene are associated with variation in body composition and fasting lipid traits in Yup'ik Eskimos. *J. Lipid Res.* 2012.** 53: **175–184.**

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Obesity is associated with a series of metabolic conditions clinically referred to as metabolic syndrome, which includes hypertension, dyslipidemia, hyperglycemia, and the development of type 2 diabetes (T2D). Approximately sixty percent of obese individuals have metabolic complications (1); however, "healthy obese" individuals have been identified with excessive accumulation of body fat that does not translate to dyslipidemia and insulin resistance (2, 3). For example, some Eskimo/Inuit people indigenous to Alaska are obese, but they have historically demonstrated low prevalence of insulin resistance, metabolic syndrome, and T2D (4–7). Specifically, Yup'ik Eskimo peoples living in Southwest Alaska have obesity prevalence comparable to the general US population, yet the prevalence of metabolic syndrome (8) and T2D (9) is significantly less than that observed in the general US population (10, 11). Although the mechanisms that allow

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Abbreviations: BMI, body mass index; CPT1, carnitine palmitoyltransferase 1; DHA, docosahexaenoic acid; EPA, eicosapentaenoic; HC, hip circumference; HWE, Hardy-Weinberg equilibrium; LCFA, long-chain fatty acid; LD, linkage disequilibrium; MAF, minor allele frequency; PBF, percentage body fat; RBC, red blood cell; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; ThC, thigh circumference; WC, waist circumference.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of three tables.

Yup'ik Eskimo people to carry excess body fat without developing features of metabolic syndrome and T2D are unknown, dietary and genetic factors are likely to be relevant (12, 13). Because weight loss as a treatment for obesity-related comorbidities is difficult to achieve and maintain (14–17), understanding the underlying mechanisms that protect this population from features of metabolic syndrome despite their adiposity would have implications for treatment of obesity without the necessity of weight loss.

It has been proposed that the "healthy obesity" observed in Yup'ik Eskimo individuals is in part related to exposure to a diet rich in n-3 polyunsaturated fatty acids (n-3 PUFA) (18, 19). n-3 PUFAs consumed by Yup'ik Eskimo people are principally composed of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and their PUFA intake is 20 times greater than the current mean intake of the general US population (4.1 ± 0.5 g/day versus 0.05 g/day in men; 2.8 ± 0.3 g/day versus 0.09 g/day in women) (20, 21). Cross-sectional studies in a Yup'ik Eskimo population offer a unique opportunity to examine the association of elevated n-3 PUFA exposure with body composition, fasting lipids, and lipoprotein levels. Studies in both animals and humans have demonstrated that EPA and DHA impact body composition and circulating fasting lipid levels by modulating gene expression to favor increased fatty acid oxidation and reduction of fat deposition (22). Evidence that elevated n-3 PUFA consumption has a direct influence on "healthy" obesity remains inconclusive (12, 13) and warrants experimental designs that evaluate gene-diet interactions that may mediate this effect in populations with elevated daily dietary intake of n-3 PUFA.

Mitochondrial carnitine palmitoyltransferase 1 (*CPT1*), a member of the carnitine palmitoyltransferase family, is a gene that controls fatty acid oxidation in skeletal, adipose, and liver tissue (23). Fatty acid oxidation is often impaired in the obese condition (24, 25), which may contribute to hepatic steatosis, hepatic insulin resistance, and impaired hepatic lipid handling (26). CPT1 as a major control point for fatty acid oxidation may, therefore, be a key player in "healthy obesity," especially if certain single nucleotide polymorphisms (SNP) are resistant to impaired fatty acid oxidation, which often accompanies obesity. Interestingly, as n-3 PUFA increases mitochondrial fatty acid oxidation by stimulating the activity of *CPT1* (27), the interaction between n-3 PUFAs and SNPs in CPT1 may improve lipid profiles.

Mammalian tissues express three *CPT1* isoforms: *CPT1A* (liver), *CPT1B* (muscle), and *CPT1C* (brain), which are encoded on separate genes (28–30). In the presence of L-carnitine, *CPT1* facilitates the transfer of long-chain fatty acids (LCFA) across the mitochondrial membrane for β-oxidation (31). Mitochondrial β-oxidation of dietary and endogenous LCFA is tightly regulated through allosteric inhibition of *CPT1* by malonyl-CoA, an intermediate in fatty acid synthesis (32). In liver cells, the partnership between malonyl-CoA and CPT1A has been shown to be a key regulatory point that modulates the oxidation of dietary and endogenous LCFA (33). Although *CPT1A* is a candidate gene for obesity (34) and *CPT1A* SNPs are associated with elevated fasting HDL-cholesterol levels (35), it is unknown whether the interaction between n-3 PUFA intake and *CPT1A* SNPs influence changes in body composition and fasting lipids.

In this study, we tested the hypothesis that SNPs within or near the *CPT1A* gene are associated with body composition and fasting lipid phenotypes in a large cross-sectional cohort of Yup'ik Eskimo peoples, a population whose daily dietary intake involves a 30-fold range of exposure of n-3 PUFA, and we examined whether these associations were modified by n-3 PUFA intake.

METHODS

Subjects and study design

The Center for Alaska Native Health Research (CANHR) studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik Eskimo peoples (9). A community-based participatory research framework guides all CANHR investigations; participant ascertainment is open to all members of the community meeting a specified age minimum. Recruitment of Yup'ik Eskimo participants was initiated in 2003 and continues in 11 Southwest Alaska communities. All residents 14 years of age and older are invited to participate, and the resulting distribution of age in our study sample reflects the age distribution among eligible participants according to 2000 US census data. Participants sign informed-consent documents before entering the study using protocols that were approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon-Kuskokwim Health Corporation Human Studies Committee. The analyses in this report were performed on 1,141 nonpregnant Yup'ik Eskimo participants with ages that ranged between 14 and 94 years at the time of enrollment.

Anthropometric and biochemical measurements

Anthropometric measurements were obtained by trained staff using protocols from the NHANES III Anthropometric Procedures Manual (36) as previously described (8). These measurements included height, weight, and four circumferences (waist, hip, triceps, and thigh). Percentage body fat was measured by electrical bioimpedance using a Tanita TBF-300A body composition analyzer (Tanita Corp., Arlington Heights, IL). Blood samples were collected from participants after an overnight fast, and lipoprotein measures, including total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, apolipoprotein A-I, and plasma triglycerides levels, were assayed as previously described by Boyer et al. (8).

Biomarker for marine n-3 PUFA intake: analysis of RBC nitrogen stable isotope ratio

n-3 PUFA intake was assessed in Yup'ik Eskimo individuals using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC) as previously described (37). RBC aliquots were autoclaved for 20 min at 121°C to destroy blood-borne pathogens, and samples were weighed into 3.5 × 3.75 mm tin capsules and freeze dried to a final mass of 0.2–0.4 mg. Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer

via the Conflo III interface (Thermo-Finnigan Inc., Breman, Germany). Isotope ratios were analyzed relative to IAEA-certified reference materials calibrated to atmospheric nitrogen, for which $^{15}N/^{14}N = 0.0036765$. By convention and for ease of interpretation, isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \cdot 1000‰$. We concurrently prepared and ran multiple laboratory standards (peptone, $\delta^{15}N = 7.00$) to assess analytical accuracy and precision; these were analyzed after every eighth sample and gave values of $\delta^{15}N = 7.01 \pm 0.24‰$ (mean ± SD). The range of isotopic variation in our dataset (9‰) was very large relative to analytical precision (0.2‰). We modeled the effects of n-3 PUFA intake as a categorical variable, and they were included in the association analysis. This categorical variable is hereafter referred to as $\delta^{15}N$.

SNP selection and genotyping

A comprehensive list of DNA variants were selected for genotyping within and near (5 kb upstream and 5 kb downstream) the *CPT1A* gene collected from HapMap data, release 3, National Center for Biotechnology Information (NCBI) B36, dbSNP 126 (38). Given that no publically available genotypic information exists on Yup'ik Eskimo people, we referenced the Caucasian (CEU) and Han Chinese (CHB) populations in HapMap using the Seattle SNPs database (http://pga.mbt.washington.edu/) to identify potential genetic variants that may be common in our study population. A set of 27 maximally informative tagging SNPs (tSNP) were selected to represent common linkage disequilibrium clusters with the LDselect algorithm as implemented in the MultiPop-TagSelect program, using thresholds of $r^2 = 0.80$ and minor allele frequency (MAF) >1% (39, 40). We chose to relax our MAF criteria to include SNPs with MAF > 0.01 to genotype tagging SNPs in the *CPT1A* gene that may be common (MAF ⩾ 0.05) in Yup'ik Eskimos despite being rare (MAF < 0.05) in CEU and CHB populations. We also included the nonsynonymous P479L (rs80356779) *CPT1A* SNP for genotyping based on previous associations with elevated plasma HDL-cholesterol and apolipoprotein A-I levels in the Greenland Inuit (35). Genotyping of the 28 SNPs, including P479L, was carried out by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight spectrometry on a Sequenom iPLEX platform at the Broad Institute (41). Linkage disequilibrium (LD) among SNPs was based on pairwise haplotype frequencies calculated using the hapfreq command in the FBAT program (42).

Quality control of phenotypic and genotypic data

Simple linear models were fit to each of the outcome variables using all of the covariates (age, sex, community membership) included in the association models, and the distributions of the residuals were examined for normality with the R statistical programming language (v2.10.1, R Development Core, 2009). Box-Cox transformations were applied to traits whose residuals did not follow a normal distribution (43). Family data was extracted from a Progeny database (Progeny Software LLC, South Bend, IN) and merged into a single extended pedigree using PedMerge (44). Genotypic data were tested for Mendelian inconsistencies using PEDCHECK (45). In this sample, Illumina IV linkage panel (Illumina, Inc., San Diego, CA) genotypes were available from an ongoing linkage study and were used to construct principal components of ancestry (PCA) using the PCA program in the EIGENSTRAT analysis package (46). The second PCA discriminated the individuals in the study into two groups that correspond to the proximity of the community to the coast. On the basis of this observation, we defined a dichotomous community group variable. We assessed Hardy-Weinberg equilibrium (HWE) using PLINK (v1.07) (47) and determined allele frequencies for each SNP using the FREQ module in the program Statistical Analysis for Genetic Epidemiology (S.A.G.E., 2009). The present study restricted analysis to only include SNPs with MAF ⩾ 5% that did not deviate from HWE after Bonferroni correction ($P < 0.002$).

Association analysis

Each SNP was tested for association with obesity-related phenotypes using the program ASSOC (48) in the Statistical Analysis for Genetic Epidemiology (S.A.G.E. 2009) software package, which can incorporate complex pedigree data, covariates, and interactions into association analysis. We included both demographic (age, community, and sex) and environmental covariates ($\delta^{15}N$) in the ASSOC analysis. Likelihood ratio statistics were calculated to compare three nested models and test the null hypothesis of no association between *CPT1A* SNPs and obesity traits after including demographic and environmental covariates. Effect sizes (β) are presented as the change in transformed phenotypes according to minor allele that was determined in a linear model adjusted for demographic and environmental covariates.

Model 1 included baseline covariates (age, sex, community membership, and $\delta^{15}N$ quartiles); Model 2 included baseline covariates and SNP to test for an additive genetic effect of SNP (defined as the number of minor alleles); and Model 3 included baseline covariates, the additive genetic effect of SNP, and interactions between the additive genetic effect and $\delta^{15}N$ quartiles. Note that Model 3 is the only model to test directly gene-diet interaction under the null hypothesis. We treated each phenotype tested as representing a separate family of null hypotheses and corrected for the number of tests within each family (49). Multiple-test correction to control the familywise error rate was calculated according to the number of nonredundant SNPs with MAF ⩾ 0.05 that were tested for association and interaction. Given the correlation among neighboring genetic markers, the effective number of nonredundant SNPs in this study was estimated using spectral decomposition of LD matrices (50, 51).

RESULTS

Characteristics of Yup'ik Eskimo participants

General clinical characteristics and descriptive statistics on Yup'ik Eskimo men and women are presented in **Table 1**. Yup'ik women in this study had a mean age of 37.6 (± 17.3) years, and men reported a mean age of 35.9 (±17.3) years. Women had significantly greater body mass index (BMI), percentage body fat (PBF), hip circumference (HC), fasting total cholesterol, HDL-cholesterol, and ApoA1 levels compared with men ($P < 0.05$). According to the standard cutoff points for overweight (BMI = 25–29.9 kg/m^2) and obese (BMI ⩾ 30 kg/m^2), 28.6% of women and 30.7% of men were overweight, whereas 37.0% of women and 7.9% of the men were classified as obese.

Distribution of $\delta^{15}N$ in study population

In 1,138 Yup'ik Eskimo participants, n-3 PUFA intake was assessed using RBC $\delta^{15}N$ as a biomarker of EPA and DHA intake. Summary statistics grouped by gender and $\delta^{15}N$ quartiles are reported in **Table 2**. The mean $\delta^{15}N$ value was 9.0‰ with a range of 6.2–15.2‰. This range was large relative to analytical precision (0.2‰) and was 3.75 times greater than the RBC (clot) $\delta^{15}N$ values previously reported for a random sample of US residents (52).

TABLE 1. Descriptive statistics of obesity-related traits in Yup'ik Eskimos

	Women	Men	*P*
Variables			
No. of participants	601	539	
Age (yr)	37.6 ± 17.3	35.9 ± 17.4	0.1113
Height (cm)	156.1 ± 6.2	167.7 ± 7.0	<0.0001
Weight (kg)	69.8 ± 16.7	73.0 ± 15.6	0.0003
Obesity measures			
BMI (kg/m^2)	28.7 ± 6.8	25.9 ± 4.8	<0.001
Percentage body fat (%)	35.1 ± 8.9	21.1 ± 8.0	<0.0001
Waist circumference (cm)	90.4 ± 15.9	89.2 ± 14.0	0.2102
Hip circumference (cm)	104.1 ± 12.8	96.7 ± 8.3	<0.0001
Thigh circumference (cm)	51.1 ± 5.6	50.2 ± 5.4	0.0071
Lipid measures			
Cholesterol (mg/dl)	216.4 ± 44.6	208.9 ± 48.0	0.0088
HDL (mg/dl)	64.8 ± 18.5	56.2 ± 15.4	<0.0001
Apolipoprote in A-I (mg/dl)	170.8 ± 26.7	159.5 ± 26.8	<0.0001
LDL (mg/dl)	134.9 ± 36.5	135.9 ± 40.2	0.5500
VLDL (mg/dl)	16.9 ± 8.8	17.3 ± 10.6	0.4760
Triglyceride (mg/dl)	83.6 ± 42.8	84.8 ± 52.5	0.5967

Values are mean ± SD. Differences by gender are derived using Student t-test.

According to the linear relationship between RBC δ^{15}N and RBC EPA reported elsewhere for this population (37), the corresponding mean EPA (% RBC fatty acids) was 2.66% with a range of ~0–9.1%. Measurement of δ^{15}N by gender yielded means of 9.1‰ for females and 8.8‰ for males. The mean RBC δ^{15}N values by quartile were 7.3‰, 8.2‰, 9.1‰, and 11.0‰ in quartiles 1–4, respectively. These values correspond to EPA (% RBC fatty acids) quartile means of: 0.9%, 1.8%, 2.8%, and 4.7% (37). The standard deviation of δ^{15}N in this sample did not differ by gender (1.5‰ for both females and males).

Genetic variation in the *CPT1A* gene

DNA was available in 1,141 Yup'ik Eskimo participants, and the mean number of individuals successfully genotyped was 1,078 (range of 986–1,137, depending on the SNP). Twenty-eight *CPT1A* SNPs were genotyped with a mean success rate of 94.7% (range 76.1–99.7%). In this sample, 4 SNPs were monomorphic, 12 SNPs had MAF < 0.05 and MAF > 0.01, and 12 SNPs had MAF ⩾ 0.05. Genotyping results for SNPs with MAF ⩾ 0.05 are presented in **Table 3**. The rs2924697 SNP (MAF = 0.28) was the only polymorphism with MAF ⩾ 0.05 that deviated significantly from Hardy-Weinberg proportions and was excluded from the analysis. The nonsynonymous P479L SNP was common in our sample, and the major L479 allele had a frequency of 0.74. We selected the 11 *CPT1A* SNPs with MAF ⩾ 0.05 that did not deviate from HWE proportions for genetic analysis (Table 3). The spectral decomposition of LD matrix (50) estimated that 8 of the 11 markers with MAF ⩾ 0.05 were nonredundant genetic markers, and we corrected our analysis for eight tests, setting the per-test α level to <0.0063 (two-tailed).

Association between fasting lipid parameters and *CPT1A* SNPs

The results of association analysis between fasting lipid traits and *CPT1A* SNPs with MAF ⩾ 0.05 are summarized in **Table 4**. HDL-cholesterol was significantly associated with seven SNPs: rs2278908 (P = 0.0007, β = −2.3, SE = 0.7), rs3019598 (P = 0.0014, β = −2.2, SE = 0.7), P479L (P = 0.0001, β = −1.0, SE = 0.3), rs11228372 (P = 0.0013, β = −1.2, SE = 0.4), rs11228373 (P < 0.0001, β = −1.3, SE = 0.3), rs3019594 (P < 0.0001, β = −1.4, SE = 0.3), and rs597316 (P = 0.0014, β = −2.2, SE = 0.7). The rs11228373 and rs3019594 SNPs were also significantly associated with ApoA1 (P = 0.0014, β = −1.1, SE = 0.4 and P = 0.0008, β = −1.2, SE = 0.4, respectively) and total cholesterol (P = 0.0063, β = −0.7, SE = 0.2 and P = 0.0031, β = −0.7, SE = 0.2, respectively) (Table 4). Note that rs11228373 and rs3019594 are in moderately strong LD (r^2 = 0.75). The P479L variant was also associated with HDL-cholesterol (P = 0.0001) and was not in strong LD with either the rs11228373 (r^2 = 0.58) or rs3019594 (r^2 = 0.61) SNP (supplementary Table I). Our model predicted that individuals homozygous for the common allele (L479) of P479L had elevated fasting HDL-cholesterol levels compared with individuals homozygous for the P479L minor allele (P479). After adjusting Model 2 for BMI, the *CPT1A* SNPs (rs2278908, rs3019598, P479L, rs11228373, rs3019594, and rs597316) associated with fasting total cholesterol, HDL-cholesterol, ApoA1 were still significant (supplementary Table II).

Association between *CPT1A* SNPs and obesity phenotypes

The results of association analysis between obesity traits and *CPT1A* SNPs with MAF ⩾ 0.05 are summarized in

TABLE 2. Distribution of the RBC nitrogen stable isotope ratio (δ^{15}N), a concentration biomarker for long chain n-3 polyunsaturated fatty acid (n-3 PUFA) intake in Yup'ik Eskimos

		Sex		Quartiles of δ^{15}N[a]			
	Total	Women	Men	Q1	Q2	Q3	Q4
No. of participants	1138	598	540	272	278	290	298
Mean ± SD (‰)	9.0 ± 1.5	9.1 ± 1.5	8.8 ± 1.5	7.3 ± 0.3	8.2 ± 0.2	9.1 ± 0.3	11.0 ± 1.0
Maximum	15.2	15.2	13.5	7.8	8.6	9.8	15.2
Minimum	6.2	6.3	6.2	6.2	7.8	8.6	9.8
Range (‰)	9.0	8.9	7.3	1.6	0.81	1.2	5.4

Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \cdot 1000‰$.

[a]The relationship between δ^{15}N and EPA follows the linear model: EPA (%RBC fatty acid) = 1.04 · δ^{15}N – 6.7‰, as previously described for this population (37).

TABLE 3. SNPs genotyped within and near the *CPT1A* gene with MAF ⩾ 0.05

SNP[a]	Allele[b]	MAF[c]	Genotype			Individuals Genotyped[d]	HWE *P*[e]
			AA	AB	BB		
rs2278908	C/T	0.06	2	40	1024	1066	0.1004
rs2278907	A/G	0.09	9	54	1074	1137	0.0270
rs3019598	G/A	0.05	2	37	1011	1050	0.0664
P479L (rs80356779)	A/G[f]	0.26	44	272	759	1075	0.1080
rs2305508	C/T	0.47	158	507	407	1072	0.7469
rs4930248	T/C	0.10	10	88	974	1072	0.0369
rs3794020	C/T	0.34	103	447	461	1011	1
rs2924697	G/C	0.28	1	533	452	986	0
rs11228372	G/A	0.14	7	158	906	1071	1
rs11228373	G/C	0.20	27	192	913	1132	0.0077
rs3019594	C/T	0.21	26	185	861	1072	0.0120
rs597316	G/C	0.05	1	42	1026	1069	0.4096

AA, homozygous recessive for minor allele; AB, heterozygous; BB, homozygous dominant allele.

[a]Seattle SNPs Genome Variation Server on March 2008 (dbSNP build 126) Version 5.01.

[b]Major/ Minor allele.

[c]MAF computed using FREQ module in S.A.G.E.

[d]Number of individuals genotyped for each *CPT1A* SNP.

[e]Computed using PLINK.

[f]The P479L SNP is an A→G missense mutation at nucleotide position c.1436 of *CPT1A*, which results in the substitution of a conserved proline (P479) for a leucine (L479) at position 479 in the CPT1A polypeptide.

Table 5. Thigh circumference (ThC) was associated with seven SNPs: rs2278908 (P = 0.0024, β = 2.1, SE = 0.7), rs2278907 (P = 0.0002, β = 1.9, SE = 0.5), P479L (P < 0.0001, β = 1.2, SE = 0.3), rs4930248 (P = 0.0042, β = 1.3, SE = 0.5), rs11228372 (P = 0.0011, β = 1.3, SE = 0.4), rs11228373 (P = 0.0006, β = 1.1, SE = 0.3), and rs3019594 (P = 0.0001, β = 1.3, SE = 0.3). Hip circumference was associated with five SNPs: rs2278907 (P = 0.0057, β = 1.2, SE = 0.4), P479L (P < 0.0001, β = 0.9, SE = 0.2), rs11228372 (P = 0.0034, β = 0.9, SE = 0.3), rs11228373 (P = 0.0063, β = 0.7, SE = 0.3), and rs3019594 (P = 0.0005, β = 0.9, SE = 0.3).

The P479L SNP and rs3019594 (r^2 = 0.61 between these SNPs) were SNPs most significantly associated with both thigh circumference and hip circumference (P < 0.0001 and P = 0.0034, respectively). The P479L SNP was the only SNP associated with all obesity measures, which included BMI (P = 0.0021, β = 0.7, SE = 0.2), percentage body fat (P = 0.0007, β = 0.0007, SE = 0.2), hip circumference (P < 0.0001, β = 0.9, SE = 0.2), thigh circumference (P < 0.0001, β = 1.2, SE = 0.3), and waist circumference (WC; P = 0.0006, β = 1.0, SE = 0.3). Individuals homozygous for the common P479L allele (L479) had a lower percentage body fat, smaller BMI, and reduced thigh, hip, and waist circumferences compared with P479 homozygotes (**Table 6**).

DISCUSSION

CPT1A has been implicated as candidate obesity gene in a meta-analysis of whole-genome linkage studies (34); however, the contribution of *CPT1A* polymorphisms to variation in the metabolic consequences of obesity and obesity phenotypes remains unclear. Our results demonstrate that *CPT1A* polymorphisms are associated with obesity and fasting lipid phenotypes in this Yup'ik Eskimo study population and may influence the "healthy obesity" phenotype. Specifically, the P479L SNP was associated with all measures of body composition (BMI, PBF, HC, ThC, and WC) and fasting HDL-cholesterol levels. We found that individuals homozygous for the major L479 allele of the P479L variant had reduced body fat and central adiposity relative to individuals homozygous for the minor P479 allele. These data indicate that individuals carrying both copies of the L479 allele of the nonsynonomous P479L variant in *CPT1A* have reduced adiposity and elevated HDL-cholesterol, even after controlling for BMI. Interestingly, when we investigated whether the P479L association with HDL was mediated by other obesity phenotypes, we found the L479 allele was still significantly associated with HDL-cholesterol after correction for either PBF or WC (data not shown). We hypothesize that the L479 allele may contribute to "healthy obesity" observed in Yup'ik Eskimo people by modulating hepatic lipid oxidation.

Three studies have previously investigated the influence of *CPT1A* polymorphisms on obesity and lipid phenotypes in humans (35, 53, 54). Hirota and colleagues (54) found no association between *CPT1A* polymorphisms and obesity or fasting lipid phenotypes in Japanese individuals with T2D. In a cross-sectional cohort of French-Canadians, Robitaille et al. reported an association between the nonsynonymous A275T (rs17610395) SNP with BMI (P = 0.05) and waist circumference (P = 0.008) only after accounting for dietary fat intake (53). Finally, in Greenland Inuit, Rajakumar et al. showed the L479 allele in the nonsynonymous P479L variant was associated with elevated fasting HDL-cholesterol and ApoA1 levels (35).

The present study found an association between SNPs (rs2278908, rs3019598, and rs597316) investigated by Hirota et al. with fasting HDL-cholesterol and replicated the P479L association with fasting HDL-cholesterol and ApoA1 levels reported by Rajakumar et al. We have shown that these SNPs were still significantly associated with HDL-cholesterol and ApoA1 after controlling for BMI, PBF, or WC. Furthermore, we used a log likelihood ratio test to determine whether the P479L SNP association with HDL-cholesterol and ApoA1 was independent of the rs11228373 and rs3019594 SNPs. We found that both rs11228373 and rs3019594 SNPs were still significant predictors for HDL-cholesterol and ApoA1 levels, even when P479L was already in the model. Given that the rs11228373 and rs3019594 SNPs were in moderately strong linkage disequilibrium with P479L as measured by r^2 (r^2 = 0.58 and 0.61, respectively), we cannot rule out the possibility that the apparent association with P479L is not due to a true association with either rs11228373 or rs3019594 or both.

We extend the findings of Rajakumar et al. to show the L479 allele of P479L is also associated with reduced adiposity in an independent Eskimo/Inuit population. Interestingly, our analysis did not replicate the A275T (rs17610395) association with BMI and WC reported by Robitaille et al. because this SNP was not included in the analysis due to a low MAF (MAF = 0.02). Factors that may

TABLE 4. Association of *CPT1A* SNPs with fasting lipid phenotypes

	Lipid Measures					
SNP	Chol	HDL	ApoA1	LDL	VLDL	TG
rs2278908	0.0842 (β = −0.9, SE = 0.5)	**0.0007 (β = −2.3, SE = 0.7)**	0.0501 (β = −1.5, SE = 0.8)	0.9124 (β = −0.1, SE = 0.7)	0.9449 (β = −0.1, SE = 0.8)	0.8209 (β = 0.2, SE = 0.8)
rs2278907	0.1478 (β = −0.6, SE = 0.4)	0.0335 (β = −1.0, SE = 0.5)	0.4893 (β = −0.4, SE = 0.6)	0.7316 (β = −0.2, SE = 0.5)	0.4551 (β = −0.4, SE = 0.6)	0.7217 (β = −0.2, SE = 0.5)
rs3019598	0.1357 (β = −0.8, SE = 0.6)	**0.0014 (β = −2.2, SE = 0.7)**	0.2382 (β = −1.0, SE = 0.8)	0.9588 (β = 0.0, SE = 0.7)	0.9073 (β = 0.1, SE = 0.8)	0.7069 (β = 0.3, SE = 0.8)
P479L (rs80356779)	0.0834 (β = −0.4, SE = 0.2)	**0.0001 (β = −1.0, SE = 0.3)**	0.0077 (β = −0.8, SE = 0.3)	0.6629 (β = −0.1, SE = 0.3)	0.6355 (β = 0.1, SE = 0.3)	0.1407 (β = 0.4, SE = 0.3)
rs2305508	0.0321 (β = −0.4, SE = 0.2)	0.6052 (β = 0.1, SE = 0.2)	0.0520 (β = 0.5, SE = 0.2)	0.0250 (β = −0.5, SE = 0.2)	0.7030 (β = −0.1, SE = 0.2)	0.2284 (β = −0.3, SE = 0.2)
rs4930248	0.0912 (β = −0.6, SE = 0.4)	0.2834 (β = −0.5, SE = 0.4)	0.9245 (β = 0.1, SE = 0.5)	0.4413 (β = −0.3, SE = 0.4)	0.8735 (β = −0.1, SE = 0.5)	0.9755 (β = 0.0, SE = 0.5)
rs3794020	0.5539 (β = −0.1, SE = 0.2)	0.2261 (β = 0.3, SE = 0.2)	0.0132 (β = 0.7, SE = 0.3)	0.2244 (β = −0.3, SE = 0.2)	0.7530 (β = 0.1, SE = 0.3)	0.6833 (β = −0.1, SE = 0.3)
rs11228372	0.0139 (β = −0.7, SE = 0.3)	**0.0013 (β = −1.2, SE = 0.4)**	0.0150 (β = −1.1, SE = 0.4)	0.2982 (β = −0.4, SE = 0.4)	0.6986 (β = −0.2, SE = 0.4)	0.6246 (β = 0.2, SE = 0.4)
rs11228373	**0.0063 (β = −0.7, SE = 0.2)**	**<0.0001 (β = −1.3, SE = 0.3)**	**0.0014 (β = −1.1, SE = 0.4)**	0.3095 (β = −0.3, SE = 0.3)	0.8199 (β = −0.1, SE = 0.4)	0.3369 (β = 0.3, SE = 0.3)
rs3019594	**0.0031 (β = −0.7, SE = 0.2)**	**<0.0001 (β = −1.4, SE = 0.3)**	**0.0008 (β = −1.2, SE = 0.4)**	0.2377 (β = −0.4, SE = 0.3)	0.6516 (β = 0.2, SE = 0.4)	0.1691 (β = 0.5, SE = 0.3)
rs597316	0.2712 (β = −0.6, SE = 0.6)	**0.0014 (β = −2.2, SE = 0.7)**	0.0449 (β = −1.6, SE = 0.8)	0.8213 (β = −0.2, SE = 0.7)	0.2500 (β = 0.9, SE = 0.8)	0.0417 (β = 1.6, SE = 0.8)

Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. Estimates of effect size (β) are reported using transformed phenotypes. Results are significant at $P < 0.0063$ (highlighted in bold). Multiple-test correction for eight tests for a phenotype was estimated using the spectral decomposition of LD matrix (50).

ApoA1, apolipoprotein A-I; Chol, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; VLDL very low density lipoprotein.

TABLE 5. Association of *CPT1A* SNPs with obesity phenotypes

	Obesity Measures				
SNP	BMI	PBF	HC	ThC	WC
rs2278908	0.5137	0.4006	0.0179	**0.0024**	0.5103
	(β = 0.4, SE = 0.6)	(β = 0.4, SE = 0.5)	(β = 1.4, SE = 0.6)	**(β = 2.1, SE = 0.7)**	(β = 0.5, SE = 0.8)
rs2278907	0.3778	0.4339	**0.0057**	**0.0002**	0.6956
	(β = 0.4, SE = 0.42)	(β = 0.3, SE = 0.4)	**(β = 1.2, SE = 0.4)**	**(β = 1.9, SE = 0.5)**	(β = 0.2, SE = 0.5)
rs3019598	0.7691	0.6930	0.0578	0.0089	0.9006
	(β = 0.2, SE = 0.6)	(β = 0.2,SE = 0.5)	(β = 1.1, SE = 0.6)	(β = 1.9, SE = 0.7)	(β = 0.1, SE = 0.8)
P479L (rs80356779)	**0.0021**	**0.0007**	**<0.0001**	**<0.0001**	**0.0006**
	(β = 0.7, SE = 0.2)	**(β = 0.7, SE = 0.2)**	**(β = 0.9, SE = 0.2)**	**(β = 1.2, SE = 0.3)**	**(β = 1.0, SE = 0.3)**
rs2305508	0.7108	0.6326	0.7604	0.1701	0.4912
	(β = −0.1, SE = 0.2)	(β = −0.1, SE = 0.2)	(β = 0.1, SE = 0.2)	(β = 0.3, SE = 0.2)	(β = −0.2, SE = 0.2)
rs4930248	0.2369	0.1874	0.0191	**0.0042**	0.1503
	(β = 0.4, SE = 0.4)	(β = 0.4, SE = 0.3)	(β = 0.9, SE = 0.4)	**(β = 1.3, SE = 0.5)**	(β = 0.7, SE = 0.5)
rs3794020	0.4386	0.2276	0.3897	0.8763	0.1390
	(β = −0.2, SE = 0.2)	(β = −0.2, SE = 0.2)	(β = −0.2, SE = 0.2)	(β = 0.0, SE = 0.2)	(β = −0.4, SE = 0.3)
rs11228372	0.0277	0.0244	**0.0034**	**0.0011**	0.0294
	(β = 0.7, SE = 0.3)	(β = 0.6, SE = 0.3)	**(β = 0.9, SE = 0.3)**	**(β = 1.3, SE = 0.4)**	(β = 0.9, SE = 0.4)
rs11228373	0.1605	0.2517	**0.0063**	**0.0006**	0.1095
	(β = 0.4, SE = 0.3)	(β = 0.3, SE = 0.2)	**(β = 0.7, SE = 0.3)**	**(β = 1.1, SE = 0.3)**	(β = 0.5, SE = 0.3)
rs3019594	0.0294	0.0312	**0.0005**	**<0.0001**	0.0154
	(β = 0.6, SE = 0.3)	(β = 0.5, SE = 0.2)	**(β = 0.9, SE = 0.3)**	**(β = 1.3, SE = 0.3)**	(β = 0.8, SE = 0.3)
rs597316	0.3342	0.6079	0.0731	0.0161	0.2518
	(β = 0.6, SE = 0.6)	(β = 0.3, SE = 0.5)	(β = 1.1, SE = 0.6)	(β = 1.8, SE = 0.7)	(β = 0.9, SE = 0.8)

Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. Estimates of effect size (β) are reported using transformed phenotypes. Results are significant at $P < 0.0063$ (highlighted in bold). Multiple-test correction for eight tests for a phenotype was estimated using the spectral decomposition of LD matrix (50).

account for differences in results reported in the present study may include, but are not limited to, differences in statistical analysis, small sample size, and population stratification (55). Our study, however, benefited from a sample size large enough to detect significant SNP associations, and we used a statistical approach that accounts for family structure while allowing for covariates.

Our Yup'ik Eskimo study population was ideally suited to investigate the contribution of n-3 PUFA and genetic factors to "healthy obesity" due to the 30-fold range of EPA and DHA consumption (20), which can be precisely estimated in large samples using nitrogen stable isotope ratios from red blood cell samples (37). When we examined whether the interaction between n-3 PUFA intake and *CPT1A* SNPs modifies the association with "healthy obesity" phenotypes (supplementary Table III), we did not find significant gene-diet interactions that modified the association with body composition. Interestingly, interactions between n-3 PUFA intake and rs3794020 and rs2305508 were associated with HDL-cholesterol and ApoA1 levels, whereby n-3 PUFA intake enhanced the positive association of rs3794020 and rs2305508 minor alleles on these traits. Although n-3 PUFA interactions with genetic factors have received considerable attention, our results should be interpreted with caution given the sample size and nominal significance.

We hypothesize that the observed association between the P479L variant on body composition and fasting lipid phenotypes in the presence of n-3 PUFA intake may, in part, explain the presence of a "healthy obese" phenotype among Yup'ik Eskimos. In humans, low rates of endogenous lipid oxidation are associated with obesity (25), and mechanisms that alter an individual's metabolic profile in favor of fatty acid oxidation have been suggested to reduce

TABLE 6. Obesity-related trait distribution within P479L (rs80356779) genotypes in Yup'ik Eskimos people

Obesity Measures	L479/L479	L479/P479	P479/P479	*P*
BMI (kg/m^2)	26.2 (24.4–28.1)	28.0 (25.3–31.3)	31.1 (26.2–37.8)	**0.0021**
Percentage body fat (%)	27.8 (24.9–30.7)	30.0 (25.8–34.3)	33.7 (26.8–41.0)	**0.0007**
Waist circumference (cm)	87.1 (82.7–91.9)	91.9 (85.2–99.8)	100.4 (88.1–116.4)	**0.0006**
Hip circumference (cm)	98.2 (95.1–101.7)	101.6 (96.8–107.3)	106.6 (98.0–118.2)	**<0.0001**
Thigh circumference (cm)	49.8 (48.0–51.7)	53.4 (50.6–56.4)	57.9 (52.9–63.5)	**<0.0001**
Lipid Measures	L479/L479	L479/P479	P479/P479	*P*
Cholesterol (mg/dl)	211.0 (197.4–225.6)	199.6 (181.6–219.8)	193.9 (166.5–266.8)	0.0834
HDL (mg/dl)	58.3 (53.3–64.1)	53.3 (47.2–60.6)	49.6 (41.1–60.7)	**0.0001**
Apolipoprotein A-I (mg/dl)	164.2 (155.2–173.9)	156.1 (144.2–169.1)	150.1 (132.4–170.5)	**0.0077**
LDL (mg/dl)	134.6 (122.4–147.8)	128.1 (111.6–146.4)	125.0 (99.7–155.1)	0.6629
VLDL (mg/dl)	15.1 (12.9–18.0)	15.8 (12.5–20.4)	17.2 (11.8–26.9)	0.6355
Triglyceride (mg/dl)	71.7 (61.7–84.2)	75.4 (60.9–95.5)	83.0 (58.6–125.1)	0.1407

Values are reported as predicted mean (95% CI) obtained from ASSOC output. Association of the P479L (L479>P479) minor allele in the linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. Results are significant at $P < 0.0063$ (highlighted in bold). Multiple-test correction for eight tests for a phenotype was estimated using the spectral decomposition of LD matrix (50).

the accumulation of body fat (56). Consumption of n-3 PUFA increases hepatic fatty acid β-oxidation, primarily through activity of CPT1A (57, 58). Functional studies in fibroblast cells have demonstrated that the L479 allele of P479L variant in *CPT1A* results in a CPT1A enzyme with diminished catalytic activity compared with control cells (59). However, expression of the L479 allele in fibroblasts was also shown to abolish the ability of malonyl-CoA to inhibit CPT1A (59). Interestingly, these data are consistent with a study in rats demonstrating that malonyl-CoA-insensitive CPT1A was more effective than overexpression of wild-type CPT1A at oxidizing lipid substrates (33). Therefore, in the presence of n-3 PUFAs, there may be a net increase in the basal activity of CPT1A among individuals carrying the L479 allele, and fatty acids normally packaged in the liver as VLDL will instead be oxidized in the hepatocyte (60). Taken together, we hypothesize that the combined effects of n-3 PUFA intake and the high frequency of the P479L variant in Eskimo/Inuit populations may influence "healthy obesity" phenotypes primarily through reduced hepatic VLDL formation and subsequent reductions of plasma triglycerides and VLDL. This model is consistent with our observations that obese Yup'ik Eskimo people with high intake of n-3 PUFAs have low triglyceride levels, reduced c-reactive protein levels (13), and high circulating HDL-cholesterol levels (12), suggesting that n-3 PUFAs may protect from chronic disease in the presence of obesity.

CPT1A deficiency has been associated with risk for hypoketotic hypoglycemia, hepatic encephalopathy, and sudden infant death syndrome (61–64), as well as muscle cramps, vomiting, and occasional loss of consciousness (59, 64). Nevertheless, the high frequency of the L479 allele in Inuit and Yup'ik Eskimo people suggested to us and several others that it may confer a selective advantage (35, 60, 65, 66). We hypothesized that genetic variants in *CPT1A* may be associated with obesity because of the central role of the CPT1A enzyme in fatty acid oxidation. Our results and those of Rajakumar and colleagues (35) are consistent with a cardioprotective role of the L479 allele of P479L through its association with elevated HDL-cholesterol levels. In this study, we have also shown that genetic variants of *CPT1A* are associated with reduced adiposity, and we have replicated the association of elevated fasting HDL-cholesterol and ApoA1 levels with carriers of the L479 allele in this Yup'ik Eskimo study population. Furthermore, we found that *CPT1A* SNPs associated with HDL-cholesterol and ApoA1 levels were independent of obesity as measured by BMI, PBF, and WC. The P479L variant was not in strong LD ($r^2 > 0.8$) with any other *CPT1A* polymorphisms associated with body composition and fasting lipid parameters, suggesting that the P479L may have a causal role in "healthy obesity." Although we cannot exclude the possibility that other variants are in strong LD with the P479L, our data suggest that the P479L variant in *CPT1A* increases hepatic fatty acid oxidation and may contribute to "healthy obesity" observed in this Yup'ik Eskimo study population. Functional genomic studies of the *CPT1A* variant and its modulation by n-3 PUFA intake, in addition to further investigation of the *CPT1A* gene in epidemiological studies among Arctic populations with variable n-3 PUFA intake, will be required to validate the larger public health impact of these results. This study lays the foundation for future population-specific dietary recommendations based on gene-diet interactions.

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