Genetic Variation in Uncoupling Protein 3 Is Associated With Dietary Intake and Body Composition in Females

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The uncoupling proteins (UCPs) are a family of mitochondrial transport proteins that promote proton leakage across the inner mitochondrial membrane, uncoupling oxidative phosphorylation from adenosine triphosphate (ATP) production and releasing energy as heat. Variation in these genes may disrupt biochemical pathways influencing thermogenesis, energy metabolism, and fuel substrate partitioning and oxidation, which may in turn predispose to obesity. We genotyped polymorphisms in UCP2 and UCP3 in a sample of nondiabetic participants (n = 722) of the San Luis Valley Diabetes Study (SLVDS) and found female-specific associations between UCP3 polymorphisms and measures of dietary intake and body composition. The UCP3-5 variant was statistically significantly associated with total caloric intake ($P = .012$), fat intake ($P = .011$), fat mass ($P = .004$), and lean mass ($P = .013$), with the C allele corresponding to higher dietary intake and lower fat mass and lean mass. The UCP3p-55 and the UCP3-3 polymorphisms, which were in high linkage disequilibrium ($D' = 0.9776$), showed similar patterns of association with total caloric intake ($P = .031$ and $P = .042$, respectively) and lean mass ($P = .035$ and $P = .059$, respectively), with the rare alleles corresponding to higher total intake and lean mass. No statistically significant associations were detected between the outcome variables and polymorphisms in UCP2. Two-way analysis of covariance (ANCOVA), used to evaluate the multi-locus effects and interactions between UCP3-5 and UCP3p-55, showed association with the main effect terms, but no evidence for statistically significant interaction between UCP3-5 and UCP3p-55 in regard to dietary intake. The UCP3-5 polymorphism was the only statistically significant genetic predictor of fat mass. The lean mass model showed no statistically significant association with either UCP3 variant. These results support a role for UCP3 in fuel substrate management and energy metabolism, which may influence body weight regulation.

OBESITY IS A COMMON condition most prevalent in Westernized societies, with approximately 20% to 30% of adults affected in the United States.[1,2] Obesity is a major risk factor for common diseases, including type 2 diabetes, hypertension, atherosclerosis, and various forms of cancer. This multifactorial disorder encompasses various genetic and environmental components manifesting in imbalances in energy intake and expenditure.[3] Energy homeostasis is maintained by signals from feedback loops that regulate food intake, energy expenditure, glucose metabolism, and fat metabolism. Thus, variation in the genes controlling these pathways can influence the development of obesity and its complications.

The uncoupling proteins (UCPs) are a family of mitochondrial transport proteins that promote proton leakage across the inner mitochondrial membrane, thereby uncoupling oxidative phosphorylation from adenosine triphosphate (ATP) production and releasing energy as heat.[4] UCP2 and UCP3 are members of the UCP gene family located adjacent to one another on human chromosome 11q13[5] and a syntenic region on mouse chromosome 7.[6] Markers in these regions have been linked to resting energy expenditure in humans[7] and obesity and hyperinsulinemia in mice.[6] UCP2 is widely expressed in human tissues, while UCP3 expression is exclusive to skeletal muscle, a major site of thermogenesis, energy homeostasis, and substrate oxidation in humans.[8-10]

Variation in the UCP2 and UCP3 genes has become a focus for groups interested in genes influencing obesity and diabetes. Two variants have been identified in UCP2, an Ala55Val (C→T) substitution in exon 4 (UCP2-4)[11] and a 45–base pair insertion/deletion polymorphism in the 3′ untranslated region of exon 8 (UCP2-8).[12] Genotype/phenotype studies of both of these variants are conflicting. An association was reported between both UCP2 polymorphisms and metabolic rate in Pima Indians.[13] The UCP2-4 variant was also associated with enhanced metabolic efficiency and fat oxidation in Danish subjects,[14] while the UCP2-8 polymorphism showed association with body mass index (BMI).[13,15] No association was detected with obesity- and diabetes-related phenotypes in several other studies.[11-12,16] In UCP3, Otabe et al reported a C→T silent substitution in codon 210 in exon 5 of UCP3 (UCP3-5). The T/T genotype was weakly associated with diabetic status, but not morbid obesity in obese French subjects.[17] Lanouette et al recently reported suggestive linkage between the UCP3-5 variant and BMI, fat mass, or leptin levels in black and white individuals.[18] A T→C silent substitution identified in codon 99 in exon 3 of UCP3 (UCP3-3) was found to be associated with BMI in morbidly obese diabetics.[17,19] A third UCP3 variant, a C→T substitution at −55bp (UCP3p-55) was identified by Schrauwen et al. The T allele was associated with a 58% increase in UCP3 mRNA expression in skeletal muscle in male Pima Indians.[20] In the obese French cohort described above, T homozygosity for this variant was associated with higher BMI and resistance to beneficial effects of physical activity.[21] A contrasting study found no association between the UCP3p-55 polymorphism and BMI or percent body fat in obese and

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Table 1. Genotyping Primers and Detection Methods

Polymorphism	Primers	Genotype Detection
UCP3p-55	5′-aggactgaaccagatctggaactcactc-3′ 5′-tctggcttggcactggtcttatacac**t**c-3′	*Ava*I digestion
UCP3-3	5′-cttcgcctccatccgcatcggcctc**g**a-3′ 5′-ctgggacatgctgttctctgcggggctgc-3′	*Drd*I digestion
UCP3-5	5′-gacatcctcaaggagaagctgctgga**g**ta-3′ 5′-accacaatgtacctggcacttttactagg-3′	*Rsa*I digestion
UCP2-4	5′-tcaggggccagtgcgcgctac**g**g-3′ 5′-cagaatcatacaggccgatgcggacag-3′	*Eae*I digestion
UCP2-8	5′-ctcaggaatgtacagaacga-3′ 5′-gatgtggaagcttaaagttc-3′	Fragment size differences on 2% agarose gel

NOTE. Bases shown in bold represent the sequence changes necessary for introduction of restriction sites.

non-obese Danish subjects.[22] However, Schrauwen et al showed a negative correlation between UCP3 mRNA levels and BMI and a positive correlation between UCP3 expression and sleeping metabolic rate in Pima Indians.[23]

Other mRNA expression studies in humans and rodents suggest roles for UCP2 and UCP3 in the regulation of lipids as fuel substrate in skeletal muscle. Millet et al observed an increase in UCP2 and UCP3 mRNA expression in lean and obese humans during fasting,[24] while Boss et al observed that UCP2 and UCP3 mRNA levels correlate with increased levels of fat metabolism in humans.[25] In experiments involving fasting and refeeding of normal rats, Ucp2 and Ucp3 mRNA in skeletal muscle were upregulated during starvation, a period of fat store mobilization, and downregulated in response to refeeding, a period of fat store replenishment.[26,27]

The physiological functions of the UCPs remain elusive; however, transgenic mouse studies suggest roles for UCP2 and UCP3 in energy metabolism, fuel substrate management, and insulin action. Transgenic mice overexpressing human UCP3 in skeletal muscle are hyperphagic, but weigh less than their wild-type littermates, and exhibit increased metabolic rate.[28,29] Conversely, there are no phenotypic differences between Ucp3 knockout mice and their wild-type counterparts.[30,31] Ucp2-deficient mice showed normal body weight, but were hyperinsulinemic in comparison to wild-type mice.[32,33] In addition, normal rat islet cells overexpressing Ucp2 showed inhibition of glucose-stimulated insulin secretion and a 50% reduction in ATP content.[34,35]

It has been demonstrated that UCP2 and UCP3 are involved in energy metabolism, fuel substrate management, and insulin action; therefore, they are clear candidate genes for susceptibility to obesity and related metabolic disorders. Variation in UCP2 and UCP3 may influence a number of physiological pathways affecting energy homeostasis, including nutrient intake, which may in turn influence body composition. In the present study, we tested the hypothesis that genetic variation in the UCP2 and UCP3 genes is associated with dietary intake and body composition in nondiabetic participants in the San Luis Valley Diabetes Study (SLVDS).

MATERIALS AND METHODS

Subjects

The SLVDS is a prospective study of the natural history, incidence, and risk factors of type 2 diabetes and its complications in Hispanic and non-Hispanic white individuals living in the geographically isolated San Luis Valley of Colorado. Participants in the SLVDS were evaluated for glucose tolerance status by a 2-hour oral glucose tolerance test at baseline (1984 to 1988) and 2 follow-up visits (1988 to 1992, and 1997 to 1998).[36] Subjects for the current study were nondiabetic at the baseline visit and were seen at the second follow-up visit. Three hundred forty-five males and 377 females were genotyped for polymorphisms in UCP2 and UCP3. Only females are presented in this report because there were no statistically significant associations between the polymorphisms considered and measures of dietary intake and body composition in males, suggesting the presence of sex-specific effects. Dietary intake was determined from a single 24-hour dietary recall administered by bilingual interviewers trained and certified by the Nutrition Coordinating Center at the University of Minnesota, where information was coded and nutrient intake was estimated based on the Nutrition Coordinating Center's nutrient database (Version 14, released 1987). Total daily caloric intake and daily fat intake were estimated in calories and grams, respectively.[37] Fat mass and lean mass were estimated in grams using dual energy x-ray absorptiometry (DEXA).[38] Informed consent was obtained from all subjects and the University of Colorado Health Sciences Center Institutional Review Board approved all of the protocols.

Genotyping

Two polymorphic sites in UCP2 were genotyped in the SLVDS: a 45–base pair insertion/deletion polymorphism in the 3′ untranslated region of exon 8 (UCP2-8)[12] and a C→T substitution in exon 4 of UCP2 (UCP2-4).[11] Three single-nucleotide polymorphisms (SNPs) in UCP3 were genotyped in the SLVDS: a C→T substitution at codon 210 in exon 5 of UCP3 (UCP3-5),[17] a T→C substitution at codon 99 in exon 3 (UCP3-3),[19] and a C→T transition at −55 base pairs 5′ of the UCP3 initiation codon (UCP3p-55).[20] Genomic DNA was amplified by standard polymerase chain reaction (PCR) methods using the primers shown in Table 1 for each variant. UCP2-8 genotypes were assigned based on fragment size differences when separated on a 2% agarose gel. Each SNP was genotyped using enzyme digestion of engineered restriction sites. Restriction enzymes used are shown in Table 1. Digestion products were resolved on 2% agarose gels containing ethidium bromide and visualized under UV illumination. Fragment sizes were assigned by comparison to a known size marker.

Statistical Analyses

Allele frequencies for each polymorphic site were estimated by gene counting. Fit to the expectations of Hardy-Weinberg equilibrium was tested using chi-square tests. Linkage disequilibrium (LD), D′, was estimated between the loci.[39] Analysis of covariance (ANCOVA) was used to test single-locus effects on total caloric intake, fat intake, lean

Table 2. Characteristics of the Female Participants in the SLVDS

	Females	Non-Hispanic Females	Hispanic Females	*P* Value
N	395	223	172	
Age (yr)	62.75 (0.6)	63.97 (0.77)	61.17 (0.93)	.506
BMI (kg/m^2)	27.31 (0.27)	26.73 (0.37)	28.07 (0.4)	.491
Physical activity (kcal/kg/h)	264.70 (1.9)	265.72 (2.35)	263.34 (3.16)	.413
Smoking status (ever smoked)	38.7%	35.6%	42.8%	.937
Fat mass (kg)	28.9 (0.51)	28.6 (0.69)	29.2 (0.77)	.475
Lean mass (kg)	36.2 (0.24)	37.1 (0.33)	35.0 (0.35)	.475
Total calories (calories)	1649 (36)	1730 (44)	1538 (58)	.476
Fat intake (g)	65 (1.82)	68 (2.27)	60 (3.0)	.539

NOTE. Results are presented as mean (SE). *P* values calculated by χ^2 tests or ANOVA.

mass, fat mass, BMI, and percent body fat in females and males separately. Ethnicity, age, physical activity, and smoking status were included as covariates and selected based on statistically significant correlation with the outcome variables. Two-way ANCOVA was subsequently used to explore multi-locus effects and interactions between UCP3-5 and UCP3p-55 and total caloric intake, fat intake, lean mass, and fat mass in the females. The UCP3-3 variant was not considered in the 2-way analysis because it was in high LD with the UCP3p-55 polymorphism (D′ = 0.9776), a potentially functional site. The UCP2 variants were not pursued further in the analysis because they showed no statistically significant associations with the outcome variables in the one-way ANCOVA analyses. All statistical analyses were performed using the SPSS statistical software package version 10.1 for Windows (SPSS Inc, Chicago, IL).

RESULTS

Table 2 shows the characteristics of the female participants in the SLVDS. The females were on average 63 years of age and moderately overweight ($25 < \text{BMI} < 30$). There were no evidence of statistically significant differences in characteristic means between ethnic groups. Table 3 shows allele frequencies for each polymorphic site and pairwise LD, measured as D′. There was no statistically significant deviation from Hardy-Weinberg equilibrium for any of the polymorphisms. For the analysis, individuals of UCP3p-55 genotypes C/T and T/T were pooled and individuals of UCP3-3 genotypes T/C and C/C were pooled due to the low frequency of rare homozygous individuals.

Table 4 shows genotype group means and *P* values for the ANCOVA of the individual polymorphisms versus total caloric intake, fat intake, lean mass, fat mass, BMI, and percent body fat in the SLVDS females. The UCP3p-55 polymorphism showed statistically significant association with total caloric intake ($P = .031$) and lean mass ($P = .035$), with the T allele corresponding to higher dietary intake and lean mass. The UCP3-3 variant, which was in high LD with the UCP3p-55 polymorphism (D′ = 0.9776), showed a similar pattern of association with the outcome variables (total caloric intake: $P = .042$; lean mass: $P = .059$), with the C allele corresponding to higher dietary intake and lean mass. The UCP3-5 variant was statistically significantly associated with total caloric intake ($P = .012$), fat intake ($P = .011$), lean mass ($P = .013$), fat mass ($P = .004$), BMI ($P = .023$), and percent body fat ($P = .049$), with the C allele corresponding to higher dietary intake and lower fat and lean mass, BMI, and percent body fat. There were no statistically significant associations observed between the UCP2 variants and any of the outcome variables. In males, no statistically significant associations were detected between any of the polymorphisms and the outcome variables (data not shown).

Table 3. Allele Frequencies and Pairwise LD

Polymorphism	Common Allele Frequency	Linkage Disequilibrium (D′)			
		UCP3-3	UCP3-5	UCP2-4	UCP2-8
UCP3p-55 (C/T)	0.782	0.9776	0.8383	0.2457	0.3467
UCP3-3 (T/C)	0.747	—	0.7401	0.2012	0.3962
UCP3-5 (T/C)	0.544		—	0.7828	0.5135
UCP2-4 (C/T)	0.590			—	0.6923
UCP2-8 (D/I)	0.669				—

Two-way ANCOVA was used to explore multi-locus effects and interactions on total caloric intake, fat intake, fat mass, and lean mass in females. BMI and percent body fat were not pursued further in the analysis since fat mass is a more biologically meaningful measure of adiposity. We chose to consider the UCP3-5 and UCP3p-55 variants in this analysis due to statistically significant associations with the outcome variables in the one-way analyses. Since the UCP3p-55 and UCP3-3 variants were in high LD (D′ = 0.9776), we chose to look only at the UCP3p-55 polymorphism because it is the potentially functional variant. The UCP2 polymorphisms were not pursued in this analysis due to lack of statistically significant associations with the outcome variables in the one-way analyses. The results of the 2-way analyses are shown in Tables 5 through 8.

The 2-way ANCOVA model for total caloric intake (Table 5) included both UCP3-5 ($P = .001$) and UCP3p-55 ($P = .011$) as statistically significant predictors; however, the interaction between those sites was not statistically significant ($P = .575$). This model explains approximately 12% of the variation in total caloric intake. Figure 1 shows the actual adjusted means for total caloric intake for SLVDS females in each UCP3-5/UCP3p-55 multi-locus genotype group. Homozygotes for either the C allele of UCP3p-55 or the T allele of UCP3-5 show an approximately 225-calorie reduction in total caloric intake when compared to carriers of the less common allele at both sites, while homozygotes for the common allele at both sites show a reduction in total caloric intake of ~561 calories when compared to carriers of the less common allele at both sites.

Table 6 shows the 2-way ANCOVA model for fat intake.

Table 4. ANCOVA of Single Locus Effects on Outcome With Adjusted Means in Females

	Predicted Total Calories	*P* Value (R^2)	Predicted Fat Intake (g)	*P* Value (R^2)	Predicted Lean Mass (kg)	*P* Value (R^2)	Predicted Fat Mass (kg)	*P* Value (R^2)	Predicted BMI (kg/m^2)	*P* Value (R^2)	Predicted % Body Fat	*P* Value (R^2)
UCP3p-55												
CC	1600 (46)	**.031** (0.08)	63.0 (2.4)	.071 (0.07)	35.9 (0.3)	**.035** (0.14)	29.0 (0.7)	.537 (0.03)	27.2 (0.4)	.589 (0.04)	42.4 (0.5)	.607 (0.05)
CT + TT	1758 (57)		69.9 (2.9)		36.9 (0.4)		29.6 (0.8)		27.6 (0.4)		42.0 (0.6)	
UCP3-3												
TT	1579 (48)	**.042** (0.07)	61.6 (2.5)	.075 (0.06)	35.9 (0.3)	.059 (0.14)	29.1 (0.7)	.982 (0.03)	27.2 (0.4)	.604 (0.03)	42.4 (0.5)	.272 (0.05)
TC + CC	1722 (51)		68.0 (2.6)		36.8 (0.4)		29.0 (0.8)		27.5 (0.4)		41.6 (0.6)	
UCP3-5												
TT	1500 (62)	**.012** (0.08)	56.9 (3.2)	**.011** (0.07)	37.4 (0.4)	**.013** (0.16)	31.5 (0.9)	**.004** (0.07)	28.4 (0.5)	**.023** (0.06)	43.3 (0.7)	**.049** (0.06)
TC	1739 (52)		68.7 (2.7)		35.8 (0.3)		27.6 (0.7)		26.6 (0.4)		41.1 (0.5)	
CC	1688 (80)		68.7 (4.1)		36.0 (0.5)		28.6 (1.2)		27.4 (0.6)		42.2 (0.9)	
UCP2-4												
CC	1640 (65)	.200 (0.08)	63.9 (3.4)	.107 (0.08)	35.9 (0.4)	.150 (0.13)	28.6 (0.9)	.462 (0.05)	27.0 (0.5)	.777 (0.04)	41.9 (0.7)	.490 (0.07)
CT	1760 (56)		70.7 (2.9)		37.0 (0.4)		29.8 (0.8)		27.5 (0.4)		42.4 (0.6)	
TT	1592 (91)		60.2 (4.7)		36.4 (0.6)		28.2 (1.3)		27.3 (0.7)		41.0 (1.0)	
UCP2-8												
DD	1698 (53)	.280 (0.07)	67.2 (2.7)	.215 (0.06)	36.1 (0.4)	.584 (0.14)	28.8 (0.8)	.832 (0.04)	27.1 (0.4)	.613 (0.04)	42.0 (0.6)	.953 (0.05)
DI	1652 (54)		65.4 (2.8)		36.4 (0.4)		29.4 (0.8)		27.7 (0.4)		42.2 (0.6)	
II	1511 (105)		56.5 (5.5)		36.8 (0.7)		29.4 (1.5)		27.5 (0.8)		41.9 (1.1)	

NOTE. Results are reported as mean (SE) and adjusted for age, ethnicity, physical activity, and smoking status. *P* values <.05 shown in bold.

This model also includes UCP3-5 ($P = .001$) and UCP3p-55 ($P = .019$) as statistically significant predictors of fat intake. Again, the interaction between UCP3-5 and UCP3p-55 was not statistically significantly associated with fat intake ($P = .727$). Roughly 10.7% of the variation in ingested fat is explained by this model. Figure 2 shows the actual adjusted means for fat intake in each UCP3-5/UCP3p-55 multi-locus genotype group in the SLVDS females. Homozygosity for either the C allele of UCP3p-55 or the T allele of UCP3-5 results in an approximately 11-g lower fat intake when compared to carriers of the less common allele at both sites, while homozygosity for the common allele at both sites results in an approximately 26-g lower fat intake when compared to carriers of the less common allele at both sites.

The 2-way ANCOVA model for fat mass is shown in Table 7. The UCP3-5 variant appears as the only statistically significant predictor of fat mass ($P = .003$), while the UCP3p-55 polymorphism and the interaction between the 2 UCP3 variants were not statistically significant. The model coefficients, however, suggest that both the UCP3p-55 and the interaction term could be important. The model predicts average fat mass in this cohort to be approximately 29 kg. In the presence of either or both variants, fat mass is predicted to be 10 to 15 kg higher. (Note that statistically this is an interaction model, since the effects of the 2 variants are not additive.)

Table 5. Two-Way ANCOVA for Total Caloric Intake (cal)

	P Value	β	R^2
Constant	<.001	3,602	
UCP3-5	.001	−556 (TT)	
		−310 (TC)	
UCP3p-55	.011	−549 (CC)	
UCP3-5 · UCP3p-55	.575	228 (TT*CC)	0.120
		348 (TC*CC)	
Ethnicity	.007	−201 (Hispanic)	
Age	<.001	−13 (per yr)	
Physical activity	.213	−1.2 (per kcal/kg/h)	
Smoking status	.235	87 (ever smoked)	

Table 8 shows the 2-way ANCOVA model for lean mass. Neither the main effect terms nor the interaction term were statistically significantly associated with lean mass. Overall, the model explains approximately 15.8% of the variation in lean mass, which is largely due to the explanatory power of age and ethnicity. Since the polymorphisms show nonsignificant *P* values and the estimated coefficients are small, this model shows little evidence for an important genetic effect on lean mass.

DISCUSSION

In this study, we investigated the effects of genetic variation in UCP2 and UCP3 on measures of dietary intake and body composition in a cohort of Hispanic and non-Hispanic participants in the SLVDS in order to explore how these "metabolic" genes may be involved in the development of overweight and/or obesity. Initially, we looked at the individual effects of each locus on total caloric intake, fat intake, lean mass, fat mass, BMI, and percentage body fat. In the female subgroup,

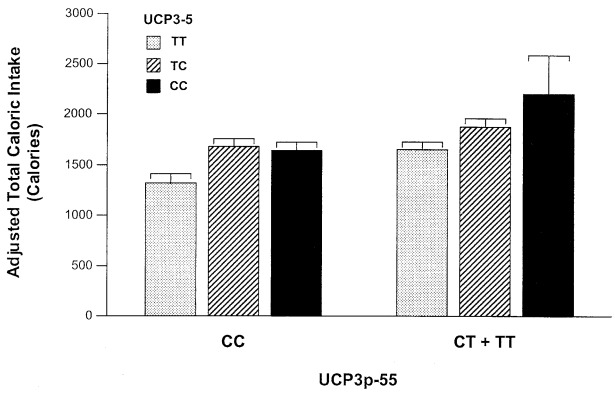

Fig 1. Bar chart of actual adjusted means for total caloric intake for SLVDS females in each UCP3-5/UCP3p-55 genotype group. Intake is measured in calories from 24-hour recall. Means are adjusted for age, ethnicity, physical activity, and smoking status. Error bars represent the standard error of the mean.

Table 6. Two-Way ANCOVA for Fat Intake (g)

	P Value	β	R^2
Constant	<.001	169	
UCP3-5	.001	−30.9 (TT)	
		−17.6 (TC)	
UCP3p-55	.019	−27.1 (CC)	
UCP3-5 · UCP3p-55	.727	13.8 (TT*CC)	0.107
		16.2 (TC*CC)	
Ethnicity	.026	−8.6 (Hispanic)	
Age	<.001	−0.7 (per yr)	
Physical activity	.089	−0.09 (per kcal/kg/h)	
Smoking status	.204	4.9 (ever smoked)	

Table 7. Two-Way ANCOVA for Fat Mass (g)

	P Value	β	R^2
Constant	<.001	29,499	
UCP3-5	.003	14,137 (TT)	
		10,115 (TC)	
UCP3p-55	.138	11,645 (CC)	
UCP3-5 · UCP3p-55	.216	−12,420 (TT*CC)	0.072
		−11,694 (TC*CC)	
Ethnicity	.394	951 (Hispanic)	
Age	.040	−98 (per yr)	
Physical activity	.113	−23 (per kcal/kg/h)	
Smoking status	.019	−2,558 (ever smoked)	

we found that the rare C allele of UCP3-5 was statistically significantly associated with higher total caloric intake and fat intake and lower lean mass, fat mass, BMI, and percentage body fat. For the UCP3p-55 and the UCP3-3 polymorphisms, which were in high LD ($D' = 0.9776$), the rare alleles were associated with increased total caloric intake and higher lean mass. The 2 UCP2 variants showed no statistically significant association with dietary intake in this cohort. In the SLVDS males, no statistically significant associations were observed between the polymorphisms and the outcome variables, suggesting a sex-specific effect. Due to the close proximity of the UCP2 and UCP3 genes and the functional evidence linking UCP2 to obesity and diabetes phenotypes, we cannot rule out the possibility that UCP3 variants are marking functional changes in the UCP2 gene. However, we only performed further analysis in UCP3 due to lack of evidence of association between the UCP2 variants and measures of dietary intake and body composition in this cohort.

Statistically significant association between the individual UCP3 polymorphisms and several of the outcome variables prompted us to explore the relationships among multiple sites within the UCP3 region to establish whether the variants exert a cumulative effect or mark a single functional change in UCP3 affecting dietary intake and body composition. We used 2-way ANCOVA to consider the multi-locus effects and interactions among the UCP3p-55 and UCP3-5 polymorphisms. We found that homozygosity for either the common C allele of UCP3p-55 or the common T allele of UCP3-5 is associated with reductions in total daily caloric intake (~225 calories) and daily fat intake (~11 g) when compared to carriers of the rarer allele at both sites. Homozygosity for the common allele at both sites is associated with more than twice those reductions in intake. This analysis showed no evidence for statistically significant interaction between UCP3-5 and UCP3p-55 in regard to dietary intake. The UCP3-5 variant was identified as the only statistically significant predictor of fat mass, suggesting an independent effect of this locus on body composition measurements. Alternatively, we may be lacking power to detect the effects of the UCP3p-55 variant on fat mass due to the small number of subjects carrying the rarer allele. In the 2-way ANCOVA model for lean mass, no evidence of statistically significant association was detected for the UCP3 variants. Thus, through univariate analysis we detected statistically significant associations between the UCP3-5 variant and total caloric intake, fat intake, fat mass, BMI, percentage body fat, and lean mass such that higher dietary intake corresponds with lower body mass. Furthermore, multivariate analysis of total caloric intake and fat intake showed statistically significant association with models containing both the UCP3-5 and UCP3p-55 loci; however, each locus appears to influence the phenotypes independently (eg, no evidence for interaction between the loci).

To further investigate the implications of this study, we considered the phenotype displayed by the Ucp3 overexpressing mouse reported by Clapham et al[28] in relation to the UCP3-5 variant in the female cohort of the SLVDS. The Ucp3 overexpressing mouse shows a hyperphagic phenotype similar to the phenotype of increased dietary intake in carriers of the

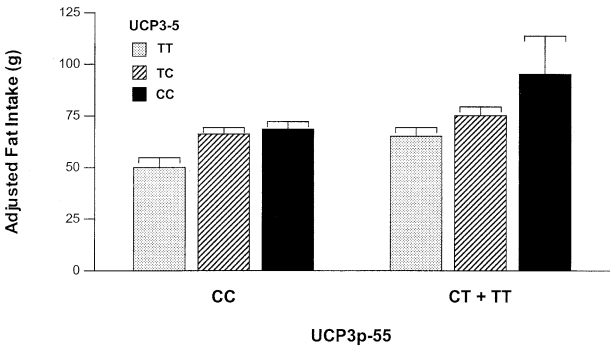

Fig 2. Bar chart of actual adjusted means for fat intake for SLVDS females in each UCP3-5/UCP3p-55 genotype group. Intake is measured in grams from 24-hour recall. Means are adjusted for age, ethnicity, physical activity, and smoking status. Error bars represent the standard error of the mean.

Table 8. Two-Way ANCOVA for Lean Mass (g)

	P Value	β	R^2
Constant	<.001	46,838	
UCP3-5	.076	2,381 (TT)	
		418 (TC)	
UCP3p-55	.649	328 (CC)	
UCP3-5 · UCP3p-55	.450	−1,943 (TT*CC)	0.158
		−566 (TC*CC)	
Ethnicity	<.001	−2,185 (Hispanic)	
Age	<.001	−123 (per yr)	
Physical activity	.934	−0.5 (per kcal/kg/h)	
Smoking status	.077	−888 (ever smoked)	

UCP3-5 C allele. Despite an increase in appetite in the Ucp3 overexpressing mouse, fat mass is reduced while plasma free fatty acids and triglycerides remain unchanged, theoretically to fuel increased metabolic rates. In the female cohort of the SLVDS, the UCP3-5 polymorphism was statistically significantly associated with fat mass measured by DEXA ($P = .004$). Individuals carrying the C allele at the UCP3-5 site showed an approximately 3.6 kg lower fat mass than T homozygotes, despite an increase in total caloric and fat intake similar to the transgenic mouse. Also, no statistically significant change in plasma free fatty acids and triglycerides was observed across genotype groups in the SLVDS female cohort, suggesting an increase in lipid metabolism. The transgenic mouse also experiences lower plasma cholesterol levels and increased insulin sensitivity in comparison to wild-type littermates. In the SLVDS female cohort, plasma cholesterol levels were lower (mean cholesterol in mg/dL: TT = 211, TC = 203, CC = 199; $P = .088$) and insulin sensitivity was higher (mean homeostasis model assessment of insulin resistance [HOMA IR]: TT = 15, TC = 13, CC = 13; $P = .195$) in carriers of the C allele compared to T homozygotes; however, statistical significance was not reached. In addition, the increase in insulin resistance observed in T homozygotes of the SLVDS female cohort corroborates the finding by Otabe et al of association between this variant and diabetic status.[17] The similarities observed between the phenotypes of the overexpressing Ucp3 mouse and the genotype-phenotype correlations at the UCP3-5 polymorphic site in this cohort are consistent with the concept that the UCP3-5 C allele may be a marker for increased UCP3 expression or a more functionally active form of the UCP3 protein.

The results of this study also show consistencies with UCP3 mRNA expression studies in humans that support the hypothesis that the UCP3-5 variant is a marker for differential UCP3 expression. Schrauwen et al showed that UCP3 mRNA expression is negatively correlated with BMI and positively correlated with sleeping metabolic rate in Pima Indians.[23] In our study, carriers of the UCP3-5 C allele exhibit less fat and lean mass despite higher caloric intake and fat intake. Assuming that the UCP3-5 C allele is a marker for higher mRNA expression, it is consistent that these individuals show lower fat and lean mass. Since an increase in UCP3 expression is positively associated with metabolic rate, it would be reasonable to hypothesize that the increases in total caloric and fat intake are used to fuel increased metabolic rates as opposed to storage in adipose tissue. In addition, UCP3 mRNA expression is positively correlated with situations that promote fat metabolism,[25] which further corroborates the finding of lower fat mass in carriers of the UCP3-5 C allele.

In this study, we used both univariate and multivariate approaches to explore the effects of UCP2 and UCP3 on dietary intake and body composition in a relatively homogeneous population. The UCP gene family has been highly studied for its role in body composition and energy metabolism, whereas the effects of the UCP genes on dietary intake are unstudied. We provide evidence that genetic variation in the UCP3 gene is associated with overall caloric intake, nutrient selection, and body composition in female participants in the SLVDS. The UCP3-5 variant was independently associated with total caloric intake, fat intake, fat mass, BMI, percentage body fat, and lean mass such that higher dietary intake corresponds with lower body mass. In regard to dietary intake, 2-way ANCOVA between UCP3-5 and UCP3p-55 showed association with the main effect terms, but no evidence for statistically significant interaction between the loci. The comparison between the UCP3-5 variant and phenotypes of the Ucp3 overexpressing mouse are consistent with the concept that variation in UCP3 marks increased activity of the UCP3 gene. This increase in UCP3 expression or functionality may result in increased metabolism, which requires additional fuel that appears to be supplied in part by an increase in dietary intake. This study supports a role for UCP3 in fuel substrate management and energy metabolism, which may play a role in body weight regulation. Further functional studies of the variation in this region could clarify the observed effects on dietary intake and uncover clues to the physiological role of the UCP2 and UCP3 genes.

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REFERENCES

1. Lönnqvist F, Nordfors L, Schalling M: Leptin and its potential role in human obesity. J Intern Med 245:643-652, 1999

2. Friedman JM: Obesity in the new millennium. Nature 404:632-634, 2000

3. Kopelman PG: Obesity as a medical problem. Nature 404:635-643, 2000

4. Klaus S, Casteilla L, Bouillaud F, et al: The uncoupling protein UCP: A membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. Int J Biochem 23:791-801, 1991

5. Pecqueur C, Cassard-Doulcier AM, Raimbault S, et al: Functional organization of the human uncoupling protein-2 gene, and juxtaposition to the uncoupling protein-3 gene. Biochem Biophys Res Commun 255:40-46, 1999

6. Fleury C, Neverova M, Collins S, et al: Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. Nat Genet 15:269-272, 1997

7. Bouchard C, Perusse L, Chagnon YC, et al: Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. Hum Mol Genet 6:1887-1889, 1997

8. Boss O, Samec S, Paoloni-Giacobino A, et al: Uncoupling protein-3: A new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408:39-42, 1997

9. Gong DW, He Y, Karas M, Ret al: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β3-adrenergic agonists, and leptin. J Biol Chem 272:24129-24132, 1997

10. Vidal-Puig A, Solanes G, Grujic D, et al: UCP3: An uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 235:79-82, 1997

11. Urhammer SA, Dalgaard LT, Sorensen TIA, et al: Mutational analysis of the coding region of the uncoupling protein 2 gene in obese NIDDM patients: Impact of a common amino acid polymorphism on juvenile and maturity onset forms of obesity and insulin resistance. Diabetologia 40:1227-1230, 1997

12. Otabe S, Clement K, Rich N, et al: Mutation screening of the human UCP2 gene in normoglycemic and NIDDM morbidly obese patients: Lack of association between new UCP2 polymorphisms and obesity in French Caucasians. Diabetes 47:840-842, 1998

13. Walder K, Norman FA, Hanson RL, et al: Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima Indians. Hum Mol Genet 7:1431-1435, 1998

14. Astrup A, Toubro S, Dalgaard LT, et al: Impact of the v/v 55 polymorphism of the uncoupling protein 2 gene on 24-h energy expenditure and substrate oxidation. Int J Obes Relat Metab Disord 23:1031-1034, 1999

15. Cassell PG, Neverova M, Janmohamed S, et al: An uncoupling protein 2 gene variant is associated with a raised body mass index but not type II diabetes. Diabetologia 42:688-692, 1999

16. Dalgaard LT, Sørensen TIA, Andersen T, et al: An untranslated insertion variant in the uncoupling protein 2 gene is not related to body mass index and changes in body weight during a 26-year follow-up in Danish Caucasian men. Diabetologia 42:1413-1416, 1999

17. Otabe S, Clement K, Dubois S, et al: Mutation screening and association studies of the human uncoupling protein 3 gene in normoglycemic and diabetic morbidly obese patients. Diabetes 48:206-208, 1999

18. Lanouette CM, Chagnon YC, Rice T, et al: Uncoupling protein 3 gene is associated with body composition changes with training in HERITAGE study. J Appl Physiol 92:1111-1118, 2002

19. Urhammer SA, Dalgaard LT, Sorensen TIA, et al: Organisation of the coding exons and mutational screening of the uncoupling protein 3 gene in subjects with juvenile-onset obesity. Diabetologia 41:241-244, 1998

20. Schrauwen P, Xia J, Walder K, et al: A novel polymorphism in the proximal UCP3 promoter region: Effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians. Int J Obes Relat Metab Disord 23:1242-1245, 1999

21. Otabe S, Clement K, Dina C, et al: A genetic variation in the 5′ flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 43:245-249, 2000

22. Dalgaard LT, Sørensen TIA, Drivsholm T, et al: A prevalent polymorphism in the promoter of the UCP3 gene and its relationship to body mass index and long term body weight change in the Danish population. J Clin Endocrinol Metab 86:1398-1402, 2001

23. Schrauwen P, Xia J, Bogardus C, et al: Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. Diabetes 48:146-149, 1999

24. Millet L, Vidal H, Andreelli F, et al: Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. J Clin Invest 100:2665-2670, 1997

25. Boss O, Hagen T, Lowell BB: Uncoupling proteins 2 and 3: Potential regulators of mitochondrial energy metabolism. Diabetes 49:143-156, 2000

26. Samec S, Seydoux J, Dulloo AG: Role of UCP homologues in skeletal muscles and brown adipose tissue: Mediators of thermogenesis or regulators of lipids as fuel substrate? FASEB J 12:715-724, 1998

27. Samec S, Seydoux J, Dullo AG: Post starvation gene expression of skeletal muscle uncoupling protein 2 and uncoupling protein 3 in response to dietary fat levels and fatty acid composition: A link with insulin resistance. Diabetes 48:436-441, 1999

28. Clapham JC, Arch JRS, Chapman H, et al: Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature 406: 415-418, 2000

29. Moore GBT, Himms-Hagen J, Harper ME, et al: Overexpression of UCP-3 in skeletal muscle of mice results in increased expression of mitochondrial thioesterase mRNA. Biochem Biophys Res Commun 283:785-790, 2001

30. Gong DW, Monemdjou S, Gavrilova O, et al: Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein-3. J Biol Chem 275:16251-16257, 2000

31. Vidal-Puig AJ, Grujic D, Zhang CY, et al: Energy metabolism in uncoupling protein 3 gene knockout mice. J Biol Chem 275:16258-16266, 2000

32. Arsenijevic D, Onuma H, Pecqueur C, et al: Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 26:435-439, 2000

33. Zhang CY, Baffy G, Perret P, et al: Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. Cell 105:745-755, 2001

34. Chan CB, MacDonald PE, Saleh MC, et al: Overexpression of uncoupling protein 2 inhibits glucose-stimulated insulin secretion from rat islets. Diabetes 48:1482-1486, 1999

35. Chan CB, De Leo D, et al: Increased uncoupling protein-2 levels in β-cells are associated with impaired glucose-stimulated insulin secretion: Mechanism of action. Diabetes 50:1302-1310, 2001

36. Hamman RF, Marshall JA, Baxter J, et al: Methods and prevalence of non-insulin-dependent diabetes mellitus in a biethnic Colorado population: The San Luis Valley Diabetes Study. Am J Epidemiol 129:295-311, 1989

37. Marshall JA, Kamboh MI, Bessesen DH, et al: Associations between dietary factors and serum lipids by apolipoprotein E polymorphism. Am J Clin Nutr 63:87-95, 1996

38. Marshall JA, Grunwald GK, Donahoo WT, et al: Percent body fat and lean mass explain the gender difference in leptin: Analysis and interpretation of leptin in Hispanic and non-Hispanic white adults. Obes Res 8:543-552, 2000

39. Devlin B, Risch N: A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 29:311-322, 1995