Racial differences in the relation between uncoupling protein genes and resting energy expenditure¹–³

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ABSTRACT
Background: Lower resting energy expenditure (REE) in African American women may contribute to their obesity. The identification of uncoupling protein (UCP) genes has fueled a search for genes involved in energy metabolism in humans.
Objective: We examined variation in REE in relation to variation in UCP1, UCP2, and UCP3 in 141 women aged 18–21 y.
Design: Standard methods were used for REE measurements and genetic analysis. Body composition was determined with the use of dual-energy X-ray absorptiometry. Multivariate analysis was used to examine the effect of genotypes on REE and on fat mass in relation to other potentially confounding variables.
Results: REE was 295 kJ/d lower in African American women than in white women. No significant variation in REE was seen for UCP1, UCP2, and UCP3 (p=55; exon 3a; and exon 3b) variants after adjustment for other variables including smoking status. For the UCP3 exon 5 variant, REE was significantly (Ρ=0.019) lower in African American women with the CC genotype than in those with the TT genotype. In African American women, there was a significant trend (Ρ=0.012) toward lower REE and a weak but nonsignificant trend (Ρ=0.1) toward greater fat mass across the 3 genotypes (TT, CT, and CC).
Conclusions: The significant and dose-dependent relation between lower REE and the C allele suggests that it may be a thrifty allele. The presence of this parsimonious energy metabolism in African American women, possibly linked to UCP3, may be implicated in their susceptibility to obesity. The absence of a UCP3 effect in white women is intriguing and needs to be explored to further understand possible interactions between UCP3 and other genes. Am J Clin Nutr 2002;75:714–9.

KEY WORDS Uncoupling protein genes, UCP1, UCP2, UCP3, African American women, white women, thrifty gene, energy metabolism, obesity, genetics

INTRODUCTION

African American women are particularly vulnerable to obesity, and almost one-half of them >20 y of age are overweight (1). Several reports indicate that African American women generally have lower values of total and resting daily energy expenditure (REE) than do white women (2–10). The presence of lower REE in African American women has been viewed as a manifestation of their genetic predisposition to obesity. However, the specific gene responsible for this racial difference in energy metabolism remains elusive.

The identification of genes that code for the uncoupling proteins (UCPs), a family of inner mitochondrial membrane transporters that dissipate the proton gradient and release stored energy in the form of heat, has opened an exciting area in the search for genetic modulators of energy metabolism (11). Although a polymorphism in the 5′-flanking region of UCP1 correlated significantly with a gain in percentage of body fat over time (12), a higher weight gain in morbidly obese adult subjects, and a lower body weight loss after energy restriction (13), the role of UCP1 in the regulation of human energy balance is debatable because it is specifically expressed in brown adipose tissue, of which humans have very little (14).

Unlike UCP1, UCP2 is widely expressed (11) and UCP3 is predominantly expressed in human skeletal muscle (15, 16), a major tissue contributing to nonshivering thermogenesis in humans (15–17). UCP2 and UCP3 have been localized within 150 kilobases of each other on chromosome 11q13 (16, 18). As uncouplers of oxidative phosphorylation and ATP synthesis (19, 20), UCP2 and UCP3 are biologically plausible candidate genes to potentially influence energy metabolism and body weight.

Allelic variation at the UCP2 and UCP3 loci is reported to be associated with REE (21, 22), rates of fat oxidation and respiratory quotients (17, 23), and obesity (21, 24–27) in populations with a marked susceptibility to obesity. Yet, other studies, which

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primarily examined white obese and nonobese populations, failed to find such relations between UCP2 or UCP3 and REE (21, 28–30) or obesity (31–33). Thus, the role of UCP2 and UCP3 in energy metabolism and human obesity remains unclear.

To date, no studies have examined the variants of the UCP genes in relation to REE in both African American and white females during late adolescence, a time when the racial differences in adiposity and REE first become manifest. The primary aim of the present study was to examine variation in REE in relation to genetic variation in UCP1, UCP2, and UCP3 in a biracial cohort of young women. A second aim was to investigate whether any of these variants were linked to adiposity.

SUBJECTS AND METHODS

Study population

One hundred fifty-two women (77 African American, 75 white) aged 18–21 y were recruited from race-specific random lists drawn from a roster of ∼700 women who were enrolled since age 9–10 y in the National Heart, Lung, and Blood Institute Growth and Health Study (NGHS), a longitudinal study of obesity development during adolescence (34). The initial recruitment for the NGHS cohort in Cincinnati was via public and parochial schools chosen from census tracts that had the least racial disparity in the proportion of African American and white children and in income and education between African American and white residents. Initial NGHS eligibility was limited to girls and their parents who declared themselves as being either black or white and who lived in racially concordant households.

Exclusion criteria were active dieting, any abrupt change in lifestyle during the past 2 wk, being ≤4 mo postpartum, taking medications (which may affect heart rate or energy metabolism), and having a chronic illness. There were no racial differences in the proportion of ineligible women or of those who refused or could not be contacted. Informed consent was obtained from each participant, and the study was approved by the respective institutional review boards at Cincinnati Children’s Hospital Medical Center, the University of Vermont, and the University of Pittsburgh.

Measurement of REE

REE was measured under controlled conditions with the use of indirect calorimetry with the DeltaTrac Metabolic Monitor II (SensorMedics, Yorba Linda, CA). All study subjects were admitted for an overnight stay at the Clinical Research Center of the Cincinnati Children’s Hospital. After the subjects had fasted overnight and while they were supine in their beds and still drowsy, their REE values were measured between 0600 and 0700 overnight and while they were supine in their beds and still drowsy, their REE values were measured between 0600 and 0700 (21, 28–30) later. Analysis of test-retest conditions yielded a CV of 5%. Thus, the average of the 2 REE measurements was used for data analysis.

Clinical measurements

At the time of each admission to the Clinical Research Center, subjects completed a questionnaire regarding current menstrual status, recent changes in body weight, changes in diet and physical activity, use of medications including contraceptives, and smoking history. Current smoking status was ascertained by a yes or no response. At the time of the first REE measurement, body composition was assessed with the use of a QDR-2000 dual-energy X-ray absorptiometry densitometer (Hologic Inc, Waltham, MA) in the pencil-beam mode with total body software (version 5.60; 36). Estimates of fat mass, total fat-free mass (tissue fat-free mass and bone mineral content), and percentage of body fat were derived from the dual-energy X-ray absorptiometry measures.

Genotyping

DNA was extracted from buffy coats or blood clots collected when the study participants were 15–21 y of age. Genotyping was done for 141 (73 African American, 68 white) women by using standard polymerase chain reaction methods. The UCP1 variants consist of a G-to-A substitution (Ala→Thr) in exon 64 of exon 2 (UCP1 exon 2) and an A-to-T substitution (Met→Leu) in codon 229 of exon 5 (UCP1 exon 5) (37). Two polymorphic sites in UCP2 were examined: a C-to-T substitution (Ala→Val) in exon 55 of exon 4 (UCP2 exon 4) (38) and a 45-base-pair insertion or deletion in the 3′-untranslated region of exon 8 (UCP2 exon 8) (32). Four UCP3 variants were examined: a C-to-T substitution at base pair upstream from exon 1 (UCP3 p→55), a T-to-C substitution (Tyr→Tyr) in codon 99 of exon 3 (UCP3 exon 3a) (33), a G-to-A substitution (Val→Ile) in codon 102 of exon 3 (UCP3 exon 3b) (33), and a C-to-T substitution (Tyr→Tyr) in exon 210 of exon 5 (UCP3 exon 5) (33). Allele frequencies were estimated by gene counting.

Statistical analysis

Independent t tests were used to examine racial differences in age, height, weight, and body composition. Chi-square tests were used to examine racial differences in smoking status and the distribution of the UCP1, UCP2, and UCP3 genotypes. Linkage disequilibria between UCP gene sites were calculated and tested with the use of the identifiable haplotypes (double heterozygotes were omitted from the calculations). The disequilibria (D) were calculated with the use of the following nonstandardized disequilibrium equation:

\[
D = \frac{\text{frequency of AB haplotype} - (\text{frequency of A allele} \times \text{frequency of B allele})}{\text{frequency of A allele} \times \text{frequency of B allele}}
\]

The test of significance for the disequilibria is the chi-square test of association applied to the 2 × 2 table of identifiable haplotypes (38).

Data were analyzed for each UCP gene variant with separate analysis of covariance (ANCOVA) models to examine the effect of each variant on REE values after adjustment for race (white as the reference), total fat-free mass, fat mass, and smoking status as covariates. Significant results by ANCOVA were followed by post hoc Tukey-Kramer tests for all pairwise comparisons. The effect of race interaction with UCP genotypes was also examined, and when appropriate, race-specific models were generated. A similar analysis was conducted with fat mass as the outcome and race, UCP genotypes, and fat-free mass as predictors. The Bartholomew trend test was used to examine REE across the UCP3 genotypes (39). Descriptive statistics and ANCOVA models were generated using SAS software (40). Statistical significance was set at \( P \leq 0.05 \).
RESULTS

The characteristics of the study population by race are shown in Table 1. The 2 races did not differ significantly in mean age and height. However, the African American women were heavier and fatter than were the white women. Significantly more white women (28.2%) than African American women (7.9%) were current smokers.

TABLE 2
Genotype frequencies of the uncoupling protein (UCP) gene variants by race

<table>
<thead>
<tr>
<th>UCP genotype</th>
<th>African American</th>
<th>White</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1 exon 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1.6 [1]</td>
<td>0 [0]</td>
<td>0.54</td>
</tr>
<tr>
<td>AG</td>
<td>14.3 [9]</td>
<td>10.9 [6]</td>
<td>0.17</td>
</tr>
<tr>
<td>GG</td>
<td>84.1 [53]</td>
<td>89.1 [49]</td>
<td>0.13</td>
</tr>
<tr>
<td>UCP1 exon 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>92.3 [60]</td>
<td>90.3 [56]</td>
<td>0.69</td>
</tr>
<tr>
<td>AT</td>
<td>7.7 [5]</td>
<td>9.7 [6]</td>
<td>0.11</td>
</tr>
<tr>
<td>UCP2 exon 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>29.6 [21]</td>
<td>24.6 [16]</td>
<td>0.70</td>
</tr>
<tr>
<td>CT</td>
<td>54.9 [39]</td>
<td>60.0 [39]</td>
<td>0.66</td>
</tr>
<tr>
<td>TT</td>
<td>15.5 [11]</td>
<td>15.4 [10]</td>
<td>0.80</td>
</tr>
<tr>
<td>UCP2 exon 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5.5 [4]</td>
<td>3.0 [2]</td>
<td>0.09</td>
</tr>
<tr>
<td>ID</td>
<td>27.4 [20]</td>
<td>55.2 [37]</td>
<td>0.001</td>
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<tr>
<td>DD</td>
<td>67.1 [49]</td>
<td>41.8 [28]</td>
<td>0.001</td>
</tr>
<tr>
<td>UCP3 p−55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>73.7 [42]</td>
<td>64.3 [36]</td>
<td>0.49</td>
</tr>
<tr>
<td>CT</td>
<td>22.8 [13]</td>
<td>28.6 [16]</td>
<td>0.21</td>
</tr>
<tr>
<td>TT</td>
<td>3.5 [2]</td>
<td>7.1 [4]</td>
<td>0.15</td>
</tr>
<tr>
<td>UCP3 exon 3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>36.5 [23]</td>
<td>5.2 [3]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CT</td>
<td>38.1 [24]</td>
<td>29.3 [17]</td>
<td>0.001</td>
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<tr>
<td>TT</td>
<td>25.4 [16]</td>
<td>65.5 [38]</td>
<td>0.001</td>
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<tr>
<td>UCP3 exon 3b</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>3.1 [2]</td>
<td>0 [0]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AG</td>
<td>27.7 [18]</td>
<td>0 [0]</td>
<td>0.001</td>
</tr>
<tr>
<td>GG</td>
<td>69.2 [45]</td>
<td>100.0 [57]</td>
<td>0.001</td>
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<tr>
<td>UCP3 exon 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>8.2 [6]</td>
<td>22.0 [15]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CT</td>
<td>32.9 [24]</td>
<td>57.4 [39]</td>
<td>0.001</td>
</tr>
<tr>
<td>TT</td>
<td>58.9 [43]</td>
<td>20.6 [14]</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001.

The genotype frequencies of the variants in UCP1, UCP2, and UCP3 by race are shown in Table 2. There was a significant difference between the 2 races in the genotype frequencies of UCP2 exon 8 and the UCP3 exon 3 and exon 5 variants. After adjustment for race, total fat-free mass, fat mass, and smoking status, there was no significant variation in REE across any of the genotypes of the UCP1, UCP2, and UCP3 variants, except for UCP3 exon 5 (Table 3).

Because there was a significant (P = 0.02) interaction between race and UCP3 exon 5, race-specific REE values (adjusted for body composition and smoking) by UCP3 exon 5 genotype are shown in Figure 1. For the African American women, those with the CC genotype had significantly lower REE values (582 kJ/d) than did those with the TT genotype. Although REE values were lower (418 kJ/d) in the African American women with the CC genotype than in those with the CT genotype, this difference was not significant. However, the trend toward lower REE values across the 3 genotypes was significant (P = 0.012). In contrast, there was no significant variation in REE across the UCP3 exon 5 genotypes in the white women. In addition, REE values for the African American women with the CC genotype were significantly lower (674 kJ/d) than those for the white women with the same genotype.
In the African American women, fat mass was greater in those with the CC genotype (33.7 ± 5.0 kg) than in those with either the CT (23.9 ± 2.5 kg) or TT genotype (27.5 ± 1.9 kg) (data not shown); however, the trend test was not significant but suggestive \((P = 0.1)\). In the white women, there was no significant association between fat mass and the UCP3 exon 5 genotypes.

Single marker association between the variable sites of UCP2 and UCP3 was examined in both races, with the exception of UCP3 exon 3b, which was examined only in the African American women because all of the white women had the GG genotype. Pairwise linkage disequilibrium values for UCP2 and UCP3 sites in the white and African American women are shown in Tables 4 and 5, respectively. The overall patterns of linkage disequilibrium in the white and African American women were similar but not identical.

**DISCUSSION**

It is now generally accepted that REE values are lower in African American women than in white women (2–10). The presence of this racial difference in REE in the face of the striking susceptibility of African American women to obesity has raised a question regarding a genetic mechanism underlying these observed racial differences in energy metabolism. Because the UCP genes are implicated in thermogenesis, the examination of UCP genes may be particularly relevant in the search for a thrifty gene.

In the present study, we examined the relation between REE and the UCP gene polymorphism in a biracial cohort of women aged 18–21 y. Our findings are consistent with those of other published reports (2–10) that also showed lower REE values in African American women. Additionally, we derived a better estimate of the racial difference in REE values between African American and white women because we adjusted for potentially confounding factors such as smoking. Several salient findings from our study suggest that a UCP gene, in particular the UCP3 exon 5 variant, may be the candidate gene for the observed lower REE in African American women. First, there was a significant association between REE and the C allele in the UCP3 exon 5 variant in African American women: women with the CC genotype; , CT genotype; , TT genotype. There was a significant \((P = 0.02)\) interaction between race and UCP3 exon 5. \(†\)Significantly different from African American women with the TT genotype, \(P = 0.019. \)Significantly different from African American women with the CC genotype, \(P = 0.017.

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>UCP2 exon 4</th>
<th>UCP2 exon 8</th>
<th>UCP3 exon 3a</th>
<th>UCP3 exon 5</th>
<th>UCP3 p–55</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP2 exon 4</td>
<td>–0.162(^1)</td>
<td>–0.065</td>
<td>0.113(^2)</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>UCP2 exon 8</td>
<td>–0.048(^3)</td>
<td>–0.077</td>
<td>–0.160(^4)</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>UCP3 exon 3a</td>
<td>–0.082(^5)</td>
<td>–0.160(^6)</td>
<td>–0.160(^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP3 exon 5</td>
<td>–0.082(^5)</td>
<td>–0.160(^6)</td>
<td>–0.160(^6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(1 P < 0.001.\)

\(2 P < 0.01.\)

\(3 P = 0.05.\)

\(4 P < 0.05.\)

\(5 P < 0.05.\)

\(6 P < 0.05.\)
type had lower REE than did those with the $CT$ or $TT$ genotype. Second, the relation between the $C$ allele and REE was dose-dependent. Third, there was a trend, albeit not significant, toward an association between greater fat mass and the $CC$ genotype in African American women. None of these 3 findings was present in white women.

The $UCP3$ exon 5 variation is a silent mutation that is not expected to alter the function of the $UCP3$ protein. Our results showed a similar but not identical pattern of linkage disequilibrium in white and African American women (Tables 4 and 5, respectively), suggesting that the nonfunctional $UCP3$ exon 5 site, which is significantly associated with REE in African American women but not in white women, may be in linkage disequilibrium for a functional variant elsewhere in the $UCP2$ and $UCP3$ gene regions in African Americans but not in whites. The failure to observe a significant association in white women may be due to an absence (or low frequency) of this postulated functional allele in whites or to a difference in the pattern of linkage disequilibrium in African Americans than in whites in this genomic region. This observation of racial differences in the $UCP3$ exon 5 gene effect is made more complex by the overall small sample size in the present study and the significant difference in $UCP3$ exon 5 allele frequencies between the 2 races.

Although it is tempting to speculate that the lower REE values present in African American women may be a manifestation of a thrifty gene, with $UCP3$ as a plausible candidate, corroboration from a larger study is needed because of the relative infrequency of the $C$ allele in African American women. Despite a suggestion of greater fat mass with the $C$ alleles, the absence of statistical significance in this relation in our study again illustrates the need for further evaluation of the role of $UCP3$ with a larger sample of African Americans. The results of our study do, however, offer a tantalizing suggestion that the exon 5 variant may be a candidate thrifty allele of the $UCP3$ gene, perhaps serving as the genetic modulator of the markedly higher susceptibility of African American women than of white women to obesity. Thus, the high prevalence of obesity in African American women in the United States today may be the result of their contemporary lifestyle of relatively high energy intake and physical inactivity in the presence of an underlying genetic propensity for efficient energy conservation.

REFERENCES

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