Racial differences in the relation between uncoupling protein genes and resting energy expenditure[1–3]

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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 ($P = 0.019$) 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 ($P = 0.012$) toward lower REE and a weak but nonsignificant trend ($P = 0.1$) 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.

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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 with the room temperature set at ≈21.7 °C. After 15 min of acclimation, data were collected for 45 min. Energy expenditure was calculated from the equation of Weir (35). A second measurement, which followed the same protocol as the first, was made 10–14 d ($\bar{x}$: 11 d) 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 codon 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 codon 55 of exon 4 (*UCP2* exon 4) (37) 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 −55 base pairs upstream from exon 1 (*UCP3* p−55) (24), 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 codon 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:

$$D = \text{frequency of } AB \text{ haplotype} - (\text{frequency of } A \text{ allele} \times \text{frequency of } B \text{ allele}) \quad (1)$$

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 predictor variables. 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 $P \leq 0.05$.

TABLE 1
Characteristics of the study population by race[1]

	African American (n = 73)	White (n = 68)
Age (y)	19.5 ± 0.8	19.4 ± 0.7
Height (cm)	164.3 ± 6.7	165.0 ± 5.1
Weight (kg)	75.7 ± 22.5	67.1 ± 13.3[2]
BMI (kg/m^2)	28.1 ± 7.8	24.7 ± 5.1[2]
Fat-free mass (kg)	48.0 ± 7.7	45.3 ± 5.6[3]
Fat mass (kg)	26.8 ± 16.6	21.1 ± 9.2[3]
REE (kJ/d)[4]	6061 ± 88	6356 ± 76[2]
Current smokers (%)	7.9	28.2[5]

[1] $\bar{x}$ ± SD. REE, resting energy expenditure.
[2,3,5] Significantly different from African American: [2] $P < 0.01$, [3] $P < 0.05$, [5] $P = 0.001$.
[4] Adjusted for total fat-free mass, fat mass, and smoking status.

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. REE was significantly lower (295 kJ/d) in the African American women than in the white women.

TABLE 2
Genotype frequencies of the uncoupling protein (UCP) gene variants by race[1]

UCP genotype	African American	White	P[2]
	%		
UCP1 exon 2			
AA	1.6 [1]	0 [0]	0.54
AG	14.3 [9]	10.9 [6]	
GG	84.1 [53]	89.1 [49]	
UCP1 exon 5			
AA	92.3 [60]	90.3 [56]	0.69
AT	7.7 [5]	9.7 [6]	
UCP2 exon 4			
CC	29.6 [21]	24.6 [16]	0.79
CT	54.9 [39]	60.0 [39]	
TT	15.5 [11]	15.4 [10]	
UCP2 exon 8			
II	5.5 [4]	3.0 [2]	0.004
ID	27.4 [20]	55.2 [37]	
DD	67.1 [49]	41.8 [28]	
UCP3 p−55			
CC	73.7 [42]	64.3 [36]	0.49
CT	22.8 [13]	28.6 [16]	
TT	3.5 [2]	7.1 [4]	
UCP3 exon 3a			
CC	36.5 [23]	5.2 [3]	<0.0001
CT	38.1 [24]	29.3 [17]	
TT	25.4 [16]	65.5 [38]	
UCP3 exon 3b			
AA	3.1 [2]	0 [0]	<0.0001
AG	27.7 [18]	0 [0]	
GG	69.2 [45]	100.0 [57]	
UCP3 exon 5			
CC	8.2 [6]	22.0 [15]	<0.0001
CT	32.9 [24]	57.4 [39]	
TT	58.9 [43]	20.6 [14]	

[1] n in brackets. *I*, insertion; *D*, deletion.
[2] Chi-square test.

TABLE 3
Adjusted resting energy expenditure (REE) by uncoupling protein (UCP) genotype[1]

UCP genotype	REE
	kJ/d
UCP1 exon 2	
AA	5334 ± 586
AG	6058 ± 155
GG	6092 ± 59
UCP1 exon 5	
AA	5991 ± 176
AT	6050 ± 54
UCP2 exon 4	
CC	6159 ± 100
CT	6205 ± 84
TT	6213 ± 134
UCP2 exon 8	
II	6427 ± 238
ID	6180 ± 88
DD	6205 ± 80
UCP3 p−55	
CC	6284 ± 75
CT	6280 ± 109
TT	6322 ± 230
UCP3 exon 3a	
CC	6138 ± 126
CT	6008 ± 92
TT	6075 ± 84
UCP3 exon 3b	
AA	6096 ± 414
AG	6155 ± 147
GG	6033 ± 167

[1] $\bar{x}$ ± SEE. Adjusted for total fat-free mass, fat mass, race, and smoking status. *I*, insertion; *D*, deletion. There was no significant variation across any of the genotypes (Tukey-Kramer post hoc comparison test).

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.

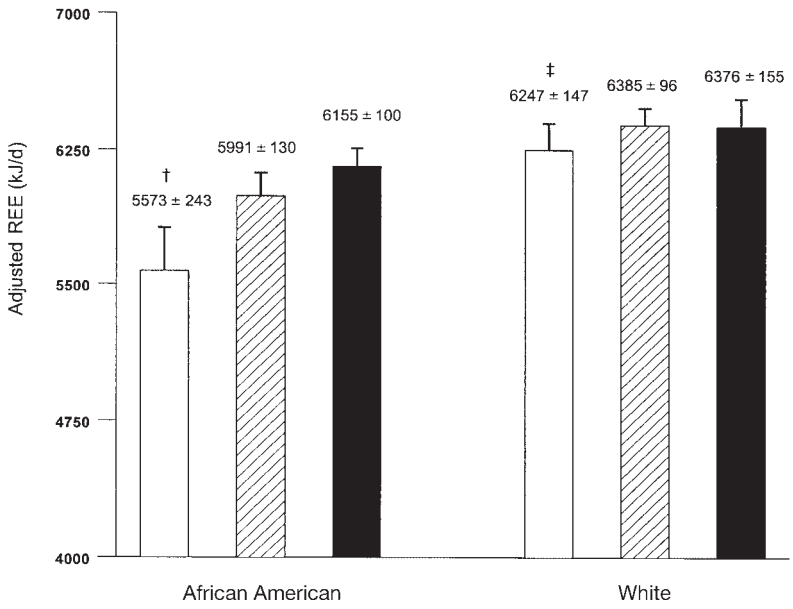

FIGURE 1. Mean (±SEE) race-specific resting energy expenditure (REE) by uncoupling protein 3 (*UCP3*) exon 5 genotype and by race. Values were adjusted for total fat-free mass, fat mass, and smoking status. □, *CC* genotype; ▨, *CT* genotype; ■, *TT* genotype. There was a significant ($P = 0.02$) interaction 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, $P = 0.017$.

In the African American women, fat mass was greater in those with the *CC* genotype ($\bar{x}$ ± SD: 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* geno-

TABLE 4
Pairwise linkage disequilibrium values for uncoupling protein 2 (*UCP2*) and *UCP3* sites in white women

	UCP2 exon 8	*UCP3* exon 3a	*UCP3* exon 5	*UCP3* p−55
UCP2 exon 4	−0.162[1]	−0.065	0.113[2]	0.074
UCP2 exon 8		−0.048[3]	−0.077	0.007
UCP3 exon 3a			−0.08[2]	−0.160[1]
UCP3 exon 5				0.079[2]

[1] $P < 0.001$.
[2] $P < 0.01$.
[3] $P = 0.05$.

TABLE 5
Pairwise linkage disequilibrium values for uncoupling protein 2 (*UCP2*) and *UCP3* sites in African American women

	UCP2 exon 8	*UCP3* exon 3a	*UCP3* exon 3b	*UCP3* exon 5	*UCP3* p−55
UCP2 exon 4	−0.095[1]	−0.001	−0.069[2]	−0.017	0.047
UCP2 exon 8		−0.05	−0.016	−0.032	−0.004
UCP3 exon 3a			−0.014	−0.078[3]	−0.03[3]
UCP3 exon 3b				−0.024	0.012
UCP3 exon 5					0.016[2]

[1] $P < 0.001$.
[2] $P < 0.05$.
[3] $P \leq 0.01$.

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

1. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults: The National Health and Nutrition Examination surveys, 1960–1991. JAMA 1994;272:205–11.
2. Albu J, Shur M, Curi M, Murphy L, Heymsfield SB, Pi-Sunyer FX. Resting metabolic rate in obese, premenopausal black women. Am J Clin Nutr 1997;66:531–8.
3. Carpenter WH, Fonong T, Toth MJ, et al. Total daily energy expenditure in free-living older African-Americans and Caucasians. Am J Physiol 1998;274:E96–101.
4. Chitwood LF, Brown SP, Lundy MJ, Dupper MA. Metabolic propensity toward obesity in black vs white females: responses during rest, exercise and recovery. Int J Obes Relat Metab Disord 1996;20:455–62.
5. Forman JN, Miller WC, Szymanski LM, Fernhall B. Differences in resting metabolic rates of inactive obese African-American and Caucasian women. Int J Obes Relat Metab Disord 1998;22:215–21.
6. Foster GD, Wadden TA, Vogt RA. Resting energy expenditure in obese African American and Caucasian women. Obes Res 1997;5:1–8.
7. Jakicic JM, Wing RR. Differences in resting energy expenditure in African-American vs Caucasian overweight females. Int J Obes Relat Metab Disord 1998;22:236–42.
8. Kushner RF, Racette SB, Neil K, Schoeller DA. Measurement of physical activity among black and white obese women. Obes Res 1995;3(suppl):S261–5.
9. Weyer C, Snitker S, Bogardus C, Ravussin E. Energy metabolism in African Americans: potential risk factors for obesity. Am J Clin Nutr 1999;70:13–20.
10. Gannon B, DiPietro L, Poehlman ET. Do African Americans have lower energy expenditure than Caucasians? Int J Obes Relat Metab Disord 2000;24:4–13.
11. Fleury C, Neverova M, Collins S, et al. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 1997;15: 269–72.
12. Oppert JM, Vohl MC, Chagnon M, et al. DNA polymorphism in the uncoupling protein (UCP) gene and human body fat. Int J Obes Relat Metab Disord 1994;18:526–31.
13. Clement K, Ruiz J, Cassard-Doulcier AM, et al. Additive effect of A→G (-3826) variant of the uncoupling protein gene and the Trp64Arg mutation of the β3-adrenergic receptor gene on weight gain in morbid obesity. Int J Obes Relat Metab Disord 1996;20:1062–6.
14. Lean M. Brown adipose tissue in humans. Proc Nutr Soc 1989;48: 243–56.
15. 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 1997;408:39–42.
16. Solanes G, Vidal-Puig A, Grujic D, Flier JS, Lowell BB. The human uncoupling protein-3 gene: genomic structure, chromosomal localization and genetic basis for short and long form transcripts. J Biol Chem 1997;272:25433–6.
17. Argyropoulos G, Brown AM, Willi SM, et al. Effects of mutations in the *UCP3* gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. J Clin Invest 1998;102:1345–51.
18. Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β3-adrenergic agonists, and leptin. J Biol Chem 1997;272:24129–32.
19. Ricquier D. Uncoupling protein-2 (UCP2): molecular and genetic studies. Int J Obes Relat Metab Disord 1999;23(suppl):S38–42.
20. Boss O, Muzzin P, Giacobino JP. The uncoupling proteins, a review. Eur J Endocrinol 1998;139:1–9.
21. Walder K, Norman RA, Hanson RL, et al. Association between uncoupling protein polymorphisms (*UCP2-UCP3*) and energy metabolism/obesity in Pima Indians. Hum Mol Genet 1998;7:1431–5.
22. Bouchard C, Perusse L, Chagnon YC, Warden C, Ricquier D. Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. Hum Mol Genet 1997; 6:1887–9.
23. Astrup A, Toubro S, Dalgaard LT, Urhammer SA, Sorensen TI, Pedersen O. 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 1999;23:1030–4.
24. 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 2000;43:245–9.
25. Cassell PG, Neverova M, Janmodamed S, et al. An uncoupling protein 2 gene variant is associated with a raised body mass index but not type II diabetes. Diabetologia 1999;42:688–92.
26. Cassell PG, Saker PJ, Huxtable SJ, et al. Evidence that single nucleotide polymorphism in the uncoupling protein 3 (*UCP3*) gene influences fat distribution in women of European and Asian origin. Diabetologia 2000;43:1558–64.
27. Evans D, Minouchehr S, Hagemann G, et al. Frequency of and interaction between polymorphisms in the β3-adrenergic receptor and in uncoupling proteins 1 and 2 and obesity in Germans. Int J Obes Relat Metab Disord 2000;24:1239–45.
28. Yanovski JA, Diament AL, Sovik KN, et al. Associations between

uncoupling protein 2, body composition, and resting energy expenditure in lean and obese African American, white, and Asian children. Am J Clin Nutr 2000;71:1405–12.
29. Klannemark M, Orho M, Groop L. No relationship between identified variants in the uncoupling protein 2 gene and energy expenditure. Eur J Endocrinol 1998;139:217–23.
30. Chung WK, Luke A, Cooper RS, et al. Genetic and physiologic analysis of the role of uncoupling protein 3 in human energy homeostasis. Diabetes 1999;48:1890–5.
31. Dalgaard LT, Sorensen TI, Andersen T, Hansen T, Pedersen O. 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 1999;42:1413–6.
32. Otabe S, Clement K, Rich N, et al. Mutation screening of the human uncoupling protein 2 gene in normoglycemic and NIDDM morbidly obese patients: lack of association between new *UCP2* polymorphisms and obesity in French Caucasians. Diabetes 1998;47:840–2.
33. Urhammer SA, Dalgaard LT, Sorensen TI, et al. Organization of the coding exons and mutational screening of the uncoupling protein 3 gene in subjects with juvenile-onset obesity. Diabetologia 1998;41:241–4.
34. The National Heart, Lung, and Blood Institute Growth and Health Study Research Group. Obesity and cardiovascular disease risk factors in black and white girls: the NHLBI Growth and Health Study. Am J Public Health 1992;82:1613–9.
35. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1–9.
36. Hologic Inc. Hologic QDR-2000 operators manual and user's guide. Document 080–0384, revision A. Waltham, MA: Hologic Inc, 1992.
37. Urhammer SA, Fridberg M, Sorensen TI, et al. Studies of genetic variability of the uncoupling protein 1 gene in Caucasian subjects with juvenile-onset obesity. J Clin Endocrinol Metab 1997;82:4069–74.
38. Speiss EB. Genes in populations. 2nd ed. New York: John Wiley & Sons, 1989.
39. Bartholomew DJ. A test of homogeneity for ordered alternatives. Biometrika 1959;46:36–48.
40. SAS Institute Inc. SAS/STAT user's guide, version 8.1 edition. Cary, NC: SAS Institute Inc, 1999.