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Uncoupling protein 3 gene is associated with body composition changes with training in HERITAGE study

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Lanouette, Christian-Marc, Yvon C. Chagnon, Treva Rice, Louis Pérusse, Patrick Muzzin, Jean-Paul Giacobino, Jacques Gagnon, Jack H. Wilmore, Arthur S. Leon, James S. Skinner, D. C. Rao, and Claude Bouchard. Uncoupling protein 3 gene is associated with body composition changes with training in HERITAGE study. *J Appl Physiol* 92: 1111–1118, 2002; 10.1152/japplphysiol.00726.2001.—The uncoupling protein 3 (UCP3) is a mitochondrial membrane transporter mainly expressed in skeletal muscle that we have shown to be associated with obesity. We have analyzed UCP3 polymorphisms, Val102Ile, Tyr210Tyr, and a new microsatellite GAIVS6 located in the sixth intron, among 276 black and 503 white subjects from the HERITAGE Family Study. Linkage and association studies were undertaken with body composition variables measured in a sedentary state (baseline) and after 20 wk of endurance training (changes). Allele and genotype frequencies were found to be significantly different between whites and blacks. Suggestive linkages ($0.009 \leq P \leq 0.033$) with Tyr210Tyr were found in blacks and whites for baseline body mass index, fat mass, or leptin level and with GAIVS6 in whites for changes in fat mass and percent body fat. Associations were also found in whites between GAIVS6 and changes in the sum of eight skinfold thicknesses ($P = 0.0006$), with a borderline result for body mass index ($P = 0.06$). We concluded that UCP3 could be involved in body composition changes after regular exercise.

microsatellite; polymorphism; fat; association; linkage

UNCOUPLING PROTEIN (UCP)1 is located in the inner membrane of mitochondria, where it uncouples phosphorylation from the cellular respiratory chain, producing heat instead of ATP (19). It is exclusively expressed in brown adipose tissue that is present in newborn humans but is practically absent in adults. Four other UCPs have been recently uncovered. UCP2 is expressed in most human tissues (14), whereas UCP3 is mainly expressed in human skeletal muscle (4). UCP4 is exclusively expressed in human and rodent brain tissue (30), whereas UCP5 or brain mitochondrial carrier protein 1 transcripts are present in multiple human and mouse tissues, with a higher abundance in the brain and testis (40). The UCP1 gene is located on chromosome 4q31, UCP2 and UCP3 on chromosome 11q13 with only 7 kilobases separating each other (27), and UCP4 at 6p11.2-q12 (22). The chromosome location of UCP5 has not been reported yet. High homology between the amino acid sequence of the different UCPs is observed. UCP2 and UCP3 have, respectively, 55% (14) and 56% (4) homology with UCP1 and 73% homology between them (4). There are two isoforms of UCP3: a long form ($UCP3_L$) and a short form ($UCP3_S$), which lacks the sixth potential transmembrane region. Studies using transfected yeast and mammalian cells (see Ref. 24 for review), reconstituted systems (12), and muscle mitochondria of transgenic mice (11, 15, 39) have shown that UCP3 behaves as a functional UCP. A recent study also showed that UCP3 protein increases thermogenesis in yeast cells (17), although the matter remains controversial in mammalian cells because the same conditions that upregulate UCP3 expression do not change mitochondrial membrane potential (8).

Skeletal muscle plays an important role in energy homeostasis and substrate oxidation, and this tissue is a major site of thermogenesis in humans (2). Skeletal muscle may contribute to as much as 40% of the whole body adrenaline-induced thermogenesis (34). Mice overexpressing human UCP3 in muscle were shown to be

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hyperphagic but lean (11). Although no phenotypic difference between UCP3 knockout and control mice was observed (15, 39), alternative compensatory mechanisms cannot be excluded.

Because of its potential role in energy metabolism and its expression in skeletal muscle, UCP3 is a good candidate gene for the regulation of body composition and its response to training. It was shown that treadmill running in rats rapidly induces skeletal muscle UCP3 mRNA expression and that this induction results in a corresponding increase in rat UCP3 protein (41). Tsuboyama-Kasaoka et al. (38) reported that a single 1-h bout of exercise in rats increases UCP3 expression in the gastrocnemius and quadriceps muscles and that this increase disappeared rapidly within 24 h after the exercise bout. In humans, acute exercise was shown to have no effect on $UCP3_S$ and $UCP3_L$ expression (31) or to increase transiently UCP3 (28). In contrast, training was also found to decrease UCP3 expression in rats (4) and in humans (31).

Otabe et al. (26) found that the effects of regular physical activity on the body weight of obese and normal-weight subjects was influence by a C→T polymorphism in the 5′ flanking region (−55 C/T) of the UCP3 gene. The same −55 C/T polymorphism was found to be associated with a decreased risk of developing Type 2 diabetes in a French cohort (23) and with an increased waist-to-hip ratio in European and South Indian women (9). In the Québec Family Study (QFS), we also reported strong associations between a UCP3 microsatellite marker and obesity and body composition-related phenotypes (20).

In the present study, we investigated the linkage and association between three UCP3 polymorphisms and body composition-related phenotypes and their change in response to chronic endurance training in the HERITAGE Family Study.

MATERIALS AND METHODS

Subjects. The aims, design, and measurement protocol of the HERITAGE Family Study cohort have been previously described (5). The study includes volunteer black and white nuclear families from five centers: Québec City; Phoenix, AZ; Minneapolis, MN; Austin, TX; and Indianapolis, IN. Participants were required to be sedentary at baseline and in good health to be eligible for the study. Individuals with a resting systolic blood pressure of >159 mmHg and/or diastolic blood pressure of >99 mmHg, as well as those taking antihypertensive medication, were excluded. All exclusion criteria have been described before (5). A total of 276 black subjects, including 77 parents (52 women and 25 men) and 199 offspring (133 women and 66 men) from 94 families, and 502 whites with 190 parents (93 women and 97 men) and 312 offspring (164 women and 148 men) from 99 families were available for the present study. Written informed consent was obtained from each participant. The study was approved by the internal review board of each participating institution.

Phenotype measurements. Subjects were tested for a battery of anthropometric and physiological variables before and after a 20-wk exercise training program that is described later. Body mass index (BMI; kg/m^2) was derived from height and weight measured using a stadiometer and a balance beam scale. Skinfold thicknesses were measured twice at eight different sites (biceps, triceps, medial calf, thigh, subscapular, suprailiac, abdominal, and midaxillary) with an Harpenden caliper after the procedure recommended by Lohman (21). A third measurement was taken if the first two differed by >1.0 mm. The two measurements (the two closest when three measurements were taken) were averaged and used as the final value. The sum of the eight skinfold thicknesses (SF8) was used as an indicator of the amount of subcutaneous fat. Percent body fat (%Fat) was estimated from body density measurements obtained by underwater weighing and the equations of Siri (3) and Lohman (21) for white men and women, respectively, and of Schutte et al. (33) and Ortiz et al. (25) for black men and women, respectively. Fat mass (FM) and fat-free mass (FFM), both in kilograms, were calculated from %Fat and body weight. Leptin (Lep) level (ng/ml) was evaluated by a RIA (Linco Research, St. Charles, MO), in which the lowest quantity detectable was 0.5 ng/ml in plasma. In this study, baseline data (before the exercise program) and changes (posttraining data minus baseline) were analyzed.

Training program. The subjects were trained on cycle ergometers, three times a week for 20 wk, using the same standardized protocol. The subjects exercised at a heart rate (HR) corresponding to 55% of their baseline maximal oxygen consumption for 30 min per session at the beginning, increasing progressively toward a HR associated with 75% of their baseline maximal oxygen consumption for 50 min. This level was maintained during the last 6 wk of training. The intensity and duration of the training program were adjusted every 2 wk. Training intensities at each exercise session were adjusted individually by a computer system. HR was monitored during all training sessions with a computerized cycle ergometer system (Universal FitNet System), which adjusted the ergometer resistance to maintain target HR. More details about the training program can be found elsewhere (35, 36).

UCP3 genetic markers. Genomic DNA was prepared from permanent lymphoblastoid cells by the proteinase K and phenol-chloroform technique. DNA was dialyzed four times against Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 6 h at 4°C and ethanol precipitated. Genotyping of a dinucleotide GA microsatellite located in intron 6 of the UCP3 gene (GAIVS6) was done as previously described (20). Briefly, PCR was performed using 50 ng of genomic DNA, 40 nM of untagged primer, 10 nM of infrared tagged M13 primer (LiCor, Lincoln, NE), 125 μM 2-deoxynucleotide 5′-triphosphate, and 0.3 units of *Taq* polymerase in PCR buffer (Boehringer Mannheim) in a final volume of 10 μl. The upstream primer tailed with a M13 sequence (lower case) and downstream primer were 5′-cacgacgttgtaaaacgacTAGAACTGTGAGAATTCGCTGC-3′ and 5′-ACATCAGGTGGAGTGCTAGG-3′, respectively. PCR conditions consisted of one cycle at 93°C for 5 min and 30 cycles at 94°C for 20 s and at 60°C for 1 min (Ericomp, San Diego, CA). PCR products were analyzed using automatic DNA sequencers and the genotyping software SAGA (LiCor).

Two others polymorphisms were studied: a valine to isoleucine substitution at amino acid 102 [V102I(G→A)] and a C-to-T nucleotide transition in tyrosine 210 codon [Y210Y(C→T)] (10). For the V102I(G→A) polymorphism, a genomic DNA fragment was amplified by PCR using forward 5′-GCATCGGCCTCTATGACTAC-3′ and reverse 5′-CTTGACCCGCACACTTTCAGCCAC-3′ primers. PCR conditions were one cycle at 94°C for 5 min; 40 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 45 s; and one cycle at 72°C for 10 min. The 100-bp PCR product was digested with 5 units of Tth111 I for 5 h at 65°C. V102(G) allele produced fragments of 80 and 20 bp, and I102(A) allele produced fragments of 100

bp. For the Y210Y(C→T) variant, forward 5′-TCAAGGAGAAGCTGCTGGAGT-3′ and reverse 5′-TACTAGGCACTGCTTCTCTCTCTG-3′ primers were used with PCR conditions of one cycle at 94°C for 5 min; 40 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 45 s; and one cycle at 72°C for 10 min. The 130-bp PCR product was digested with 5 units of *Rsa* I at 37°C overnight. The Y210(C) allele exhibited fragments of 110 and 20 bp, and the Y210(T) allele exhibited a fragment of 130 bp. DNA fragments were resolved on a 3% agarose gel and visualized with ethidium bromide. All PCR reactions were performed in a GeneAmp PCR system 9600 (Perkin Elmer, Foster City, CA) using *Taq* DNA polymerase purchased from QIAGEN (Santa Clara, CA).

Statistical analysis. For linkage studies, phenotypic variables were adjusted within race, gender, and age groups for age and age^2 using a regression procedure. Lep was also further adjusted for FM. The response phenotypes (delta score) were calculated by subtracting the baseline value from the posttraining value. Response phenotypes were further adjusted for corresponding baseline values and Lep for changes in FM. Residuals were then standardized to a mean of 0 and a standard deviation of 1. The sib-pair linkage analysis was performed on nuclear families using the SIBPAL version 3.0 software from the SAGE package, with the *t*-statistic and the degrees of freedom adjusted for the nonindependence of sib-pairs.

For the association analyses, phenotypes were compared among genotypes using the covariance analysis with the same covariates as for linkage. The mixed procedure was used to correct for nonindependence among family members. That is, the main effects of genotype, age, and gender (etc.) were included in the model, and all analyses were conducted separately by race. Because the frequencies of allele 236 bp of GAIVS6 was <1%, it was grouped with other genotypes for analysis. The heterozygotes for this allele were grouped with the homozygotes for the other allele. For example, subjects with 236/238-bp genotype were grouped with 238/238-bp genotypes. No homozygote for this allele was observed. Alleles 244 and 256 observed in the QFS (20) were not observed in HERITAGE. The SAS package (versions 6.12 and 6.8) for PC was used for the analysis. Probability values (*P*) of linkage and association tests were adjusted, where indicated, for multiple tests using the Bonferroni correction in which the adjusted *P* value = $1 - (1 - P)^{\text{number of traits}}$.

RESULTS

We have studied the impact of the three UCP3 gene polymorphisms, V102I(G→A), Y210Y(C→T), and GAIVS6, on body composition-related phenotypes in the HERITAGE Family Study, before and after a 20-wk exercise-training period. Descriptive statistics of the phenotypes investigated are shown in Table 1 for whites and blacks, by generation and by gender. Lean, overweight, and obese individuals are present in both white and black groups in the following proportions: 50% normal weight (BMI <25), 31% overweight (BMI between 25 and 30), and 19% obese (BMI >30) in whites, and 36, 32, and 32%, respectively, in black subjects. These proportions were significantly different between blacks and whites [χ^2 = 18.34 with 2 degrees of freedom (df) and $P = 0.001$]. Black subjects had higher mean values than whites for adiposity phenotypes, except for male parents in whom the opposite was observed.

Allele and genotype frequencies for the three polymorphisms are presented in Table 2 and are significantly different between blacks and whites ($P = 0.001$ for each of the three polymorphisms). Polymorphisms were in Hardy-Weinberg equilibrium in whites [GAIVS6: $\chi^2 = 7.33$, 6 df, $P = 0.29$; V102I(G→A): $\chi^2 = 0.002$, 1 df, $P = 0.96$; Y210Y(C→T): $\chi^2 = 0.005$, 1 df, $P = 0.94$] and blacks [GAIVS6: $\chi^2 = 8.90$, 6 df, $P = 0.18$; V102I(G→A): $\chi^2 = 3.64$, 1 df, $P = 0.06$; Y210Y(C→T): $\chi^2 = 0.90$, 1 df, $P = 0.34$]. Only three white subjects were carriers of the variant allele for the V102I(G→A) polymorphism. The three polymorphisms were in linkage disequilibrium with each other in blacks ($P < 0.001$), as well as in whites ($P < 0.001$).

Some evidence of linkage ($0.003 \leq P \leq 0.03$) with baseline body composition phenotypes was found in whites with Y210Y(G→A) and for changes after train-

Table 1. *Number of subjects, mean, and range by race and gender for each phenotype*

	Parent						Adult Offspring					
	Male			Female			Male			Female		
Phenotype	*n*	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*	Mean	Range
						Blacks						
Age, yr	25	50.9	39–66	51	47.2	37–65	66	28.4	16–46	132	27.7	16–48
BMI, kg/m^2	25	26.6	19–39	52	29.5	21–43	64	27.5	18–43	133	27.7	18–45
SF8, mm	21	119.9	45–263	37	215.6	128–323	57	122.0	35–277	101	163.9	59–320
FM, kg	22	19.9	10–35	35	31.1	13–56	64	21.4	6–55	109	26.8	8–61
Fat, %	22	24.6	17–34	35	39.2	22–51	64	22.9	9–41	109	35	16–54
Leptin, ng/ml	22	6.8	1–25	43	28.5	7–82	63	7.5	<1–35	116	27.3	3–104
FFM, kg	22	59.7	49–69	35	46.6	35–61	64	66.0	46–93	109	46.2	33–61
						Whites						
Age, yr	97	53.6	44–64	93	52.1	42–65	148	24.4	17–40	164	25.5	17–41
BMI, kg/m^2	97	28.3	21–41	93	27.4	19–48	147	25.7	17–44	164	23.7	17–39
SF8, mm	84	146.1	56–340	80	196.1	82–355	139	121.9	38–262	161	148.8	47–319
FM, kg	91	24.6	8–58	84	26.8	35–57	139	17.3	<1–53	163	18.1	3–59
Fat, %	91	27.6	11–42	84	36.6	20–54	139	19.8	<1–43	163	26.8	7–53
Leptin, ng/ml	96	9.0	2–30	87	23.7	5–70	145	5.9	<1–30	158	15.3	2–82
FFM, kg	91	62.3	47–80	84	44.4	35–57	139	64.4	45–87	163	46.1	34–65

n, No. of subjects; BMI, body mass index; SF8, sum of 8 skinfold thicknesses; FM, fat mass; FFM, fat-free mass.

Table 2. *χ^2 test and allele and genotype frequencies for 3 different polymorphisms in the UCP3 gene between blacks and whites in the HERITAGE Family Study*

	V102I (G→A)			Y210Y (C→T)		
	Blacks	Whites	*P*	Blacks	Whites	*P*
Genotype						
1/1	0.74 (201)	0.99 (499)	0.001	0.03 (9)	0.25 (125)	0.001
1/2	0.25 (68)	0.01 (3)		0.35 (95)	0.50 (251)	
2/2	0.01 (1)	0.0 (0)		0.62 (172)	0.25 (125)	
Allele						
1	0.87	0.99	0.001	0.20	0.50	0.001
2	0.13	0.01		0.80	0.50	
	GAIVS6					
	Blacks	Whites	*P*			
Genotype						
238–238	0.27 (70)	0.54 (242)	0.001			
238–240	0.50 (128)	0.17 (77)				
238–242	0.05 (15)	0.20 (88)				
240–240	0.12 (31)	0.03 (13)				
240–242	0.05 (15)	0.04 (20)				
242–242	0.01 (1)	0.02 (10)				
Allele						
238	0.55	0.72	0.001			
240	0.39	0.14				
242	0.06	0.14				

Wild-type (1) and variant (2) alleles. Nos. in parentheses, no. of subjects. UCP3, uncoupling protein 3.

ing with GAIVS6 (Table 3). After Bonferroni correction for multiple comparisons, all linkage results did not remains significant. Linkage analysis with V102I(G→A) was not performed in whites, because all but three subjects were homozygous for the wild-type allele. Weak linkages were also found among blacks between Y210Y and bBMI and bSF8 ($P = 0.02$ and 0.03, respectively).

Association results are shown in Table 4 and Fig. 1. Evidence of association was observed between the GAIVS6 polymorphism and training-induced changes in SF8 and, to a lower extent, BMI among whites (Table 4 and Fig. 1). Subjects with the 240–240 genotype showed significantly greater reduction in SF8, with borderline value for BMI and a similar but nonsignificant pattern of variation for FM and %Fat. Carriers of the 238-bp allele showed a smaller reduction for those phenotypes, whereas subjects with genotypes including the 242 bp (240–242 and 242–242), but not the 238 bp, showed generally the smallest drop or even a gain in response to training. When association analysis was performed after genotype grouping, according to apparent allelic dominance (allele 238 bp over 242 and 240 bp, and 242 bp over 240 bp), resulting in carriers of allele 238 bp vs. other genotypes with allele 242 bp vs. homozygotes for the allele 240 bp, stronger associations were observed (Fig. 1). No other associations in whites and blacks were observed for the

Table 3. *Sib-pair linkage results between allelic variations in the UCP3 gene and body composition-related phenotypes*

	GAIVS6				V102I (G→A)			Y210Y (C→T)			
	Blacks		Whites		Blacks		Whites	Blacks		Whites	
Phenotype	*P* value	*n*	*P* value	*n*	*P* value	*n*	NA	*P* value	*n*	*P* value	*n*
bBMI	0.340	90	0.822	273	0.986	101		0.022*	104	0.033*	357
bSF8	0.286	46	0.495	163	0.946	55		0.026*	56	0.487	198
bFM	0.616	71	0.465	258	0.869	77		0.084	80	0.015*	336
b%Fat	0.748	71	0.862	258	0.651	77		0.070	80	0.245	336
bLep	0.882	71	0.835	261	0.946	80		0.508	51	0.025*	258
bFFM	0.219	71	0.205	258	0.821	77		0.284	80	0.076	336
dBMI	0.536	80	0.190	249	0.093	91		0.387	94	0.562	327
dSF8	0.623	43	0.490	227	0.517	55		0.815	56	0.627	299
dFM	0.680	64	0.013*	229	0.055	70		0.696	73	0.513	301
d%Fat	0.405	64	0.009*	229	0.148	70		0.648	73	0.495	301
dLep	0.853	50	0.888	228	0.121	57		0.732	59	0.061	283
dFFM	0.493	64	0.598	229	0.633	70		0.869	73	0.665	301

n, No. of sib-pairs. Lep, leptin; NA, not available; b, baseline; d, delta (after Bonferroni correction, results do not remain significant). *Significant results, $P \leq 0.05$.

Table 4. *Covariance analysis in whites of changes after training in body composition-related phenotypes for the GAIVS6 polymorphism of the UCP3 gene*

Phenotype	Genotype	*n*	Mean	SE	*P*
BMI, kg/m^2	238–238	230	−0.082	0.05	0.06
	238–240	77	−0.121	0.08	
	238–242	84	−0.156	0.08	
	240–240	13	−0.447	0.21	
	240–242	19	+0.224	0.17	
	242–242	10	+0.402	0.23	
SF8, mm	238–238	208	−6.84	1.07	0.0006*
	238–240	71	−5.01	1.70	
	238–242	75	−9.07	1.45	
	240–240	13	−16.86	3.23	
	240–242	16	+1.64	3.45	
	242–242	10	−5.26	3.35	
FM, kg	238–238	218	−0.77	0.12	0.42
	238–240	71	−0.65	0.19	
	238–242	81	−0.88	0.28	
	240–240	13	−1.32	0.34	
	240–242	17	−0.12	0.48	
	242–242	8	−0.15	0.95	
%Fat	238–238	218	−0.93	0.13	0.58
	238–240	71	−0.90	0.20	
	238–242	81	−0.81	0.25	
	240–240	13	−1.44	0.38	
	240–242	17	−0.23	0.53	
	242–242	8	−0.68	0.83	

n, No. of subjects. *$P \leq 0.01$ after Bonferroni correction.

GAIVS6 polymorphism or for the other UCP3 polymorphisms studied (data not shown). When analysis was undertaken by gender, results remained significant only among women, with changes in BMI, SF8, and FM ($0.01 \leq P \leq 0.03$) (data not shown), but the number of subjects was low (between 2 and 5) for some genotype groups.

DISCUSSION

UCP3 is a protein mainly expressed in skeletal muscle, an important tissue in thermogenesis and in the adaptation to training. In brief, we have observed associations in whites between the GAIVS6 polymorphism in the UCP3 gene and changes in body composition-related phenotypes after a 20-wk exercise-training program.

Subjects homozygous for the 240-bp allele had a greater loss of adiposity than did subjects with other genotypes. Our laboratory previously reported associations with BMI, sum of six skinfolds, FM, and %Fat for the same polymorphism in the QFS (20). For instance, in QFS, the 240/240-bp genotype was associated with a higher adiposity, in contrast to the 238-bp allele carriers who were leaner. Also, in both the QFS and HERITAGE studies, the 240/240-bp genotype was the genotype showing the greatest response, with either baseline body composition in QFS or body mass and subcutaneous fat changes with training in the HERITAGE Family Study. Carriers of the 238-bp allele had lower values for body composition phenotypes after training compared with baseline but not as much as homozygotes 240/240 bp. Carriers of the 242-bp allele showed an inverse effect of training, with the 240/242- and 242/242-bp genotypes showing an increase in BMI after training. From these observations, we conclude that the 240/240-bp genotype is more responsive to biochemical, physiological, and genetic environments. We also conclude that the allele 238 appears to be dominant over alleles 242 and 240, and allele 242 appears to be dominant over allele 240. Interestingly, the effect is observed only in women when the analyses were done by gender, but this result has to be confirmed because of the low number of subjects (between 2 and 5) in some genotype groups.

However, in contrast to QFS, we did not observe significant differences across GAIVS6 genotypes for body composition before training among whites. Part of the subjects in QFS were ascertained for obesity because two individuals in a family had to be obese for that family to be included. Therefore, the genetic background favors obesity, because subjects were selected according to the presence of obesity in the family. In

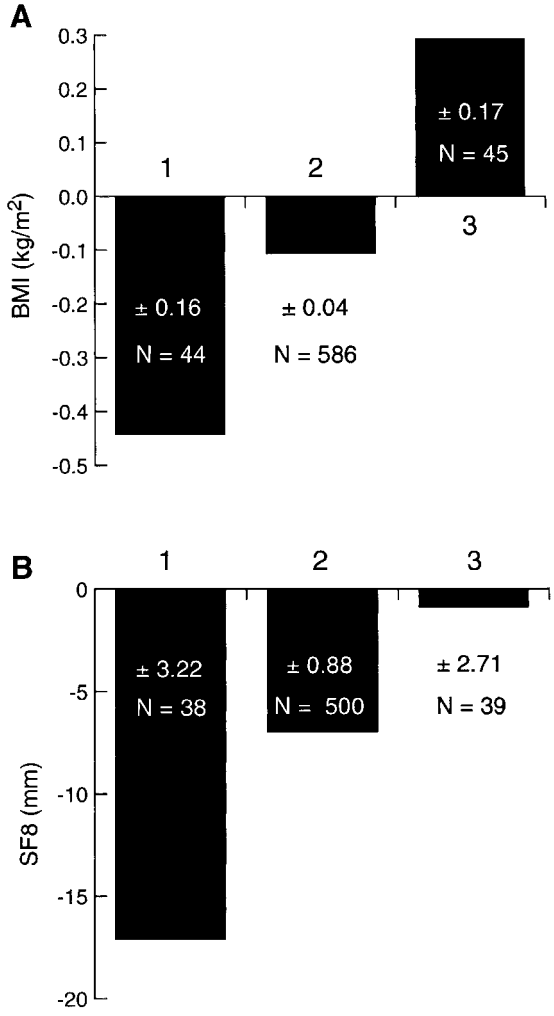

Fig. 1. Changes after training when genotypes of GAIVS6 are grouped by apparent allelic dominance. *A*: body mass index (BMI). *B*: sum of 8 skinfold thicknesses (SF8). *Genotype 1* = 240–240; *genotype 2* = 238–238, 238–240, 238–242; *genotype 3* = 240–242, 242–242. *N*, no. of subjects. $P = 0.01$ (*A*); $P = 0.0008$ (*B*). After Bonferroni correction: $P = 0.11$ (*A*) and $P = 0.009$ (*B*).

contrast, although the HERITAGE cohort contained overweight and obese individuals, these subjects were volunteers, were not selected for any obesity phenotypes, and did not seem to present the genetic predisposition apparently needed for the expression of the 240-bp homozygote effect on body composition in the sedentary state. This sampling strategy allowed for collection of individuals from different genetic pools, which could be enhanced by the fact that HERITAGE subjects have been sampled from largely distinct geographical areas.

It is not clear what could be the biochemical or physiological effects of the GAIVS6 polymorphism (a microsatellite located in intron 6). Obviously, GAIVS6 did not induce any change in the amino acid sequence of UCP3 protein. The UCP3 gene generates two mRNA termination variants, producing a short ($UCP3_S$) and a long ($UCP3_L$) form of the protein (14, 37). $UCP3_S$ lack the VIth potential transmembrane domain and a large part of the putative nucleotide binding domain of UCP3. It was hypothesized that the $UCP3_S$ protein was either inactive, because of the absence of the VIth transmembrane domain, or constitutively active, because of the absence of inhibition by GDP (18). In fact, Hinz et al. (18) observed that $UCP3_S$ had a higher intrinsic activity than $UCP3_L$, whereas another study (16) concluded that $UCP3_S$ had modestly reduced activity compared with $UCP3_L$. Because of its location in intron 6 near the alternative stop codon responsible for the production of $UCP3_L$ and $UCP3_S$, the GAIVS6 polymorphism could eventually modify the proportion of the two UCP3 forms and change UCP3 activity. However, it has been reported that the splice-site mutation IVS6+1G→A, also located in intron 6, has no apparent effect on the uncoupling activity of UCP3 (6).

It is also possible that the effect was caused by another polymorphism in linkage disequilibrium, with GAIVS6 located in the UCP3 promoter region, in the coding region, or with another gene located in the same region, e.g., UCP2. For instance, the Val/Ala −55 polymorphism of UCP2 has already been associated with exercise efficiency (7), but this particular polymorphism, as well as the insertion/deletion in exon 8, showed no association in HERITAGE for the body composition-related phenotypes (data not shown). On the other hand, Esterbauer et al. (13) reported that a −866 G/A polymorphism in the UCP2 gene promoter contributes to obesity in the studied population. However, the effect of this polymorphism on the response to training had not been studied yet.

In some studies, training was shown to have an effect on UCP3 expression (31, 38, 41). Here, we reported that a UCP3 polymorphism influences the response to training. Otabe et al. (26) found that a C→T change at position −55 in the 5′ region of the UCP3 gene can reduce the benefit of physical activity in obese subjects, and Schrauwen et al. (32) reported that this polymorphism was associated with an increased expression of UCP3 in skeletal muscle in male nondiabetic Pima Indians. In QFS, we genotyped 10 subjects for the −55 C/T UCP3 polymorphism and observed a possible partial linkage disequilibrium with GAIVS6. This led us to hypothesize that both polymorphisms could be related to the responsiveness to regular exercise. Complete genotyping of the −55 C/T variant in both QFS and HERITAGE cohorts should, therefore, be undertaken.

Studies with UCP3 knockout mice concluded that UCP3 does not seem to be required for body weight regulation, exercise tolerance, fatty acid oxidation, or cold-induced thermogenesis (15, 39). This discrepancy with our results might be explained by the fact that, in rodents, in contrast to humans, a possible decrease in muscle thermogenesis might be compensated for by the brown adipose tissue. The complete absence of expression of UCP3, as in knockout mice, could induce compensatory mechanisms in contrast to an allele with altered functions, such as results observed in the present study.

No association was found here with the V102I(C→T) and Y210Y(G→A) markers, as was previously observed in QFS (20) and by Argyropoulos et al. (1). Allelic frequency of V102I(C→T) was not significantly different for blacks in the HERITAGE Family Study and the American participants in the Argyropoulos study. This polymorphism was not detected in another white population (1) or in QFS, in which all subjects were white. However, three related white subjects (a father and his two children) from the HERITAGE Family Study were found to be heterozygotes; they are the first three whites reported to be carriers of this polymorphism. UCP3 effects were observed mainly among whites, with only weak linkages among blacks. Moreover, genotypic and allelic frequencies were different between blacks and whites.

We concluded that UCP3 could be involved in the body composition changes in response to training because the GAIVS6 polymorphism of the UCP3 gene has been shown to be associated with changes in BMI and SF8 in whites. Weak linkages were also found between GAIVS6 and Y210Y(G→A) polymorphisms, with some body composition-related phenotypes and their changes with training. This supports the hypothesis that UCP3 could be involved in human body composition regulation and possibly in the control of obesity, by modulating the response to regular exercise.

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