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ORIGINAL ARTICLE

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Uncoupling protein 1 and 3 polymorphisms are associated with waist-to-hip ratio

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Abstract Body weight regulation is a complex phenotype also depending on the action of uncoupling proteins (UCPs) that mediate the "uncoupling" of respiration leading to the dissipation of energy as heat. This study investigated whether genetic variants in the genes encoding UCP-1 and UCP-3 are associated with different obesity-related phenotypes in 162 whites with a wide range of body mass index. All subjects were genotyped for the polymorphisms UCP-1 A-3826G, UCP-1 Ala64Thr, and UCP-3 C-55T using a PCR-based restriction method with appropriate enzymes. The frequencies of the UCP-1 3826G, UCP-1 64Thr, and UCP-3 55T alleles were 27.2%, 12.0%, and 22.8%, respectively. No significant associations were observed between polymorphism and body mass index or obesity. However, after adjustment for gender, age, body mass index, and diabetes mellitus the waist-to-hip ratio was significantly associated with UCP-1 Ala64Thr (P=0.003) and UCP-3 C-55T (P=0.02) but not with UCP-1 A-3826G. The higher waist-to-hip ratios associated with the UCP-1 64Thr and UCP-3 55T alleles were due to higher waist circumference in these allele carriers. In conclusion, central obesity in whites as reflected by an increased waist-to-hip ratio is associated with the UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. To what extent these genotypes contribute to the overall cardiovascular risk remains to be elucidated.

Keywords Genetics · Uncoupling proteins 1 and 3 · Obesity · Waist-to-hip ratio

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Abbreviations *BMI:* Body mass index · *UCP:* Uncoupling protein

Introduction

Body weight is tightly regulated by the balance between energy intake and expenditure. Energy expenditure itself is a complex trait including the metabolic rate at rest and depends on basal metabolism and exercise-induced and adaptive thermogenesis [1]. Low rate of energy expenditure has been shown to increase the risk of developing obesity [2]. In addition to environmental factors, lifestyle, and the quantity and quality of food intake, there are genetic factors that modulate the balance between energy intake and expenditure [3, 4].

Uncoupling proteins (UCPs) are mitochondrial membrane transporters that mediate the "uncoupling" of respiration leading to the dissipation of energy as heat [5]. UCPs may increase energy expenditure and thereby decrease body weight. Five members of the UCP gene family, termed UCP-1 to UCP-5, have so far been identified in humans [6, 7], all of which exhibit structural homology and share functional properties. Whereas UCP-1 is expressed only in brown adipose tissue [5], UCP-3 is expressed in both human skeletal muscle and brown adipose tissue [8]. Several human tissues including brown and white adipose tissue, lung, liver, spleen, and macrophages express UCP-2 [9]. The UCP-1 gene resides on chromosome 4q31 [10], and the UCP-2 and UCP-3 genes constitute a cluster mapping to 11q13 [11]. Genetic polymorphisms in these three UCP genes have been variably associated with metabolic and obesity-related phenotypes. Since genetic studies on the impact of UCP-2 polymorphisms on obesity appear less convincing [12, 13, 14, 15], we investigated whether known variants in UCP-1 (A-3826G, Ala64Thr) and UCP-3 (C-55T) genes are associated with obesity-related phenotypes in a well characterized study population of white subjects.

Materials and methods

Subjects

The study enrolled 162 unrelated white subjects (45 men; 117 women) with a wide range of body mass index (BMI; range 20.2–54.9 kg/m^2), with and without hypertension and/or impaired glucose tolerance, who responded to an advertisement on the university campus of the Benjamin Franklin Medical Center in Berlin. Men and women had similar age (52.9±9.9 years), BMI (32.0±6.4 kg/m^2), hip circumference (110.7±14.2 cm), and postprandial thermogenesis (241±90 kJ/day). Other phenotypic measurements such as waist-to-hip ratio were significantly higher in men than in women except for percentage body fat, fat mass, and fasting plasma leptin levels, which were all higher in women (Table 1). Patients with congestive heart failure, abnormal renal or liver function, and intentional weight reduction during the preceding 3 months were excluded. Patients were also assessed for the intake of thyroid hormones and other medications. Obesity was defined as BMI of 30 kg/m^2 or higher [16]. All subjects were characterized for weight, height, waist and hip circumferences. Body composition was determined by bioelectrical impedance analysis (Akern-RJL BIA 101/S, Frankfurt, Germany) [17]. Subjects were subsequently asked to record their daily intake of foods using a 7-day weighed dietary record. The study protocol was approved by the local institutional ethics committee, and all subjects gave informed consent.

Measurement of energy expenditure

For the measurement of energy expenditure subjects were admitted to a metabolic ward in the evening before the test. They were given a light evening snack. After a 12-h overnight fast the resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over two 25-min periods with an open-circuit indirect calorimetric system (standardized for temperature, pressure and moisture) fitted with a face mask (Sensor Medics 2900 Z, NewMedics Medizinelektronik, Öhringen, Germany). Complete urine samples were collected during the measurement of energy expenditure for the assessment of nitrogen excretion to determine the basal substrate oxidation. From each measurement the first 5-min period was discarded. Data from the remaining 20 min were averaged and used to calculate energy expenditure and substrate oxidation based on oxygen consumption, carbon dioxide production, and urinary nitrogen excretion [18, 19].

Genotyping

Genomic DNA was prepared from peripheral blood using a DNA-selective preparation method (Qiagen, Hilden, Germany). The re-

Table 1 Characteristics of the study population: arithmetic means ±SD or geometric means (95% confidence interval)

	Men (*n*=45)	Women (*n*=117)	*P*
Age (years)	53.3±10.7	52.8±9.6	0.76
Body weight (kg)	101.2±19.3	84.7±17.6	<0.0001
Body height (cm)	177.1±7.0	162.9±6.7	<0.0001
Body mass index (kg/m^2)	32.2±6.3	31.9±6.5	0.81
Waist circumference (cm)	110.6±16.4	99.6±15.3	<0.0001
Hip circumference (cm)	112.0±14.3	110.2±14.2	0.48
Waist-to-hip ratio	0.986±0.040	0.902±0.048	<0.0001
Body fat (%)	25.9±6.7	36.6±5.3	<0.0001
Fat mass (kg)	26.8±11.1	31.7±10.3	0.01
Fat-free mass (kg)	73.7±11.3	52.9±8.6	<0.0001
Resting metabolic rate (kJ/day)	7957±1210	6296±911	<0.0001
Postprandial thermogenesis (kJ/day)	255±93	235±88	0.20
Fasting plasma leptin (ng/ml)	8.9 (7.3–10.9)	23.0 (20.4–26.1)	<0.0001
Fasting plasma insulin (mU/l)	22.7 (18.7–27.4)	14.7 (13.0–16.6)	0.002

gions encompassing the three different polymorphic sites were amplified by polymerase chain reaction as previously described [20, 21, 22]. Polymerase chain reaction products were digested using the restriction enzymes *Bcl*I, *Msp*A1, and *Bse*DI, for the polymorphisms UCP-1 A-3826G, UCP-1 Ala64Thr, and UCP-3 C-55T, respectively, and finally visualized on UV-transilluminated ethidium bromide-stained agarose gels.

Statistical analysis

We used SAS version 8.1 (SAS Institute, Cary, N.C., USA) for database management and statistical analysis. Measurements with a skewed distribution were normalized by logarithmic transformation. Comparisons of means and proportions were performed with the standard normal *Z*-test and Fisher's exact test, respectively. We employed analysis of covariance to compare adjusted continuous measurements between genotypes.

Results

UCP-1 and UCP-3 polymorphisms are associated with waist-to-hip ratio

The genotype distributions for UCP-1 A-3826G (AA 0.51, AG 0.44, GG 0.05; P=0.12) and UCP-3 C-55T (CC 0.58, CT 0.38, TT 0.04; P=0.27) were in Hardy-Weinberg equilibrium, which was not the case for UCP-1 Ala64Thr, regardless of gender, and the presence of obesity or diabetes mellitus. UCP-1 A-3826G and Ala64Thr displayed significant linkage disequilibrium with one another (P<0.0001). Genotype and allele frequencies did not differ between obese and nonobese subjects for any of the three polymorphisms investigated in the whole study population (P>0.32; Table 2).

No significant association was observed between the three genetic polymorphisms and BMI, resting metabolic rate, postprandial thermogenesis, or fasting plasma leptin and insulin levels. However, after adjustment for gender, age, BMI, and the presence of diabetes mellitus the waist-to-hip ratio was significantly associated with UCP-1 Ala64Thr (means: AlaAla 0.920, AlaThr 0.946, ThrThr 0.953; P=0.003) and UCP-3 C-55T (CC 0.920, CT 0.928, TT 0.966; P=0.02; Fig. 1), but not with UCP-1 A-3826G (AA 0.919, AG 0.931, GG 0.933; P=0.15). The associations between the waist-to-hip ratio and UCP-1 Ala64Thr and UCP-3 C-55T were due to the increased waist circumference in carriers of the UCP-1 64Thr allele (AlaThr and ThrThr 105.2 vs. AlaAla 102.2 cm, P=0.01; Table 3) and UCP-3 55T allele (CT and TT 103.6 vs. CC 101.9 cm, P=0.06).

In models comparing carriers of the UCP-1 3826G allele with UCP-1 3826A homozygotes, UCP-1 3826G allele carriers had a slightly higher waist-to-hip ratio (P=0.05; Table 3). However, when both UCP-1 variants were included in a single model, the difference in the waist-to-hip ratio between UCP-1 3826A homozygotes and G allele carries became nonsignificant (0.932 vs. 0.936; P=0.59), whereas the effect of the UCP-1 Ala64Thr polymorphism remained unaltered (0.921 vs. 0.948; P=0.004). These results suggest that the slightly higher waist-to-hip ratio in UCP-1 3826G allele carriers was actually due to linkage disequilibrium with the Ala64Thr variant.

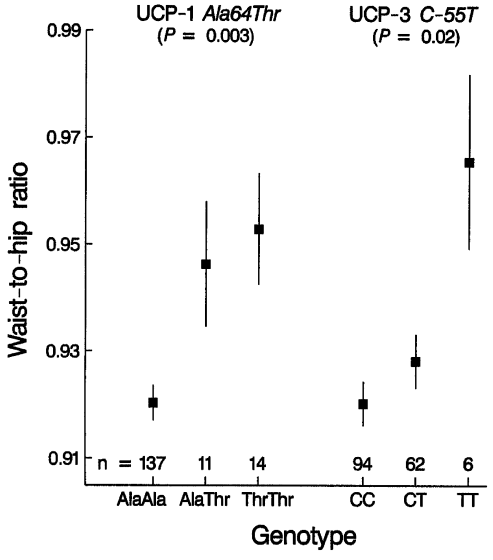

Fig. 1 Association between waist-to-hip ratio and UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. *Squares* Means adjusted for gender, age, BMI, and diabetes mellitus; *vertical lines* SEs. *P* values were based on analysis of variance; *n* the number of subjects by genotype

Table 2 Genotype frequencies for UCP-1 and UCP-3 polymorphisms; obesity defined as BMI ≥30 kg/m^2

	Genotype frequency (n)			P^a
UCP-1 A-3826G	AA	AG	GG	0.90
Nonobese (n=69)	50.7% (35)	43.5% (30)	5.8% (4)	
Obese (n=93)	50.5% (47)	45.2% (42)	4.3% (4)	
UCP-1 Ala64Thr	AlaAla	AlaThr	ThrThr	0.32
Nonobese (n=69)	84.1% (58)	4.3% (3)	11.6% (8)	
Obese (n=93)	84.9% (79)	8.6% (8)	6.5% (6)	
UCP-3 C-55T	CC	CT	TT	0.66
Nonobese (n=69)	55.1% (38)	42.0% (29)	2.9% (2)	
Obese (n=93)	60.2% (56)	35.5% (33)	4.3% (4)	

[a] *P* values based on Fisher's exact test for the comparison between obese and nonobese

Table 3 Associations between obesity-related phenotypes and the UCP-1 A-3826G and Ala64Thr polymorphisms. All analyses adjusted for gender, age, and the presence of diabetes mellitus; waist-to-hip ratio, waist circumference, and fasting plasma leptin and insulin levels also adjusted for BMI; resting metabolic rate also adjusted for fat-free mass and body fat

	UCP-1 A-3826G			UCP-1 Ala64Thr		
	AA	AG+GG	*P*	AlaAla	AlaThr+ThrThr	*P*
Body-mass index (kg/m^2)	31.9±0.7	32.1±0.7	0.81	31.9±0.5	32.3±1.3	0.83
Waist-to-hip ratio	0.919±0.004	0.931±0.004	0.05	0.920±0.003	0.950±0.008	0.0006
Waist circumference (cm)	101.9±0.6	103.4±0.6	0.09	102.2±0.5	105.2±1.1	0.01
Resting metabolic rate (kJ/day)	6705±66	6785±68	0.41	6730±51	6821±119	0.49
Postprandial thermogenesis (kJ/day)	246±10	235±11	0.43	240±8	245±18	0.79
Fasting plasma leptin (ng/ml)	17.7 (16.1–19.4)	17.7 (16.1–19.5)	0.95	17.8 (16.6–19.2)	17.0 (14.4–20.1)	0.62
Fasting plasma insulin (mU/l)	16.4 (14.5–18.5)	16.9 (14.9–19.2)	0.72	17.1 (15.5–18.8)	14.7 (11.8–18.3)	0.22

We additionally performed a gender-specific analysis of the waist-to-hip ratio in relation to the UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. In 117 women the waist-to-hip ratio was significantly related to UCP-1 Ala64Thr (P=0.002) but was only weakly associated with UCP-3 C-55T (P=0.08). In 45 men these effect sizes were similar to those in women, but due to the smaller sample size these associations did not reach statistical significance (P=0.23 and 0.59, respectively).

Discussion

The main result of the present study is the significant association between the waist-to-hip ratio and the UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. When considering UCP-1 A-3826G alone, a weak association with waist-to-hip ratio was observed, which may possibly be explained by linkage disequilibrium with UCP-1 Ala64Thr.

None of the investigated polymorphisms was associated with BMI as continuous variable or obesity as a dichotomous trait. A possible explanation is that abdominal obesity, a phenotype of visceral fat distribution measured by the waist-to-hip ratio [23], is more specific than BMI, which reflects overall obesity. Central obesity has been shown to be a better predictor of coronary events in high-risk populations than overall obesity [24]. The large prospective Atherosclerosis Risk in Communities Study [25] found the waist-to-hip ratio and not BMI to be a risk factor for incident coronary heart disease. Another study [26] reported that a waist-to-hip ratio of 0.91 or more was associated with a nearly threefold increased risk of acute coronary events, and provided additional prognostic information beyond BMI. Interestingly, BMI did not add to the predictive value of the waist-to-hip ratio in predicting these coronary events. Along similar lines, a prospective hospital-based case-control study of first myocardial infarction showed that in Indians the most predictive independent variables for acute coronary events were smoking and the waist-to-hip ratio [27].

From a genetic point of view Jee et al. [28] investigated environmental and genetic influences on waist circumference and waist-to-hip ratio in 2,507 members of 435 Korean families. Segregation analysis provided evidence of a major gene controlling waist circumference in these families. In line with the present findings, several other reports on various genetic polymorphisms have also suggested an association with abdominal fat but not with overall obesity. This indicates that genotype-phenotype associations differ in central obesity and overall obesity [29, 30]. More specifically, Perusse et al. [31] performed a genome-wide scan for different abdominal fat phenotypes (abdominal total fat, abdominal visceral fat, abdominal subcutaneous fat) assessed by computed tomography in the Québec Family Study and identified several chromosomal loci harboring genes affecting the propensity to store abdominal fat.

After weight reduction or during prolonged weight loss the UCP-3 mRNA expression decreases in obese individuals. This observation suggests that reduced UCP-3 expression contributes to the decreased energy expenditure [32, 33]. Independent studies report linkage of the resting metabolic rate and other obesity-related phenotypes to markers at the UCP-2/UCP-3 locus [11, 34]. Interestingly, this region is syntenic to a region of mouse chromosome 7, which is also linked to obesity as well as to a region of rat chromosome 1, which is linked to glucose intolerance and adiposity in a rat model of type 2 diabetes mellitus [9, 35].

Although some association studies on the impact of UCP-1 polymorphisms on metabolic phenotypes were inconclusive [36, 37, 38], other investigators have observed associations of UCP-1 variants with weight gain and resistance to weight loss [21, 39, 40]. Only a few studies have investigated UCP-1 Ala64Thr in relation to obesity or related phenotypes [20]. The latter study conducted among children and adolescents found that the 64Thr allele was more frequent in obese subjects than in a lean controls. However, correction for multiple testing removed the statistical significance. Thus the functional impact of this substitution remains unclear.

With respect to the UCP-3 gene polymorphisms some studies indicate a role for UCP-3 in energy expenditure in humans [22, 41]. More specifically, and in agreement with our present findings, Cassell et al. [42] reported a

consistent association between the UCP-3 55T allele and increased waist-to-hip ratio in women from three separate data sets. Interestingly, the C-55T polymorphism is situated in close vicinity to a putative TATA-box as well as a retinoic acid responsive element [43], indicating a potential functionality of the variant. Even when the nucleotide change does not directly involve a consensus site, it may alter the secondary DNA structure around these sequence elements affecting the access of transcription factors [44].

The present findings should be interpreted within the context of its limitations. Our study population is well characterized but relatively small, and a large proportion of subjects were obese or overweight. Therefore our findings can probably not be extrapolated to the general population. The observed associations, although highly significant, require further confirmation in larger groups of subjects.

In conclusion, central obesity in whites as reflected by an increased waist-to-hip ratio is associated with the UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. To what extent these genotypes contribute to the overall cardiovascular risk remains to be elucidated.

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