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ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 608 – 612
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Relation of −55CT polymorphism of uncoupling protein 3 gene with fat mass and insulin resistance in morbidly obese patients

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Received 6 July 2009; accepted 3 September 2009

Abstract

Some studies have pointed to a role of uncoupling protein 3 (*UCP3*) in the regulation of whole-body energy homoeostasis and regulation of fat distribution. The aim of our study was to investigate the influence of −55CT polymorphism of *UCP3* gene on fat mass and insulin resistance in morbidly obese patients. A population of 47 obese subjects (body mass index [BMI] >40 kg/m^2) was selected randomly in a prospective way. A nutritional evaluation was performed. Dietary intake and exercise were recorded. The mean age was 48.2 ± 15.4 years; and the BMI was 44.7 ± 4.7 kg/m^2, with 10 men (21.3%) and 37 women (78.7%). Thirty-two (68.1%) had the genotype −55CC (wild-type group), and 15 patients (31.9%) had −55CT (mutant-type group). In the mutant-type group, insulin (20.6±10.8 vs 31.2 ± 17.4 mIU/L, $P <$ .05), homeostasis model assessment (5.3 ± 2.7 vs 8.7 6.6, $P <$ .05), weight (114.1 ± 17.3 vs 122.8±19.1 kg, $P <$ .05), BMI (44.1 ± 4.6 vs 45.7 ± 6.3 kg/m^2, $P <$ .05), fat mass (56.3 ± 11.4 vs 61.4 ± 15.1 kg, $P <$ .05), and waist circumference (124.8 ± 12.5 vs 128.3 ± 9.1 cm, $P <$ .05) were higher than those in the wild-type group. Adiponectin levels were higher in wild-type group than mutant-type group (70.3 ± 26.1 vs 30.5 ± 32.5 ng/mL, $P <$ .05). In conclusion, mutant-type group of −55CC *UCP3* gene patients had higher weight, fat mass, and insulin resistance than wild-type group.

1. Introduction

The prevalence of obesity is rising. In Spain, approximately 0.5% of the population is morbidly obese [1]. Uncoupling protein 3 (*UCP3*) is likely to be involved in the regulation of energy balance; it is a candidate gene for the pathogenesis of morbidly obese.

Uncoupling protein 3 belongs to a family of mitochondrial transporters that could uncouple the oxidative phosphorylation by increasing the proton leak of the inner mitochondrial membrane [2]. Decreased expression or function of *UCP3* could reduce energy expenditure and increase the storage of energy as fat [3]. Some studies have pointed to a role of *UCP3* in the regulation of whole-body energy homoeostasis [4] and diet-induced obesity [5], and regulation of insulin resistance response secondary to weight loss [6].

The C/C genotype of a polymorphism in the *UCP3* promoter (−55C->T) is associated with increased expression of *UCP3* messenger RNA in muscle [7]. Other authors have reported that the −55T/T genotype is associated with increased body mass index (BMI) and interacts with physical activity [8]. It was shown that T/T genotype was associated with an atherogenic lipid profile in French white people and with a decreased risk of type 2 diabetes mellitus [9]. Recently, a study realized in Spain (North Area) has demonstrated an apparently lower risk of obesity in *UCP3* −55C/T carriers [10].

The reasons for these discrepancies are unclear; and this is an interesting topic area of investigation, with a lack of studies in morbidly obese patients. The aim of our study was to investigate the influence of −55CT polymorphism of *UCP3* gene on fat mass and insulin resistance in morbidly obese patients.

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doi:10.1016/j.metabol.2009.09.004

2. Subjects and methods

2.1. Subjects

A population of 47 obese subjects (BMI >40 kg/m^2) was selected randomly in a prospective way. These patients were studied in a nutrition clinic unit and signed an informed consent. Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol greater than 300 mg/dL, triglycerides greater than 400 mg/dL, blood pressure greater than 140/90 mm Hg, fasting plasma glucose greater than 110 mg/dL, as well as the use of sulfonylurea, thiazolidinediones, metformin, insulin secretagogues, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blocker, angiotensin-converting enzyme inhibitors, and psychoactive medications. Patients with drinking and/or smoking habit and those with uncontrolled hypothyroidism were excluded, too.

2.2. Procedure

A nutritional evaluation was performed (weight, height, waist, waist to hip ratio [WHR], bioimpedance, indirect calorimetry, and blood pressure). Basal glucose, C-reactive protein (CRP), insulin, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride blood levels were measured. Dietary intake and exercise were recorded. Genotype of *UCP3* gene −55CT polymorphism was studied.

2.3. Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, New York, NY), whereas HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. Low-density lipoprotein cholesterol was calculated using the Friedewald formula.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Insulin was measured by enzymatic colorimetry (Insulin; WAKO Pure-Chemical Industries, Osaka, Japan), and the homeostasis model assessment of insulin sensitivity (HOMA) was calculated using these values [11]. C-reactive protein was measured by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany) with a reference range of 0 to 7 mg/dL and analytical sensitivity of 0.5 mg/dL.

Resistin was measured by enzyme-linked immunosorbent assay (ELISA) (Biovendor Laboratory, Brno, Czech Republic) with a sensitivity of 0.2 ng/mL and a reference range of 4 to 12 ng/mL. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Webster, TX) with a sensitivity of 0.05 ng/mL and a reference range of 10 to 100 ng/mL. Adiponectin was measured by ELISA (R&D systems, Minneapolis, MN) with a sensitivity of 0.246 ng/mL and a reference range of 8.65 to 21.43 ng/mL.

2.4. Genotyping of UCP3 gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International, Los Angeles, CA). The polymerase chain reaction was carried out with 250 ng of genomic DNA, 0.5 μL of each oligonucleotide primer (primer forward: 5′-GAT CTG GAA CTC ACT CAC CTC-3′ and primer reverse: 5′-CTG TTG TCT CTG CTG CTT CT-3′), and 0.25 μL of each probe (wild probe: 5′-Fam-TAT ACA CAC GGG CTG ACC TGA-Tamra-3′ and mutant probe: 5′-Hex-CTT ATA CAC ACA GGC TGA CCT GA-Tamra -3′) in a 25-μL final volume (Termociclador iCycler IQ; Bio-Rad, Hercules, CA). DNA was denaturated at 95°C for 3 minutes; this was followed by 50 cycles of denaturation at 95°C for 15 seconds and annealing at 59.3°C for 45 seconds. The polymerase chain reactions were run in a 25-μL final volume containing 12.5 μL of IQTM Supermix (Bio-Rad) with Hot Start Taq DNA polymerase.

2.5. Indirect calorimetry

For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12-hour overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20-minute period with an open-circuit indirect calorimetry system (standardized for temperature, pressure, and moisture) fitted with a face mask (MedGem; Health Tech, Golden, CO); coefficient of variation was 5%. Resting metabolic rate (in kilocalories per day) and resting metabolic rate corrected by fat-free mass (in kilocalories per kilogram per day) were calculated [12].

2.6. Anthropometric measurements

Body weight was measured to an accuracy of 0.5 kg, and BMI was computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive WHR were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition [13]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat, and fat-free mass.

Blood pressure was measured twice after a 10-minute rest with a random zero mercury sphygmomanometer and averaged.

2.7. Dietary intake and habits

Patients received prospective serial assessment of nutritional intake with 3-day written food records. All enrolled subjects received instruction to record their daily dietary intake for 3 days including a weekend day. Handling of the

dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference [14]. Regular aerobic physical activity (walking was allowed; no other exercises) was maintained during the period study (2-3 h/wk).

2.8. Statistical analysis

Sample size was calculated to detect differences over 5 mIU/L of insulin levels with 90% power and 5% significance (n = 45 patients). The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a 2-tailed, paired Student *t* test. Nonparametric variables were analyzed with the Mann-Whitney *U* test. Qualitative variables were analyzed with the χ^2 test, with Yates correction as necessary, and Fisher test. Correlation analysis was performed with Pearson and Spearman tests. The statistical analysis was performed for the combined −55CT and −55TT as a mutant group and wild-type −55CC as second group. A P value < .05 was considered statistically significant.

3. Results

Forty-seven patients gave informed consent and were enrolled in the study. The mean age was 48.2 ± 15.4 years; and the mean BMI was 44.7 ± 4.7, with 10 men (21.3%) and 37 women (78.7%). Thirty-two (6 men [18.7%]/26 women [81.3%]) (68.1%) had the genotype −55CC (wild-type group), and 15 patients (4 men [26.6%]/11 women [73.4%]) (31.9%) had −55CT (mutant-type group). The −55TT genotype was not detected in our sample.

To explore the effect of this polymorphism on anthropometric parameters, means ± SD of these variables in wild and mutant groups were calculated (Table 1). In the mutant-type group, weight, BMI, fat mass, and waist circumference were higher than those in the wild-type group, showing that patients were more obese and had a central distribution of obesity in the mutant-type group.

Table 1
Changes in anthropometric variables

Characteristics	55CC n = 32	55CT n = 15
BMI (kg/m^2)	44 ± 5	46 ± 6*
Weight (kg)	114 ± 17	123 ± 19*
Fat-free mass (kg)	56 ± 16	61 ± 16
Fat mass (kg)	56 ± 11	61 ± 15*
Waist circumference (cm)	125 ± 13	128 ± 9*
WHR	0.9 ± 0.1	0.9 ± 0.2
Systolic BP (mm Hg)	137 ± 14	139 ± 9
Diastolic BP (mm Hg)	87 ± 8	86 ± 7
RMR (kcal/d)	2348 ± 730	2320 ± 478
RMRC (kcal/d)	43 ± 15	45 ± 21

BP indicates blood pressure; RMR, resting metabolic rate; RMRC, resting metabolic rate corrected by fat-free mass.

* P <.05, between groups.

Table 2
Classic cardiovascular risk factors and circulating adipocytokines

Characteristics	55CC n = 32	55CT n = 15
Glucose (mg/dL)	105 ± 19	109 ± 30
Total cholesterol (mg/dL)	205 ± 36	202 ± 34
LDL cholesterol (mg/dL)	124 ± 32	125 ± 33
HDL cholesterol (mg/dL)	54 ± 12	50 ± 9
TG (mg/dL)	134 ± 59	137 ± 34
Insulin (mIU/L)	21 ± 11	31 ± 17*
HOMA	5.3 ± 2.7	8.7 ± 6.6*
CRP (mg/dL)	9 ± 6	10 ± 7
Adiponectin (ng/mL)	70 ± 26	31 ± 33*
Resistin (ng/mL)	4.3 ± 2.3	4.4 ± 2.1
Leptin (ng/mL)	158 ± 112	144 ± 102

* P < .05, between groups.

The effects of the polymorphism on classic cardiovascular factors such as lipid profile, insulin resistance, and CRP are shown in Table 2. Insulin and HOMA were higher in mutant-type group than wild-type group. No differences were detected in other parameters. These data show a potentially high cardiovascular risk in mutant-type group patients. Adipocytokine levels are in Table 2, too. Adiponectin levels were higher in wild-type group than mutant-type group. Leptin and resistin did not have statistical differences between genotypes. The high levels of adiponectin in wild-type patients could explain the observed effects on insulin and HOMA in this group of patients.

Finally, no statistical differences were detected in exercise and in calorie, carbohydrate, fat, and protein intakes (Table 3). This dietary intake analysis shows that anthropometric and biochemical differences between wild-type and mutant-type group patients could not have been explained by dietary intake.

4. Discussion

In mutant-type group of −55CC *UCP3* gene morbidly obese patients, insulin, HOMA, fat mass, and weight were higher than those in wild-type group. Adiponectin levels were higher in wild-type group, and this could explain metabolic differences.

Table 3
Dietary intake

Characteristics	55CC n = 32	55CT n = 15
Energy (kcal/d)	1957 ± 614	1985 ± 780
Carbohydrate (g/d)	179 ± 76	183 ± 52
Fat (g/d)	77 ± 33	79 ± 41
Protein (g/d)	85 ± 23	91 ± 21
Exercise (h/wk)	2.5 ± 5.3	2.6 ± 3.2

The ubiquitous expression of *UCP2*, the expression of *UCP3* in skeletal muscle, and their homology with *UCP1* made *UCP3* attractive targets for studies on obesity patients and its relation with cardiovascular risk factors and anthropometric parameters [15,16].

Genetic polymorphisms in *UCP* genes have been variably associated with metabolic and obesity-related phenotypes. Genetic studies on the impact of *UCP2* polymorphisms on obesity appear less convincing [17]. Dalgaard et al [18] tested whether variation of the *UCP3* promoter is associated with obesity or body weight change. In other studies, no difference in genotype frequencies was observed between obese and lean subjects in a French cohort [9], too. However, Liu et al [19] found statistically significant association and linkage between −55CT and BMI; and subjects carrying the T allele had lower BMI than those without it. Furthermore, Otabe et al [8] have demonstrated that BMI was higher in TT than CC and CT patients. In the study by Casell et al [20], the WHR was higher in female subjects carrying the *UCP3* gene −55CT polymorphism, but BMI was not different in both groups. Our group had similar prevalence of mutant-type genotype to previous studies, with differences in weight and fat mass between wild- and mutant-type groups with a similar physical activity. Other study [21] has detected an association between UCP-1 and UCP-3 polymorphisms and visceral fat distribution measured as WHR, as our study. Moreover, other study has demonstrated an apparently lower risk of obesity in *UCP3* −55C/T carriers [10]; this inverse association may only occur in people with a high level of physical activity. As we can see, it is therefore unclear that the −55C/T variant has an effect on BMI or body fat content.

The reasons for these discrepancies in the literature are unclear but may have been caused by several factors. First, criteria for recruitment were different in the various studies; thus, there were differences in confounding factors such as sex, age range considered, assessed outcomes, lifestyle, presence of diabetes mellitus, duration of time of diabetes mellitus, degree of obesity [22], type of treatments in comorbidities, and the study design. Second, ethnic heterogeneity may affect *UCP* genotype and obesity. Third, genetic background with other different genetic single nucleotide polymorphisms in the *UCP* gene [23] could influence *UCP* interaction with metabolic parameters.

In our study, HOMA and insulin were higher in mutant group (T carriers) in morbidly obese patients; this is a novel result in the literature without a clear explanation. Perhaps the presence of mutant allele of *UCP* could produce a more proinflammatory state, for instance, in skeletal muscle; and adipose tissue may modify the production of cytokines, for example, adiponectin. However, a potential bias could present in our study because neither an oral glucose tolerance test nor measurement of postprandial insulin sensitivity indices has been performed. Moreover, the result has potential interesting applications because this is a high-risk group of patients (morbidly obese patients); and this association has not been explored previously.

Adiponectin is an adipocyte-derived collagen-like protein identified through an extensive search of adipose tissue. Hypoadiponectinemia increased risk of coronary artery disease together with the presence of multiple risk factors, indicating that adiponectin is a key factor of the metabolic syndrome [24]. Decreased adiponectin concentration is associated with insulin resistance and hyperinsulinemia. Adiponectin decreases glucose production in the liver and causes decreases in glucose and free fatty acid concentrations in the blood [25]. The synthesis and secretion of adiponectin are regulated by several mechanisms; the amount of *UCP3* messenger RNA associated with −55C->T could be an unknown key to explain the relationship detected in our study among genotype, insulin resistance, and adiponectin levels. Other hypothesis could be the influence of polymorphisms and hypoadiponectinemia with insulin resistance [26].

In conclusion, in mutant-type group of −55CC *UCP3* gene patients, weight, fat mass, and insulin resistance were higher than those in wild-type group. Adiponectin levels were lower in mutant-type group, too.

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