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Synergistic effect of polymorphisms in uncoupling protein 1 and β_3-adrenergic receptor genes on basal metabolic rate in obese Finns

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Summary The polymorphisms in the uncoupling protein 1 (UCP1, A to G) and β_3-adrenergic receptor (β_3-AR, Trp64Arg) genes have been suggested to be associated with an increased tendency to gain weight. We investigated the frequency of the A to G polymorphism of the UCP1 gene and its effect on basal metabolic rate (BMR) among obese Finns. We also examined the effects of the simultaneous occurrence of the polymorphisms in the UCP1 and β_3-AR genes on BMR. Altogether 170 obese subjects (29 men, 141 women, BMI 34.7 ± 3.8 kg/m^2, age 43 ± 8 years, mean ± SD) participated in the study. The A to G substitution of the UCP1 gene was verified by digestion of the PCR product with *Bcl* I. The frequency of the A to G polymorphism of the UCP1 gene in obese subjects did not differ significantly from the population-based control subjects (5 vs 1 % for homozygotes (GG) and 35 vs 42 % for heterozygotes (AG), $p = 0.077$, for trend). BMR adjusted for lean body mass, age and sex (adjBMR) was similar among the three UCP1 gene genotypes of obese subjects (AA $n = 90$, AG $n = 72$ or GG $n = 8$). However, the subjects with the polymorphisms in both UCP1 and β_3-AR genes ($n = 18$) had a 79 kcal/day (95 % CI 30–128) lower adjBMR than the subjects without these polymorphisms ($n = 76$) (1551 ± 77 vs 1629 ± 141 kcal/day, $p = 0.002$). Furthermore, adjBMR was 63 kcal/day (95 % CI 7–118 kcal/day) lower in the subjects with both polymorphisms ($n = 18$) compared with the subjects ($n = 14$) who had only the polymorphism in the β_3-AR gene (1551 ± 77 vs 1613 ± 76 kcal/day, $p = 0.028$). The A to G polymorphism of the UCP1 gene did not have an independent effect on BMR, but its simultaneous existence with the Trp64Arg polymorphism of the β_3-AR gene resulted in more lowered BMR than the Trp64Arg polymorphism of β_3-AR gene alone. [Diabetologia (1998) 41: 357–361]

Keywords Obesity, uncoupling protein 1, β_3-adrenergic receptor, basal metabolic rate, polymorphism.

Low basal metabolic rate (BMR) is one of the risk factors for weight gain [1]. BMR is mainly determined by lean body mass, age and sex [2]. However, BMR varies considerably even among the subjects of similar weight and age, suggesting that BMR is partly genetically determined [3].

Only few genetic defects have been found to be associated with BMR. One of which is the Trp64Arg polymorphism of the β_3-adrenergic receptor gene (β_3-AR) which has been shown to be related to low BMR in both Caucasian and Japanese subjects [4–6], and possibly also in Pima Indians [7]. The β_3-AR gene is expressed both in brown and white adipose tissue [8]. In brown adipose tissue, β_3-AR is the major adrenoreceptor which stimulates uncoupling protein (UCP1) by cAMP. In rodents, UCP1 alters respiration coupling and dissipates oxidation energy as heat maintaining body temperature [9]. The dysfunction of brown adipose tissue in obese rodent models con-

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Abbreviations: UCP1, Uncoupling protein 1; β_3-AR β_3-adrenergic receptor; BMR, basal metabolic rate; cAMP, cyclic adenosine monophosphate; adjBMR, basal metabolic rate adjusted for lean body mass, age and sex; UCP2, uncoupling protein 2.

nects it to the development of obesity [10]. Although the functional role of brown adipose tissue in humans is still controversial, the presence of UCP1 mRNA also demonstrates the existence of brown adipose tissue in human adults [8, 11]. Interestingly, recent studies on humans have suggested an association between the A to G substitution of the UCP1 gene and an increased capacity to gain weight [12, 13] or resistance to low calorie diet [14]. Therefore, we investigated the frequency of the A to G polymorphism of the UCP1 gene and the effects of this polymorphism on BMR in obese subjects. In addition, we investigated the possible interaction between the polymorphisms of the UCP1 and β_3-AR genes in relation to BMR.

Subjects and methods

Subjects. All subjects participating in this study were Finnish. The Finnish population is genetically homogenous, descending mainly from a small number of founders of Baltic Finnish and German origin [15]. Screening for the previously reported A to G substitution in the 5′-flanking domain of the UCP1 gene [16] and the Trp64Arg polymorphism of the β_3-AR gene [5] was performed in 170 (29 men, 141 women), unrelated obese subjects participating in a weight reduction study [17]. The subjects were recruited from primary health care in Kuopio and Helsinki. Their mean age was 43 ± 8 years and body mass index (BMI) 34.7 ± 3.8 kg/m^2.

All subjects had normal liver, kidney and thyroid functions, and none had a history of excessive alcohol intake. None of the subjects was taking drugs known to affect BMR or glucose metabolism and, none had diabetes evaluated by fasting serum glucose or an oral glucose tolerance test [17].

The frequencies of the A to G polymorphism of the UCP1 gene and Trp64Arg polymorphism of the β_3-AR gene were compared among the study subjects with a group of 112 (53 men, 59 women) population-based non-diabetic control subjects who were recruited from 180000 inhabitants of the county of Kuopio in eastern Finland [18].

The protocol was approved by the ethics committees of the Universities of Kuopio and Helsinki, and all the subjects gave their informed consent.

Analytical methods. All the measurements were done in the morning after a 12-h fast with standardized methods. The obese subjects were advised to continue their normal diet and avoid drinking alcohol and taking vigorous exercise before the visit. Weight was measured by electric scales. BMI was calculated with the following formula: BMI = weight (kg)/height^2 (m). Waist circumference was measured at the level midway between the lateral lower rib margin and the iliac crest. Hip circumference was measured at the levels of the greater trochanters through the pubic symphysis. The level of physical activity was evaluated by an interview and classified into five categories (inactive, light, moderate, high, vigorous). Subjects were classified as physically active if they took regular exercise at least once a week. Energy intake was calculated from 4-day food records in 112 obese subjects. Information on the age of onset of obesity was obtained from 160 subjects. Oral glucose tolerance test (75 g of glucose) was performed in 76 obese subjects. Body composition was determined by bioelectrical impedance (RJL Systems Inc., Detroit, Mich., USA). BMR was measured by indirect calorimetry (Deltatrac; TM Datex, Helsinki, Finland) after a 12-h fast as previously reported in detail [19]. Gas exchange was measured for 30 min, of which the first 10 min were discarded and the mean value of the last 20 min was used in calculations. Energy production rate (cal/min) was calculated according to Ferrannini [20] as follows BMR (kcal/min) = 3.91*VO_2 (ml) + 1.10*VCO_2 (ml) – 3.34*N (ml/min) and expressed as kcal/day. The urinary nitrogen was measured for 119 subjects. For each subject, the adjusted BMR (adjBMR) [1] was calculated as follows: (the group mean BMR) + (measured BMR – the predicted BMR), where the group mean BMR is the mean absolute metabolic rate calculated according to Ferrannini (kcal/day), measured BMR is the rate (kcal/day) measured in each subject, and the predicted BMR is the calculated rate (kcal/day) obtained by using the individual lean body mass, age and sex in the linear regression equation generated from the initial examinations of 170 subjects. Serum insulin was analysed by radioimmunoassay with the double antibody-polyethyleneglycol technique (CIS Bio International, Gif-sur-Yvette, France) and serum glucose by kinetic photometry with glucose dehydrogenase [21].

Determinations of the A to G polymorphism of the UCP 1 gene and Trp64Arg polymorphism of the β_3-AR gene. DNA was prepared from peripheral blood leucocytes by the proteinase K-phenol-chloroform extraction method. The UCP1 gene was amplified by polymerase chainreaction (PCR) with the forward primer = 5′-CCAGTGGT GGCTAATGAGAGAA-3′ and reverse primer = 5′-GCAC AAAGAAGAAGCAGAGAGG-3′ (product size 279 bp). PCR amplification was conducted in a 15 µl volume containing 50 ng genomic DNA, 5 pmol of each primer, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l MgCl and 0.1 % Triton X-100, 0.25 units of DNA polymerase (DynaZyme DNA Polymerase; Finnzymes, Espoo, Finland), and 200 µmol/l dNTP. PCR conditions were denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s with final extension at 72 °C for 4 min. Amplified product (279 bp) was digested with *Bcl*I, a restriction enzyme specific for the sequence T/GATCA, at 50 °C for 1 h. The digested samples were separated on a 3 % agarose gel (NuSieve GTG; FMC Bioproducts, Rockland, Me., USA). Digestion of samples with the normal sequence (AA) yields fragments of 157 bp and 122 bp in length, whereas the A to G substitution eliminates the *Bcl*I site, yielding only a 279 bp product (GG). Samples from heterozygous (AG) subjects (A to G substitution) yield the 279 bp as well as the 157 bp and 122 bp fragments.

The Trp64Arg polymorphism of the β_3-AR gene was detected by PCR-RFLP assays as previously described [5].

Statistical analysis. All calculations were performed using the SPSS/WIN program version 6.0 (SPSS Inc., Chicago, Ill., USA 1993). Data are presented as means ± SD. Statistical significance of the differences between the groups was evaluated with the chi-square test, ANOVA or Student's *t*-test, when appropriate. Food records were analysed by Nutrica computer program based on Finnish nutrient databases (Social Insurance Institution, Helsinki, Finland).

Results

Eight (5 %) of the obese subjects were homozygous (GG) and 72 (42 %) heterozygous (AG) for A to G polymorphism of the UCP1 gene, and 90 (53 %) had

Table 1. Characteristics of obese subjects with the AA, AG and GG genotypes of the uncoupling protein 1 gene

	AA genotype $n = 90$	AG genotype $n = 72$	GG genotype $n = 8$
Men/women	12/78	15/57	2/6
Age (years)	42.1 ± 8.7	43.7 ± 6.3	48.9 ± 9.0
Weight (kg)	95.6 ± 12.6	95.1 ± 13.0	96.1 ± 15.8
BMI (kg/m^2)	34.6 ± 3.4	34.9 ± 4.1	34.9 ± 4.6
Lean body mass (kg)	59.3 ± 9.2	59.6 ± 10.7	62.6 ± 12.4
Body fat (%)	37.9 ± 5.7	37.3 ± 6.6	34.8 ± 8.1
Waist (cm)	104.3 ± 11.1	106.1 ± 10.4	106.2 ± 10.9
Waist-to-hip ratio	0.91 ± 0.08	0.94 ± 0.08	0.95 ± 0.05
adjBMR (kcal/day)	1627 ± 133	1627 ± 149	1634 ± 157
Energy intake (kcal/day)	1498 ± 477	1584 ± 535	1528 ± 440
Serum glucose (mmol/l)	5.4 ± 0.6	5.7 ± 0.9	5.7 ± 0.6
Serum insulin (pmol/l)	91.7 ± 40.5	103.4 ± 53.8	82.7 ± 28.6
Respiratory quotient	0.82 ± 0.04	0.82 ± 0.05	0.80 ± 0.04
Fasting glucose oxidation[a] (μmol · min^{-1} · kg^{-1} LBM)	8.33 ± 3.16	8.10 ± 3.89	6.77 ± 4.88
Fasting lipid oxidation[a] (mg · min^{-1} · kg^{-1} LBM)	1.04 ± 0.27	1.06 ± 0.30	1.16 ± 0.33

[a] 61 subjects with the AA genotype 45 subjects with AG genotype and 6 subjects with the GG genotype.
Mean ± SD

the genotype AA. The frequencies did not differ significantly from the control subjects participating in our previous population-based study ($n = 112$, 1 (1 %) homozygous (GG), 40 (35 %) heterozygous (AG) for the A to G polymorphism of the UCP1 gene and 71 (64 %) wild type genotype (AA), $p = 0.077$ for trend). Sex distribution, age, weight, BMI, lean body mass, percentage of body fat, waist circumference, waist-to-hip ratio, adjBMR and energy intake were similar among the three polymorphic groups of the UCP1 gene (AA, AG or GG). There was no association of the A to G polymorphism of the UCP1 gene with fasting serum concentrations of glucose or insulin, respiratory quotient and nutrient oxidation rates (Table 1). None of the subjects were homozygous for the Arg-encoding allele of the β_3-AR gene. Among the obese subjects 32 (19 %) were heterozygous (Trp64Arg) for the polymorphism of the β_3-AR gene and 138 (81 %) had the wild type (Trp64Arg) [5]. The frequencies were similar to those of the control subjects (Trp64Arg heterozygotes $n = 20$ (18 %) and Trp64Trp homozygotes $n = 92$ (82 %).

The obese subjects with the simultaneous presence of the A to G polymorphism of the UCP1 gene and Trp64Arg polymorphism of the β_3-AR gene ($n = 18$) were similar to the rest of the study subjects with respect to sex distribution, weight, BMI, lean body mass, fat mass, waist circumference, waist-to-hip ratio, energy intake, fasting serum concentrations of glucose or insulin, respiratory quotient, nutrient oxidation rates, level of physical activity and age of onset of obesity (data not shown).

The A to G polymorphism of the UCP1 gene was not significantly associated with adjBMR. As reported earlier [5], BMR adjusted for lean body mass, age and gender was 60 kcal/day (95 % CI 22–98 kcal/day) lower in obese subjects with the Trp64Arg polymorphism in the β_3-AR gene ($n = 32$) than in normal Trp64Trp homozygotes ($n = 138$) (1578 ± 81 vs 1639 ± 148, $p = 0.002$). Interestingly, the obese subjects with both polymorphisms (Trp64Arg and AG or GG genotypes) ($n = 18$, 4 men, 14 women) had a 79 kcal/day (95 % CI 30–128 kcal/day) lower adjBMR than the subjects without these polymorphisms (Trp64Trp and AA genotypes) ($n = 76$, 11 men, 65 women) (1551 ± 77 vs 1629 ± 141 kcal/day, $p = 0.002$). Even after further adjustment for fat mass the subjects with both polymorphisms had 53 kcal/day (95 % CI 8–97 kcal/day) lower BMR than the subjects without (1577 ± 71 vs 1630 ± 124 kcal/day, $p = 0.021$). Also subjects with both polymorphisms ($n = 14$) whose urinary nitrogen excretion levels were available, had lower adjBMR than those who did not have these polymorphisms ($n = 57$) (1548 ± 73 vs 1634 ± 139, $p = 0.004$, difference 86 kcal/day 95 % CI 29–143). This finding was expected since urinary nitrogen has only a minor effect on calculated BMR.

In the subgroup analysis, adjBMR was compared between the obese subjects with the polymorphism in the β_3-AR gene ($n = 14$) and the subjects with polymorphisms in both β_3-AR and UCP1 genes ($n = 18$). AdjBMR was 63 kcal/day (95 % CI 7–118 kcal/day) lower in the subjects with both polymorphisms compared with the subjects who had only the polymor-

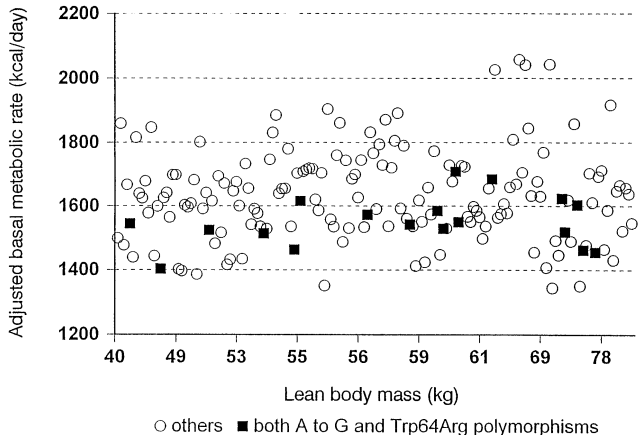

Fig. 1. BMR adjusted for lean body mass, age and sex in relation to lean body mass in obese subjects with ■ or without ○ the polymorphisms in both β_3-AR and UCP1 genes

phism in the β_3-AR gene (1551 ± 77 vs 1613 ± 76 kcal/day, $p = 0.028$).

When all obese subjects were classified as having "low" or "high" BMR according to the median of the adjBMR (1616 kcal/day), 16 out of 18 (88.9 %) obese subjects with both polymorphisms had BMR less than the median value whereas only 69 out of 152 (45.4 %) obese subjects with one or without any of these two polymorphisms had BMR less than the median value ($p < 0.001$, Fig. 1).

Discussion

The novel finding in the present study was that BMR, when adjusted for lean body mass, age and sex was on the average 80 kcal/day lower in the obese subjects with the polymorphisms in both the UCP1 (AG or GG) and the β_3-AR (Trp64Arg) genes than in the obese subjects without these polymorphisms. We have previously reported that adjBMR was a 60 kcal/day lower in the subjects with the Trp64Arg genotype than in the subjects with Trp64Trp genotype [5]. These studies confirm that gene polymorphisms may affect BMR and the present study could be an example of a synergistic effect of the polymorphisms in the UCP1 and the β_3-AR genes on lower BMR.

Both of the polymorphisms in the UCP1 and β_3-AR genes are fairly common and seem to occur at similar frequencies among the obese subjects and population-based control subjects. The frequencies were also similar to those reported in previous studies [7, 12–14, 22, 23]. This indicates that the β_3-AR and UCP1 genes are not likely to be the major genes affecting BMR. However, the polymorphisms in these two genes may increase the susceptibility to a lower BMR.

Although we could not demonstrate any independent effect of the A to G polymorphism of the UCP1 gene on BMR, the present study gives an example of two susceptibility genes and their synergistic effect on BMR. The UCP1 is a specific inner mitochondrial component of brown adipose tissue [9, 24]. In human adults, brown adipose tissue is supposed to be responsible only for 1–2 % of the energy expenditure [25], and therefore, the effect of the A to G polymorphism of the UCP1 gene on BMR is likely to be overcome by other genetic and environmental factors which also regulate BMR.

In addition to the regulation of lipolysis in white adipose tissue, β_3-adrenergic receptors activate UCP1 in brown adipose tissue. Subjects with mutations in the genes encoding these two proteins may have defects in thermogenesis, thus leading to lowered BMR. The variation in BMR is related to variability in body temperature [26] suggesting that the mechanisms controlling heat production may also affect BMR. The function of the UCP1 and β_3-adrenergic receptors may have changed due to the polymorphisms in the genes encoding these proteins. Although a recent study suggested that the Trp to Arg substitution in codon 64 had no measurable effect on the function of β_3-adrenergic receptors [27], modest functional defects may still exist. To our knowledge, no studies are available on the effects of the A to G substitution on the function of the UCP1 gene. Recently, a novel uncoupling protein, UCP2 was discovered [28]. UCP2 is widely expressed in adult human tissues, and thus may have a greater role in energy balance than UCP1.

There are no data on what happens with body weight in the subjects with polymorphisms in both β_3-AR and UCP1 genes in the long term but, despite their lower BMR, the subjects with these polymorphisms were not more obese than the other subjects in our study. The follow-up data on body weight changes would be of interest since a prospective study on Pima Indians indicated that subjects who gained more than 10 kg over a 4-year period had only 4 % lower BMR than other subjects [1]. Furthermore, in the study of Oppert et al. [12] the rare 8.3 kilobase allele of the UCP1 gene (GG genotype) was more frequent in the high gainers for percent body fat, but there was no significant difference in BMR among the subjects. In our study, the age of onset of obesity, reported level of physical activity and energy intake were similar in the groups. Longitudinal studies are needed to demonstrate whether decreased BMR in carriers of both polymorphisms will lead to greater weight gain as has been previously reported [13].

In conclusion, the obese subjects with both the A to G polymorphisms of the UCP1 gene and the Trp64Arg polymorphism of the β_3-AR gene have on the average 80 kcal/day lower basal metabolic rate than the subjects without these polymorphisms. Prospective studies are needed to evaluate the importance of this finding on weight gain.

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