# Polymorphism of the $\beta_3$-Adrenergic Receptor Gene Affects Basal Metabolic Rate in Obese Finns

**Raisa Sipiläinen, Matti Uusitupa, Sami Heikkinen, Aila Rissanen, and Markku Laakso**

**Low basal metabolic rate (BMR) is a risk factor for weight gain and obesity. The polymorphism at codon 64 of the $\beta_3$-adrenergic receptor gene has been suggested to be associated with BMR. We investigated the frequency of the Trp64Arg of the $\beta_3$-adrenergic receptor gene and the effects of this polymorphism on BMR in obese Finns. Altogether, 170 obese subjects (29 men, 141 women, BMI 34.7 ± 3.8 kg/m$^2$, mean ± SD) participated in the study. The frequency of the Trp64Arg polymorphism was 19%. None of the obese subjects were homozygous for the Arg-encoding allele. The frequency of the Trp64Arg polymorphism in obese Finns did not differ from nonobese and normoglycemic control subjects. BMR adjusted for lean body mass and age was lower in subjects with the Trp64Arg polymorphism ($n$ = 20) than in normal homozygotes Trp64Trp ($n$ = 99) (1,569 ± 73 vs. 1,635 ± 142 kcal/day, $P$ = 0.004). For the female group ($n$ = 98), the respective values were 1,501 ± 66 kcal/day vs. 1,568 ± 127 kcal/day ($P$ = 0.004). There were no significant differences in weight, BMI, waist-to-hip ratio, lean body mass, percentage of fat, and respiratory quotient between the groups with or without the Trp64Arg polymorphism. Neither serum glucose nor insulin levels differed between the two groups. We conclude that the Trp64Arg polymorphism of the $\beta_3$-adrenergic receptor gene affects basal metabolic rate in obese Finns but does not have significant effect on glucose metabolism. *Diabetes* 46:77–80, 1997**

Development of human obesity is determined by genetic and environmental factors. Several genes, loci, or chromosomal regions may be important in the pathogenesis of obesity (1). Of the genes that regulate basal metabolic rate (BMR), the $\beta_3$-adrenergic receptor gene has gained considerable attention recently. Defects in this gene may lead to insulin resistance and obesity. In a study on Pima Indians, the missense mutation in the codon 64 of the $\beta_3$-adrenergic receptor gene, Trp64Arg, was associated with an early onset of NIDDM and a tendency to have low BMR (2). According to another study, nondiabetic subjects with the Trp64Arg polymorphism had clinical features of insulin resistance syndrome, abdominal obesity, and hypertension (3). Furthermore, subjects with this polymorphism have been reported to have increased body mass index or capacity to gain weight (4,5). The effect of the codon 64 polymorphism of the $\beta_3$-adrenergic receptor gene on BMR has not been studied in obese normoglycemic Caucasian subjects. Therefore, we investigated the frequency of the Trp64Arg of the $\beta_3$-adrenergic receptor gene and the effects of this polymorphism on energy metabolism in obese Finns.

## RESEARCH DESIGN AND METHODS

All subjects participating in this study were Finnish. The Finnish population is genetically quite homogeneous, descending mainly from a small number of founders of Baltic Finnish and German origin (6).

**Subjects.** Screening for the previously reported amino acid substitution in exon 1 of the $\beta_3$-adrenergic receptor gene (3) was performed in 170 (29 men, 141 women) obese subjects participating in a weight reduction study (7). The subjects were recruited from primary health care in Kuopio and Helsinki. Their mean age was 43 ± 8 years and BMI 34.7 ± 3.8 kg/m$^2$. Of the women, 16.3% were postmenopausal.

All subjects had normal liver, kidney, and thyroid function tests, and none had a history of excessive alcohol intake. None of the subjects were taking drugs known to affect BMR or glucose metabolism and none had diabetes, evaluated by fasting serum glucose or an OGTT.

The frequency of the Trp64Arg polymorphism was compared with 82 healthy normoglycemic subjects who were the participants of our previous study aiming to investigate the relationship between insulin resistance and dyslipidemia (8). None of the control subjects had any chronic disease, any drug treatment, glucose intolerance, or hypertension.

The protocol was approved by the Ethics Committees of the Universities of Kuopio and Helsinki, and all the subjects gave informed consent.

**Analytical methods.** All the measurements were done in the morning after a 12-h fast with standardized methods. Otherwise, the obese subjects had their normal diet before the visit. It was emphasized that alcohol intake and vigorous exercise should have been avoided within the 48 h before the visit. Weight was measured by electric scales. BMI was calculated from the following formula: BMI = weight (kg)/height (m)$^2$. Waist circumference was measured at the level of midway between the lateral lower rib margin and the iliac crest. Hip circumference was measured at the levels of major trochanters through the pubic symphysis. The level of physical activity was evaluated by an interview before body composition and basal metabolic rate measurements in the beginning of the weight reduction study. The level of physical activity was asked according to the five-category list (inactive, light, moderate, high, and vigorous) in which the categories were described. We classified the subjects to inactive or active according to regular exercise at least once a week. Energy intake was calculated from the 4-day food records in 112 obese subjects. The age of onset of obesity was available 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, Detroit, MI). BMR rate was measured by indirect calorimetry (Deltatrac, TM Datex, Helsinki, Finland) after a 12-h fast as previously reported in detail (9). 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 (calories per min) was calculated according to Ferrannini (10) and expressed as kilocalories per day. The urinary nitrogen was measured from 119 subjects. For each subject, the adjusted BMR (11) was calculated as (the group mean BMR) + (measured BMR – the predicted BMR), where the group

From the Departments of Clinical Nutrition (R.S., M.U.) and Medicine (S.H., M.L.), University of Kuopio, Kuopio; and the Eating Disorder Unit (A.R.), University Hospital of Helsinki, Helsinki, Finland.

Address correspondence and reprint requests to Dr. Raisa Sipiläinen, Department of Clinical Nutrition, University of Kuopio, 70211 Kuopio, Finland. E-mail: raisa.sipilainen@uku.fi.

Received for publication 13 May 1996 and accepted in revised form 1 August 1996.

BMR, basal metabolic rate; LBM, lean body mass; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction.

TABLE 1
Characteristics of the obese subjects with Trp64Trp or Trp64Arg genotypes of the $\beta_3$-adrenergic receptor gene

| | Trp64Trp | Trp64Arg |
|---|---|---|
| Sex (M/F) | 23/115 | 6/26 |
| Age (years) | 43.6 ± 6.8 | 41.1 ± 8.0 |
| Weight (kg) | 95.4 ± 13.5 | 95.2 ± 9.8 |
| BMI (kg/m$^2$) | 34.8 ± 3.9 | 34.4 ± 3.3 |
| Lean body mass (kg) | 59.3 ± 10.2 | 60.8 ± 8.7 |
| Body fat (%) | 37.8 ± 6.0 | 36.0 ± 7.1 |
| Waist (cm) | 105.2 ± 11.2 | 104.9 ± 9.2 |
| Waist-to-hip ratio | 0.92 ± 0.08 | 0.93 ± 0.07 |
| Energy intake (kcal/day) | 1,532 ± 507 | 1,543 ± 452 |
| Hypertensives (%) | 25 | 28 |
| Impaired glucose tolerance (%) | 14 | 8 |
| Physically active (%) | 35 | 43 |
| Age of onset of obesity, ≤18 years (%) | 33 | 29 |

Data are means ± SD. Data for energy intake represent 4-day food records; 93 subjects with the Trp64Trp genotype and 19 subjects with the Trp64Arg genotype. Data under hypertensives are for subjects with systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥95 mmHg or who use antihypertensive drugs.

mean BMR is the mean calculated according to Ferrannini (kcal/d), BMR is the rate of kilocalories per day in each subject, and the predicted BMR is the calculated rate (kcal/d) obtained by using the individual lean body mass and age in the linear regression equation generated from the initial examinations of 119 subjects. Serum insulin was analyzed by radioimmunoassay with the double antibody-PEG technique (CIS bio international, B.P. 32, F-911 92 Gif-sur-Yvette Cedex, France) and serum glucose by kinetic photometry with glucose-dehydrogenase (12).

**Determination of the Trp64Arg polymorphism in exon 1 of the $\beta_3$-adrenergic receptor gene.** DNA was prepared from peripheral blood leukocytes by proteinaseK-phenol-chloroform extraction method. Exon 1 was amplified with polymerase chain reaction (PCR) with the primers BSTNUP = 5′-CGCCCAATACCGCCAACAC-3′ and BSTNDOWN = 5′-CCACCAGGAGTCCCATCACC-3′ (product size 210 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, 0.1% Triton X-100, 0.25 U of DNA polymerase (DynaZyme DNA Polymerase, Finnzymes, 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 63°C for 30 s, and extension at 72°C for 30 s with final extension at 72°C for 4 min. The single base replacement of T by C in codon 64 in exon 1 predicted an amino acid change from tryptophan (TGG) to arginine (CGG) (3). Therefore, amplified exon 1 fragments were digested with *Bst*NI, a restriction enzyme specific for the sequence CC(A/T)GG, in a 15-μl volume containing 5 μl of PCR product. The mixture was incubated at 60°C for 1 h. The digested samples were separated on a 3% agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, ME). Digestion of the normal sequence yields fragments of 97, 61, 31, 15, and 6 bp in length, whereas the Trp64Arg mutation eliminates one of the *Bst*NI sites, yielding a novel 158-bp product.

**Statistical analysis.** All calculations were performed using the SPSS/WIN program version 6.0 (SPSS, Chicago, IL). Data are presented as means ± SD. Statistical significance of the differences between groups was evaluated using the $\chi^2$ test or Student's $t$ test, when appropriate. Food records were analyzed by a Nutrica computer program based on Finnish nutrient databases (Social Insurance Institution, Helsinki, Finland).

## RESULTS

The frequency of the Trp64Arg polymorphism in obese Finns was 19% (21% for men, 18% for women). None of the obese subjects were homozygous for the Arg-encoding allele. The frequencies did not differ from nonobese and normoglycemic control subjects participating in our previous population-based study ($n$ = 82, the frequency of Trp64Arg polymorphism 13%, NS). As shown in Table 1 the subjects with the Trp64Arg polymorphism tended to be younger than subjects without it (Trp64Trp) ($P$ = 0.084). Sex distribution, weight, BMI, lean body mass, percentage of body fat, waist circumference, and waist-to-hip ratio did not differ between the two groups. There were no differences in the percentage of subjects with hypertension or impaired glucose tolerance, the onset of obesity, the level of physical activity, or energy intake between the two groups.

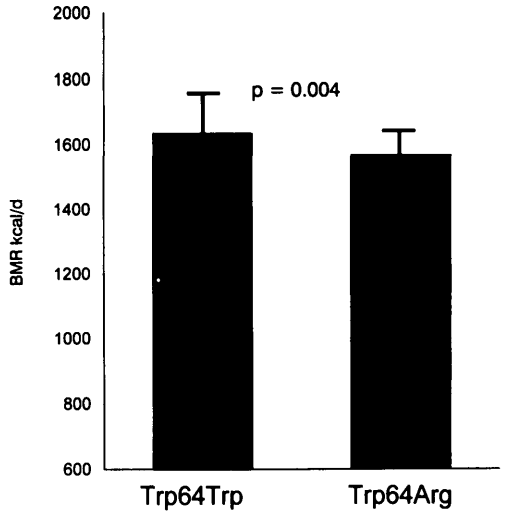


**FIG. 1. BMR adjusted for lean body mass and age in obese subjects with the Trp64Trp or Trp64Arg genotypes of the $\beta_3$-adrenergic receptor gene.**

BMR adjusted for lean body mass and age, determined among those whose urinary nitrogen excretion was available ($n$ =119), was lower in the subjects who had the Trp64Arg polymorphism than in those who did not (Trp64Trp) (1,569 ± 73 vs. 1,635 ± 142 kcal/day, $P$ = 0.004, Fig. 1). Subjects with the Trp64Arg polymorphism ($n$ = 20) expended an average of 66 kcal/day (95% CI 23–110) less than the normal homozygotes ($n$ = 99). When women were analyzed separately, those with the Trp64Arg polymorphism ($n$ = 16) had significantly lower BMR adjusted for age and lean body mass than those without it (Trp64Trp, $n$ = 82) (1,501 ± 66 vs. 1,568 ± 127 kcal/day, $P$ = 0.004), regardless of menopausal status (data not shown). The difference between these two groups was 67 kcal/day (95% CI 23–111). Furthermore, when BMR was related to lean body mass in the entire group, the ratio was lower in those with the Trp64Arg polymorphism than in the subjects without it (Fig. 2).

There was no significant association of the Trp64Arg polymorphism with fasting serum concentrations of glucose or insulin. The respiratory quotient and nutrient oxidation rates did not differ between the groups (Table 2).

## DISCUSSION

Our main finding was that BMR adjusted for lean body mass and age was on the average 66 kcal/day lower in obese subjects with the Trp64Arg genotype of the $\beta_3$-adrenergic receptor gene than in obese subjects with the Trp64Trp genotype. Furthermore, when the analysis was restricted to females, the statistically significant difference remained between the subjects with or without the Trp64Arg polymorphism. To our knowledge, this has not been reported previously in Caucasian subjects. However, the frequency of the Trp64Arg

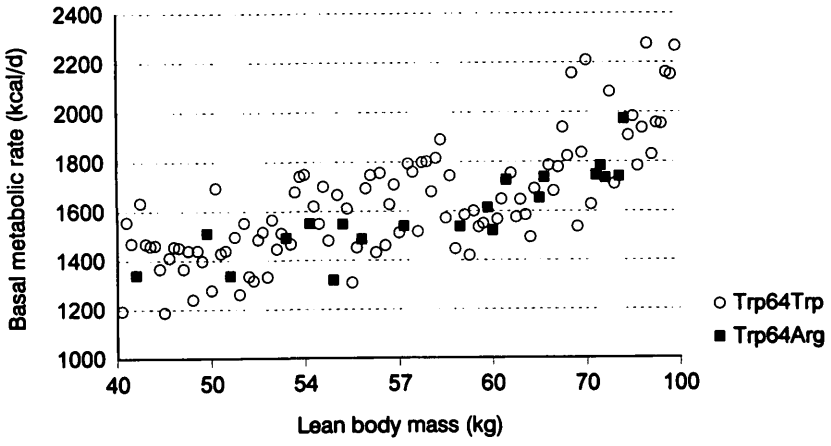


**FIG. 2. BMR in relation to lean body mass in obese subjects with the Trp64Trp ($n$ = 99) or Trp64Arg ($n$ = 20) genotypes of the $\beta_3$-adrenergic receptor gene. (27.9 ± 2.9 vs. 26.5 ± 1.9 kcal · day$^{-1}$ · kg$^{-1}$, $P$ = 0.012, difference 1.4 kcal · day$^{-1}$ · kg$^{-1}$, 95% CI 0.3–2.4)**

polymorphism was not appreciably higher in obese subjects compared with that in control subjects.

BMR accounts for 60–80% of daily energy expenditure (13). The decrease in BMR may be caused by genetic factors, for example, functional differences in the protein of Trp64Arg carriers of the $\beta_3$-adrenergic receptor gene, although the evidence that $\beta_3$-adrenergic receptors regulate BMR is limited. However, $\beta_3$-adrenergic receptors mediate the stimulation of lipolysis by catecholamines in adipose tissue (14), and therefore, low $\beta_3$-adrenergic receptor activity could promote obesity by decreasing lipolysis, which leads to the retention of lipids in fat cells. Furthermore, decreased thermogenesis in brown adipose tissue may contribute to a decrease in BMR (15). On the other hand, a recent study (16) suggested that the Trp-to-Arg substitution in codon 64 has no measurable effect on the function of the $\beta_3$-adrenergic receptor. Nonetheless, there still may be some minor functional abnormalities or interactions with other genes not known yet.

Variation in BMR is largely explained by lean body mass, age, and sex (17). In our study, there were no differences in lean body mass and percentage of body fat between the two groups. Even after BMR was adjusted for lean body mass, fat mass, and age, the subjects with the Trp64Arg polymorphism had 3% lower BMR than subjects without it (difference, 51 kcal/day; 95% CI 11–91, $P$ = 0.014). The majority of our study subjects were women. The mean difference in BMR between the two groups was 55 kcal/day, (95% CI 15–95, $P$ = 0.008). Menstrual cycle may also influence BMR, but there is no evidence that differences in the menstrual cycle could explain our results. Furthermore, 16.3% of the women were postmenopausal, and the difference in BMR between the mutated and nonmutated subjects was seen among them as well.

The reduction of BMR by 51–66 kcal/day can result in ~2.5–3.5 kg of excess weight gain per year. In agreement with this notion is a prospective study in Pima Indians, which indicated that subjects who gained >10 kg over a 4-year period had a 4% lower BMR than other subjects (17). Despite their lower BMR, the subjects with the Trp64Arg polymorphism were not more obese than the normal homozygotes (Trp64Trp). Similar results have been previously reported on obese Pima Indians with NIDDM (3). In our study, reported level of physical activity and energy intake did not differ between the groups. Longitudinal studies are needed to demonstrate whether decreased BMR in carriers of the Trp64Arg polymorphism will lead to weight gain.

The allelic frequencies of the Trp64Arg polymorphism did not differ between the obese and nonobese control subjects. This confirms the findings from a previous study where this polymorphism was not more frequent among obese subjects than in normal weight subjects (5). These findings imply that the $\beta_3$-adrenergic receptor gene is not likely to be a major determinant of obesity or BMR, but the variation in this gene may contribute to weight gain together with other genetic, environmental, and behavioral factors.

In our study, no differences were found in fasting insulin and glucose levels between the subjects with the Trp64Arg and Trp64Trp genotypes. Moreover, waist-to-hip ratio and the proportion of the subjects with impaired glucose tolerance and hypertension did not differ between the two groups. These results do not support the findings of a previous study (4), which demonstrated that the Trp64Arg polymorphism is associated with clinical features of insulin resistance syndrome. However, in our study, an OGTT was performed in only 76 (Trp64Trp, $n$ = 64; Trp64Arg, $n$ = 12) of the 170 obese subjects examined, which might weaken the conclusions made. Nonetheless, there was no preselection as to who took part in the OGTT, and therefore these results can still be valid.

We conclude that the Trp64Arg polymorphism of the $\beta_3$-adrenergic receptor gene affects basal metabolic rate in obese Finns. Prospective studies are needed to evaluate the importance of this finding on weight gain and the development of insulin resistance and diabetes.

TABLE 2
Metabolic characteristics of the obese subjects with Trp64Trp or Trp64Arg genotypes of the $\beta_3$-adrenergic receptor gene

| | Trp64Trp | Trp64Arg |
|---|---|---|
| Male/female | 23/115 | 6/26 |
| Serum glucose (mmol/l) | 5.5 ± 0.8 | 5.4 ± 0.5 |
| Serum insulin (pmol/l) | 98.0 ± 46.5 | 89.9 ± 46.2 |
| Respiratory quotient | 0.82 ± 0.04 | 0.83 ± 0.05 |
| Fasting glucose oxidation (μmol · min$^{-1}$ · kg$^{-1}$ LBM) | 8.16 ± 3.6 | 8.23 ± 3.32 |
| Fasting lipid oxidation (mg · min$^{-1}$ · kg$^{-1}$ LBM) | 1.07 ± 0.28 | 0.98 ± 0.29 |

Data are means ± SD. For fasting glucose oxidation and fasting lipid oxidation, the number of subjects with Trp64Trp genotype was 94 and Trp64Arg genotype was 19. LBM, lean body mass.

## ACKNOWLEDGMENTS

This work was supported by grants from the Research Council for Health, Academy of Finland, and by Hoffmann La Roche, Basel, Switzerland.

## REFERENCES

1. Bouchard C: Genetics of obesity: an update on molecular markers. *Int J Obes* 19 (Suppl. 3):S10–S13, 1995
2. Walston J, Silver K, Bogardus C, Knowler W, Celi F, Austin S, Manning B, Strosberg D, Stern M, Raben N, Sorkin J, Roth J, Shuldiner A: Time of onset of non-insulin dependent diabetes mellitus and genetic variation in the $\beta_3$-adrenergic receptor gene. *N Engl J Med* 333:343–347, 1995
3. Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC: Association of a polymorphism in the $\beta_3$-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 333:348–351, 1995
4. Fujisawa T, Ikegami H, Yamato E, Takekawa K, Nakagawa Y, Hamada Y, Oga T, Ueda H, Shintani M, Fukuda M, Ogihara T: Association of Trp64Arg mutation of the $\beta_3$-adrenergic-receptor with NIDDM and body weight gain. *Diabetologia* 39:349–352, 1996
5. Clement K, Vaisse C, Manning B, Basdevant A, Guy-Grand B, Ruiz J, Silver K, Shuldiner A, Froguel P, Strosberg D: Genetic variation in the $\beta_3$-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 333:352–354, 1995
6. De la Chapelle A: Disease gene mapping in isolated human population: the example of Finland. *J Med Genet* 30:857–865, 1993
7. Uusitupa M, Karhunen L, Franssila-Kallunki A, Rissanen A, Niskanen L, Kervinen K, Kesäniemi YA: Apolipoprotein E phenotype modifies metabolic and hemodynamic abnormalities to central obesity in women. *Am J Clin Nutr* 64:131–136, 1996
8. Haffner SM, Karhapää P, Mykkänen L, Laakso M: Insulin resistance, body fat distribution and sex hormones in men. *Diabetes* 43:212–219, 1994
9. Laakso M, Uusitupa M, Takala J, Majander H, Reijonen T, Penttilä I: Effects of hypocaloric diet and insulin therapy on metabolic control and mechanisms of hyperglycemia in obese non-insulin-dependent diabetic subjects. *Metabolism* 37:1092–1100, 1988
10. Ferrannini E: The theoretical bases of indirect calorimetry: a review. *Metabolism* 37:287–301, 1988
11. Ravussin E, Bogardus C: Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 49:968–975, 1989
12. Rindfrey H, Helger R, Lang H: Kinetic determination of glucose concentrations with glucose hydrogenase. *J Clin Chem Clin Biochem* 15:217–220, 1977
13. Ravussin E, Lillioja S, Andersson TE, Christian L, Bogardus C: Determinants of 24-hour energy expenditure in man: methods and results using a respiratory champer. *J Clin Invest* 78:1568–1578, 1986
14. Giacobino J-P: $Beta_3$-adrenoreceptor: an update. *J Clin Endocrinol* 132:377–385, 1995
15. Emorine LJ, Marullo S, Briend-Sutren M, Patey G, Delavier-Klutchko C, Stroberg A: Molecular characteristics of the human $\beta_3$-adrenergic receptor. *Science* 245:1118–1121, 1989
16. Candelore MR, Deng L, Tota L, Kelly L, Cascieri M, Strader C: Pharmacological characterization of a recently described human beta3-adrenergic receptor mutant. *Endocrinology* 137:2638–2641, 1996
17. Ravussin E, Lillioja S, Knowler W, Christin L, Freymond D, Abbott W, Boyce V, Howard B, Bogardus C: Reduced rate of energy expenditure as a risk factor for body-weight gain. *N Engl J Med* 318:467–472, 1988