Relation of β_3-Adrenergic Receptor Gene Mutation to Total Body Fat But Not Percent Body Fat and Insulin Levels in Thais

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A Trp64Arg mutation in the β_3-adrenergic receptor gene has been implicated in the pathophysiology of non–insulin-dependent diabetes mellitus and obesity. However, the findings have been controversial due to the use of different populations and different methods for the estimation of body fat. In the present study, the prevalence of Trp64Arg mutation of the β_3-adrenergic receptor gene was determined and its relation to body fat as assessed by dual-energy x-ray absorptiometry (DEXA) was evaluated in Thai men and women. The effect on insulin sensitivity as assessed by the serum insulin to glucose ratio was also examined. The subjects were 76 men and 135 women aged 20 to 80 years. Body fat and its regional distribution were assessed by DEXA. Mutation in the β_3-adrenergic receptor gene was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism. Data are expressed as the mean ± SEM. Fifty-nine subjects (28.0%) had the Trp64Arg mutation in the β_3-adrenergic receptor gene; 54 (25.6%) were heterozygotes and five (2.4%) were homozygotes. The gene frequency of Trp64Arg mutation was 15.2% in these subjects. In women, Trp64Arg mutation was not associated with the difference in total body fat (Trp/Arg or Arg/Arg, 19.4 ± 1.0 kg; Trp/Trp, 19.2 ± 0.6 kg) or percent body fat (Trp/Arg or Arg/Arg, 34.6% ± 1.2%; Trp/Trp, 34.3% ± 0.6%). In contrast to the findings in women, men with Trp64Arg mutation had lower total body fat after controlling for age (Trp/Arg or Arg/Arg, 13.2 ± 1.1 kg; Trp/Trp, 15.8 ± 0.7 kg; $P < .05$). However, no difference was found in percent body fat (Trp/Arg or Arg/Arg, 20.9% ± 1.3%; Trp/Trp, 23.3% ± 0.7%). No difference in the fasting insulin resistance index (FIRI) was found between subjects with and without Trp64Arg mutation. The data suggest that Trp64Arg mutation of the β_3-adrenergic receptor is common in Thais and appears to exert effects on total body fat but not percent body fat in men. Trp64Arg mutation is not associated with insulin resistance as assessed by the FIRI in Thais.

BODY FAT and its regional distribution are genetically determined.[1] The candidate genes for fatness include the recently found β_3-adrenergic receptor gene. β_3-Adrenergic receptors mediate lipolysis in white adipose tissue[2] and thermogenesis in brown adipose tissue. Moreover, the β_3-adrenergic receptor may modulate insulin sensitivity and the associated metabolic syndrome.[3] Recently, a common mutation (Trp64Arg) in the β_3-adrenergic receptor gene was described, and the mutant allele has been associated with obesity, insulin resistance, and non–insulin-dependent diabetes mellitus in a number of studies.[4-6] The effects of Trp64Arg mutation in other ethnically different populations remain to be determined. Concerning adiposity, most of the reported studies used the body mass index (BMI) as an index of fatness, and it is unclear whether more sensitive and direct methods to determine body fat will yield similar results. Moreover, males and females differ in terms of body fat, its regional distribution, and the association of fatness to the components of the metabolic syndrome.[7,8] It is unclear how gender may affect the influence of the β_3-adrenergic system on body composition and insulin sensitivity.

Therefore, the purposes of the present study were (1) to estimate the prevalence of Trp64Arg mutation of the β_3-adrenergic receptor gene in Thais, (2) to determine the effect of this mutation on body fat and insulin resistance, and (3) to examine whether there are sexual differences in the effects of Trp64Arg mutation.

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SUBJECTS AND METHODS

Subjects

Subjects residing in the Bangkok metropolitan area were recruited by leaflet or direct contact. Individuals with diabetes mellitus were excluded. Seventy-six men and 135 women participated in the study. None of the subjects were related as determined by examination of their family names, and none smoked more than 10 cigarettes per day or drank more than two glasses of beer or its equivalent more often than once per week. A medical history was obtained and a complete physical examination was performed to assess the health status. All subjects were considered healthy, with normal fasting plasma glucose (<140 mg/dL) and liver function tests. None were taking medication that may affect body composition.

All subjects provided informed consent, and the study was approved by the ethical clearance committee on human rights related to research involving human subjects of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Body Composition Assessment

Body composition was assessed by dual-energy x-ray absorptiometry (DEXA). Briefly, the proportion of fat mass to fat-free mass was determined by attenuation of the x-ray energy at measured areas containing only soft tissue (Rst) using a standard curve relating Rst to the ratio of fat mass to fat-free mass. The fat mass was then determined by the product of this ratio with the soft tissue mass. The in vivo coefficient of variation for fat mass was 2.4%.

Laboratory Assays

Fasting blood samples were obtained from subjects between 8:00 and 10:00 AM. Serum samples were frozen at −20°C until measurement. The plasma glucose level was measured by the glucose oxidase method. Serum insulin was determined by radioimmunoassay (CIS Bio International, Paris, France).

Table 1. Age and Anthropometric Characteristics of the Subjects

Characteristic	Men (n = 76)	Women (n = 135)	P
Age (yr)	52.5 ± 1.9	51.0 ± 1.2	NS
Body weight (kg)	65.3 ± 1.3	55.0 ± 0.7	<.001
Height (cm)	164.3 ± 0.7	154.5 ± 0.4	<.001
Waist circumference (cm)	83.4 ± 1.1	76.9 ± 0.8	< 001
Hip circumference (cm)	95.3 ± 0.7	93.7 ± 0.5	NS
Waist to hip ratio	0.87 ± 0.01	0.82 ± 0.01	<.001
BMI	24.1 ± 0.4	23.0 ± 0.3	<.05

Abbreviation: NS, nonsignificant.

β_3-Adrenergic Receptor Genotyping

Genomic DNA was extracted from peripheral leukocytes by phenol/chloroform extraction. DNA sequence flanking codon 64 in exon 1 of the β_3-adrenergic receptor gene was amplified by polymerase chain reaction (PCR) with the following primers: forward, 5′CCAGTGGGC-TGCCAGGGG3′; and reverse, 5′GCCAGTGGCGCCCAACGG3′.[6] The final reaction contained 0.5 μg DNA, 1 U Taq polymerase, 10 mmol/L Tris hydrochloride, pH 8.3, 50 mmol/L KCl, 1 mmol/L $MgCl_2$. The reaction was performed for 35 cycles with denaturation at 94°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 30 seconds. The final 248–base pair PCR product was then digested with *Bst*NI restriction nuclease. In the absence of the Trp64Arg mutation, a restriction site will be created. The digested material was resolved on 1.4% agarose gel with ethidium bromide staining.

Statistical Analyses

The fasting insulin resistance index (FIRI) was calculated from the formula, FIRI = [fasting plasma glucose (mmol/L) × fasting insulin (mU/L)]/25.[9] The data are expressed as the mean ± SEM. Differences between groups were assessed by Student's *t* test or one-way ANOVA as appropriate. Analysis of covariance was used to control for the effect of age on body fat.

RESULTS

The age and anthropometric characteristics of the subjects are shown in Table 1. Fifty-nine subjects (28.0%) had Trp64Arg mutation in the β_3-adrenergic receptor gene; 54 (25.6%) were heterozygotes and five (2.4%) were homozygotes. The gene frequency of Trp64Arg mutation in our subjects was 15.2% and the distribution of genotypes comformed to Hardy-Weinberg equilibrium, as there was no difference in the distribution from a binomial distribution with a frequency of one allele equal to the Trp64Arg frequency. There were positive correlations between increasing age and total body fat and percent body fat in both women (total body fat, $r = .42$, $P < .0001$; percent body fat, $r = .49$, $P < .0001$) and men (total body fat, $r = .26$, $P < .05$; percent body fat, $r = .43$, $P < .0001$). The FIRI was positively related to total body fat ($r = .34$, $P < .0001$) and percent body fat ($r = .27$, $P < .01$) in women. Likewise, there were also positive associations for total body fat ($r = .25$, $P < .05$) and percent body fat ($r = .23$, $P < .05$) with the FIRI in men.

Table 2. Genotype Distribution of Trp64Arg Mutation of the β_3-Adrenergic Receptor Gene in Thai Men and Women

Group	Trp/Trp	Trp/Arg	Arg/Arg
Men	52	22	2
Women	100	32	3
Total	152 (72.0%)	54 (25.6%)	5 (2.4%)

Table 3. Effect of Trp64Arg Mutation of the β_3-Adrenergic Receptor Gene in the Women

Parameter	Genotype: Trp/Trp	Genotype: Trp/Arg or Arg/Arg	P
Age (yr)	50.9 ± 1.4	51.4 ± 2.3	NS
Body weight (kg)	55.0 ± 0.8	54.9 ± 1.4	NS
Height (cm)	154.8 ± 0.5	154.0 ± 0.9	NS
Waist circumference (cm)	76.4 ± 1.0	77.3 ± 1.7	NS
Hip circumference (cm)	94.0 ± 0.6	92.8 ± 1.3	NS
Waist to hip ratio	0.81 ± 0.01	0.84 ± 0.01	NS
Total body fat (kg)	19.2 ± 0.6	19.4 ± 1.0	NS
Percent body fat (%)	34.3 ± 0.6	34.6 ± 1.2	NS
FIRI	5.1 ± 0.3	5.0 ± 0.4	NS

NOTE. There were no differences in body fat, anthropometric variables, and FIRI between women with and without Trp64Arg mutation.

In women, Trp64Arg mutation was not associated with a difference in body weight, total body fat, or percent body fat. Also, no difference in the waist circumference, hip circumference, waist to hip ratio, or FIRI was found (Table 3).

In contrast to the findings in women, men with Trp64Arg mutation had lower total body fat (Table 4). The effect persisted after controlling for age ($P < .05$). However, no difference in percent body fat was found. Although hip circumference was lower in men with a Trp64Arg nucleotide change, the waist to hip ratio was not significantly different. No difference in the FIRI was found.

DISCUSSION

β_3-Adrenergic receptors play an important role in the metabolism of fat tissue. For example, targeted disruption of β_3-adrenergic receptors in mice causes mild obesity.[10] Moreover, a specific β_3-adrenergic receptor agonist has an antiobesity effect[11] and increased visceral fat β_3-adrenoceptor sensitivity is associated with components of the metabolic syndrome. The relation between the BMI and Trp64Arg mutation has been described in a number of populations.[4-6] However, the association of this mutation and fatness in nondiabetic subjects is unclear. Although data in Finns showed no difference in the BMI among subjects with different genotypes,[5] there are studies

Table 4. Effect of Trp64Arg Mutation of the β_3-Adrenergic Receptor Gene in the Men

Parameter	Genotype: Trp/Trp	Genotype: Trp/Arg or Arg/Arg	P
Age (yr)	52.2 ± 2.3	53.2 ± 3.4	NS
Body weight (kg)	66.8 ± 1.7	61.5 ± 1.9	NS
Height (cm)	164.6 ± 0.9	164.0 ± 1.2	NS
Waist circumference (cm)	84.8 ± 1.2	80.1 ± 2.0	NS
Hip circumference (cm)	96.6 ± 0.9	92.4 ± 1.1	<.01
Waist to hip ratio	0.88 ± 0.01	0.87 ± 0.01	NS
Total body fat (kg)	15.8 ± 0.7	13.2 ± 1.1	<.05
Percent body fat (%)	23.3 ± 0.7	20.9 ± 1.3	NS
FIRI	3.5 ± 0.3	4.0 ± 0.8	NS

NOTE. Men with Trp64Arg mutation had lower total body fat and hip circumference, although no difference in the waist to hip ratio was found.

showing that the mutant allele is associated with greater weight gain in caucasians.[12] It is of note that most of the studies to date used the BMI as an assessment of body fat. Although the BMI is the most common measure of obesity, it is also not a true measure of fatness,[13] and part of the discordance in findings may be due to the inaccuracy inherent in the method used for assessment of body composition.

Recently, DEXA has been shown to be an accurate and convenient method to assess body composition.[14] Using DEXA to assess body fat, we found in the present study that Trp64Arg was associated with lower total fat but not with percent body fat. The relation was demonstrable only in men. The lack of effect on adiposity is not likely due to misclassification of the electrophoretic pattern, since the gene frequency was similar to that reported in other Asian populations[15,16] and conformation to the Hardy-Weinberg principle was also observed. In vitro, it is also controversial as to whether Trp64Arg mutation has any physiological effect. When expressed in a heterologous system, the mutant β_3-adrenergic receptor was functionally indistinct from the wild-type receptor,[17] although the absolute amount of intracellular cyclic adenosine monophosphate has been reported to be lower.[18]

Sexual dimorphism in body fat and its regional distribution has been observed.[19] The underlying basis for this difference is unclear, but may include hormonal and genetic factors. Findings from the present study suggest that the effect of Trp64Arg β_3-adrenergic receptor mutation on total body fat may also be sex-dependent, since body fat was found to be lower only in men with the mutant allele. However, the effect was not demonstrable for percent body fat, suggesting that Trp64Arg β_3-adrenergic receptor mutation may be related to body size rather than the degree of adiposity in our male population. The sex-specific effect has also been described in an elderly Australian population,[20] although only in women. This suggests that the effect of the β_3-adrenergic system can be sex- and body habitus–dependent. Therefore, gender and body size, as well as the ethnic and environmental background, need to be taken into account when studying the role the β_3-adrenergic system in a physiological or pathological process.

The association between insulin sensitivity and codon 64 polymorphism of the β_3-adrenergic receptor has been controversial as well. For example, lower insulin sensitivity was found in three Dutch subjects who were homozygous for Trp64Arg mutation[12] using Bergman's minimal model to assess insulin sensitivity, whereas no change in insulin sensitivity was found in heterozygotes. A study in Finns also found lower insulin sensitivity as assessed by the area under the curve for the insulin response to oral glucose, while no differences in basal or postglucose insulin concentrations were found.[5] Part of the discrepancy may be explained by the different methods to assess insulin sensitivity. Other reasons for the disagreement between findings may be related to factors that may potentially confound the assessment of insulin sensitivity such as differences in body fat, including its distribution,[21] and the androgen profile[22] of the study populations. In the present study, we did not find any difference in the FIRI among subjects with and without the mutation of either sex. Although the FIRI is not an accurate means to determine insulin sensitivity, it suggests that Trp64Arg mutation at codon 64 of the β_3-adrenergic receptor gene has a minor effect, if any, on insulin sensitivity in our population.

In conclusion, the gene frequency of Trp64Arg mutation of the β_3-adrenergic receptor gene in Thais is similar to that in other Asian populations. The effect of the mutation in terms of total body fat is found only in men, but no effect on percent body fat was found. This suggests that the Trp64Arg mutation is not related to adiposity in Thais.

REFERENCES

1. Bouchard C, Prusse L, LeBlanc C, et al: Inheritance of the amount and distribution of human body fat. Int J Obes 12:205-215, 1988
2. Giaconino JP: β_3-Adrenoceptor: An update. Eur J Endocrinol 132:377-385, 1995
3. Hoffstedt J, Wahrenberg H, Thorne A, et al: The metabolic syndrome is related to β_3-adrenoceptor sensitivity in visceral adipose tissue. Diabetologia 39:838-844, 1996
4. Walston J, Silver K, Bogardus C, et al: Time of onset of non–insulin-dependent diabetes mellitus and genetic variation in the β_3-adrenergic-receptor gene. N Engl J Med 333:343-347, 1995
5. Widen E, Lehto M, Kanninen T, et al: Association of a polymorphism in the β_3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333:348-351, 1995
6. Clement K, Vaisse C, Manning BSJ, et al: Genetic variation in the β_3-adrenergic receptor and increased capacity to gain weight in patients with morbid obesity. N Engl J Med 333:352-354, 1995
7. Margolis CF, Sprecher DL, Simbartl LA, et al: Male-female differences in the relationship between obesity and lipids/lipoproteins. Int J Obes 20:784-790, 1996
8. Bouchard C, Despres JP, Maurige P: Genetic and nongenetic determinants of regional fat distribution. Endocr Rev 14:72-93, 1993
9. Duncan MH, Singh BM, Wise PH, et al: A simple measure of insulin resistance. Lancet 346:120-121, 1995
10. Susulic VS, Frederich RC, Lawitts J, et al: Targeted disruption of the β_3 adrenergic receptor gene. J Biol Chem 270:29483-29492, 1995
11. Connacher AA, Bennet WM, Jung RT: Clinical studies with the β-adrenoceptor agonist BRL 26830A. Am J Clin Nutr 55:258-261, 1992 (suppl)
12. Urhammer S, Clausen JO, Hansen T, et al: Insulin sensitivity and body weight changes in young white carriers of the codon 64 amino acid polymorphism of the β_3-adrenergic receptor gene. Diabetes 45:1115-1120, 1996
13. Bouchard C: Current understanding of the etiology of obesity: Genetic and nongenetic factors. Am J Clin Nutr 53:1561S-1565S, 1991 (suppl)
14. Jensen MD, Kanaley JA, Roust LR, et al: Assessment of body composition with use of dual-energy x-ray absorptiometry: Evaluation and comparison with other methods. Mayo Clin Proc 68:867-873, 1993
15. Fujusawa T, Ikegami H, Yamato E, et al: Association of Trp64Arg mutation of the β_3-adrenergic-receptor with NIDDM and body weight gain. Diabetologia 39:349-352, 1996
16. Kadowaki H, Yasuda K, Iwamoto K, et al: A mutation in the β_3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 215:555-560, 1995
17. Candelore MR, Deng L, Tota LM, et al: Pharmacological

characterization of a recently described human beta 3-adrenergic receptor mutant. Endocrinology 137:2638-2641, 1996

18. Pietri-Rouxel F, Manning B, Gros J, et al: The biochemical effect of the naturally occurring Trp64 → Arg mutation on human beta3-adrenoceptor activity. Eur J Biochem 247:1174-1179, 1997

19. Enzi G, Gasparo M, Biondetti PR, et al: Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. Am J Clin Nutr 44:739-746, 1986

20. Kurabayashi T, Carey DGP, Morrison NA: The β_3-adrenergic receptor gene Trp64Arg mutation is overrepresented in obese women: Effects on weight, BMI, abdominal fat, blood pressure and reproductive history in an elderly Australian population. Diabetes 45:1358-1363, 1996

21. Kissebah AH, Vydelingum N, Murray R, et al: Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 54:254-260, 1982

22. Evans DJ, Hoffman RG, Kalkhoff RK, et al. Relationship of androgenic activity of body fat topography, fat cell morphology and metabolic aberrations in premenopausal women. J Clin Endocrinol Metab 57:304-310, 1983