Diabetologia (1997) 40: 469–472

Diabetologia

Rapid communications

A mutation of the β3-adrenergic receptor is associated with visceral obesity but decreased serum triglyceride

H. Kim-Motoyama[1], K. Yasuda[2], T. Yamaguchi[3], N. Yamada[1], T. Katakura[4], Alan R. Shuldiner[5], Y. Akanuma[2], Y. Ohashi[3], Y. Yazaki[1], T. Kadowaki[1]

[1] Third Department of Internal Medicine, University of Tokyo, Tokyo, Japan
[2] The Institute for Diabetes Care and Research, Asahi Life Foundation, Tokyo, Japan
[3] Epidemiology and Biostatistics, School of Health Sciences and Nursing, Faculty of Medicine, University of Tokyo, Tokyo, Japan
[4] Nippon Express Health Insurerance Society Tokyo Hospital, Tokyo, Japan
[5] Divisions of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD 21218

Summary The Trp64Arg mutation of the β3-adrenergic receptor (β3AR) is prevalent in several ethnic groups and is associated with weight gain, and some features of syndrome X such as insulin resistance and dyslipidaemia. Nevertheless, it is not known at present whether this mutation is associated with visceral obesity, which is an important risk factor for the development of hypertension, dyslipidaemia, insulin resistance, non-insulin-dependent diabetes mellitus, and atherosclerosis. To investigate whether this mutation may contribute to visceral obesity, we studied the relationships between β3AR genotypes and clinical phenotypes. The Trp64Arg allele of β3AR was examined in 278 Japanese men with respect to variables relating to visceral obesity assessed by computerised tomography. To detect the Trp64Arg mutation, polymerase chain reaction-restriction fragment length polymorphism analysis using Bst NI digestion was performed. This mutation was more frequently observed in subjects with higher body mass index (BMI) ($p = 0.02$). Moreover, in 120 subjects with a moderate degree of obesity ($22 \leqq \text{BMI} < 26.4$ kg/m^2), the mutation (homozygotes and heterozygotes) was associated with visceral obesity (higher ratio of visceral to subcutaneous fat area; V/S) ($p = 0.03$). Furthermore, the Trp64Arg allele was more frequent in subjects with lower serum triglyceride levels ($p = 0.02$) and the Trp64Arg homozygotes, but not heterozygotes, exhibited lower triglyceride levels. Thus, this mutation appears to be associated with visceral obesity but with lower serum triglyceride. It is suggested that those with the mutation may describe a subset of subjects characterized by decreased lipolysis in visceral adipose tissue. [Diabetologia (1997) 40: 469–472]

Keywords β3-adrenergic receptor, body mass index, visceral obesity, triglyceride, lipolysis.

The sympathetic nervous system plays a major role in energy expenditure. By catecholamine stimulation, lipolysis in white adipose tissue and thermogenesis in brown adipose tissue and skeletal muscle are induced. These effects are mediated by several subtypes of catecholamine receptors namely α2, β1, β2 and β3 adrenergic receptors. Among these the β3 adrenergic receptor (β3AR) is important for mediating the stimulation of lipolysis by catecholamines, because this subtype is almost exclusively expressed in adipose tissue and moreover, it resists ligand-dependent desensitization via β adrenergic receptor kinase.

RNA encoding the β3AR is expressed in both white and brown adipose tissues, especially in visceral adipose tissue in humans [1]. White adipocytes derived from subcutaneous fat tissue express few β3ARs and do not respond or minimally respond to β3-selective agonists in humans, while adipocytes derived from intra-abdominal or visceral depots express more β3ARs and are responsive to β3-selective agonists. Thus, one can hypothesize that molecular

Received: 6 December 1996
and in revised form: 17 January 1997

Corresponding author: Dr. T. Kadowaki, Third Department of Internal Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan

Abbreviations: β3AR, β3-adrenergic receptor; W/H ratio, waist/hip ratio; V/S ratio, visceral to subcutaneous fat ratio; NIDDM, non-insulin-dependent diabetes mellitus; CT, computer tomography.

defects in the β3AR may predispose to impaired lipolysis in visceral fat, leading to increased visceral adiposity. It has been recently proposed that body fat distribution such as the waist to hip circumference ratio (W/H ratio) or visceral to subcutaneous fat ratio at umbilical levels (V/S ratio), may be a more important indicator for metabolic disorders and also for the risk of cardiovascular diseases than body mass index (BMI) [2]. In fact, it has been reported that visceral obesity is an important risk factor for the development of the insulin resistance syndrome, non-insulin-dependent diabetes mellitus (NIDDM), and atherosclerosis [2, 3]. Several family studies including the comparison of monozygotic and dizygotic twins after overfeeding suggest that the genetic backgrounds contribute to regional adiposity. However, the genetic factors for body fat distribution are not clear at present.

It has been reported that the Trp64Arg mutation of the β3AR is quite prevalent in several ethnic groups and is associated with weight gain in part due to decreased basal metabolic rate, insulin resistance, and some features of the insulin resistance syndrome (syndrome X) [4–7].

Nevertheless, it is not known whether this mutation is associated with visceral obesity. To investigate whether this mutation may contribute to visceral obesity evaluated by computed tomography (CT), the relationships between β3AR genotype and clinical phenotypes were studied.

Patients and methods

Subjects. The subjects were 278 Japanese men aged 21–65 years (51.3 ± 8.8 years, mean ± SD) who underwent a medical checkup in a company-based clinic. They included 161 subjects with normal glucose tolerance (NGT), 74 subjects with impaired glucose tolerance (IGT), and 43 subjects with NIDDM. NIDDM and IGT were diagnosed according to the World Health Organization criteria [6]. However, none required insulin injection or oral hypoglycaemic agents.

Procedure. Informed consent was obtained from all the subjects studied. Following 12 h fast, venous blood samples were obtained. Body height, body weight, plasma glucose and insulin levels during a 75-g oral glucose load, HbA_{1c}, V/S ratio, waist to hip (W/H) ratio, serum total cholesterol, triglyceride, HDL-cholesterol, γ-glutamyl transpeptidase, uric acid, and systolic and diastolic blood pressures were studied in all the subjects. The V/S ratio was defined as the ratio of visceral fat area (V) to subcutaneous fat area (S) by CT scan at the level of the umbilicus. All CT scans were performed with the patient in the supine position using a Toshiba Electric CT/T scanner (Tokyo, Japan). Visceral fat area was measured as the same density area as the subcutaneous fat layer. W/H ratio was defined as the ratio of waist circumference at umbilical level to maximum hip circumference. Genomic DNA was purified from peripheral blood leukocytes. To detect the Trp64Arg mutation, polymerase chain reaction-restriction fragment length polymorphism analysis using Bst NI digestion was performed as previously described [5].

Table 1. Characteristics of non-diabetic subjects according to the V/S ratio

Characteristics	V/S < 0.4 $n = 22$	0.4 ≦ V/S < 0.8 $n = 129$	V/S ≧ 0.8 $n = 70$	*P*-Value
Waist/hip ratio	0.87 ± 0.00	0.88 ± 0.04	0.88 ± 0.05	NS
Fasting insulin (pmol/l)	41.6 ± 3.6	44.5 ± 1.4	49.5 ± 2.2	0.05
γ-GTP (μkat/l)	0.70 ± 0.18	0.98 ± 0.08	1.12 ± 0.10	NS
T-cholesterol (mmol/l)	5.20 ± 0.18	5.48 ± 0.07	5.46 ± 0.10	NS
Triglyceride (mmol/l)	0.93 ± 0.24	1.55 ± 0.10	1.71 ± 0.13	0.02
HDL (mmol/l)	1.45 ± 0.07	1.40 ± 0.03	1.40 ± 0.04	NS
Systolic blood pressure (mm Hg)	124 ± 3.6	125 ± 1.5	127 ± 2.0	NS
Diastolic blood pressure (mm Hg)	76 ± 2.4	81 ± 1.0	83 ± 1.3	0.03

Continuous data are expressed as least square mean ± SEM
V/S, Visceral fat area/subcutaneous fat area; HDL, high density lipoprotein-cholesterol; γ-GTP, γ-glutamyl transpeptidase. *P* value was tested by analysis of covariance between V/S ratio and other parameters adjusted by body mass index and age

Statistical analysis

Differences between group means were tested by Student's *t*-test or Wilcoxon's test for variables not normally distributed. An analysis of covariance was performed to test whether there were interactions between genotype and BMI. A multivariate regression analysis was performed to test whether there were interactions between V/S ratio and other parameters. The Mantel-Haenszel chi-square test was used to compare allele frequencies between groups. A trend test was performed to study the effects of three genotypes on V/S ratio or visceral fat area. All statistical analyses were carried out with the Statistical Analysis Systems package (SAS Institute, Cary, N.C., USA).

Results

The overall frequency of the Trp64Arg β3AR allele was 22.5% consistent with our previous report on Japanese subjects [7]. The Trp64Arg β3AR mutation was observed more frequently in subjects with higher BMI (15.5% in 58 subjects with BMI < 22 kg/m^2, 25.2% in 159 subjects with BMI between 22 and 26.4, and 22.1% in 61 subjects with BMI ≧ 26.4 kg/m^2; $p = 0.02$). There was no difference in allele frequency according to the glucose tolerance (NGT 22.7%, IGT 22.1%, and NIDDM 24.4%). It is possible that the effects of this mutation are modified by diet therapy or the metabolic disorder in the diabetic subjects. Therefore, phenotypic traits were compared in non-diabetic subjects according to V/S ratio by analysis of covariance adjusted by BMI and age (Table 1). Subjects with higher V/S ratio showed higher fasting insulin levels ($p = 0.05$), higher triglyceride

Table 2. Characteristics of non-diabetic subjects according to the β3AR genotype and BMI

Characteristics	β3AR Genotype	Normal subjects BMI < 22 $n = 53$	Moderately obese 22 ≦ BMI < 26.4 $n = 120$		Severely obese BMI ≧ 26.4 $n = 48$
V (mm^2)	Trp/Trp	5605 ± 2818 $n = 37$	8532 ± 3251 $n = 66$		12375 ± 3952 $n = 28$
	Trp/Arg	3796 ± 2189 $n = 16$	9428 ± 3115 $n = 49$		10998 ± 2990 $n = 18$
	Arg/Arg	– $n = 0$	10325 ± 5636 $n = 5$		11876 ± 10137 $n = 2$
S (mm^2)	Trp/Trp	7553 ± 3002 $n = 37$	12497 ± 3530 $n = 66$		20926 ± 8871 $n = 28$
	Trp/Arg	7315 ± 2991 $n = 16$	12230 ± 2969 $n = 49$		20285 ± 8198 $n = 18$
	Arg/Arg	– $n = 0$	10362 ± 1847 $n = 5$		17808 ± 2587 $n = 2$
V/S	Trp/Trp	0.75 ± 0.27 $n = 37$	0.70 ± 0.26 $n = 66$		0.63 ± 0.20 $n = 28$
	Trp/Arg	0.54 ± 0.27 $n = 16$	0.80 ± 0.28 $n = 49$	$p = 0.039$[a]	0.59 ± 0.19 $n = 18$
	Arg/Arg	– $n = 0$	0.96 ± 0.38 $n = 5$		0.63 ± 0.48 $n = 2$
Triglyceride (mmol/l)	Trp/Trp	1.34 ± 1.00 $n = 37$	1.57 ± 1.21 $n = 66$		1.96 ± 0.91 $n = 28$
	Trp/Arg	0.94 ± 0.41 $n = 16$	1.47 ± 0.84 $n = 49$		1.97 ± 2.26 $n = 18$
	Arg/Arg	– $n = 0$	1.13 ± 0.33 $n = 5$		0.96 ± 0.32 $n = 2$

Data are expressed as mean ± SD
Trp/Trp, Normal homozygotes; Trp/Arg, Trp64Arg heterozygotes; Arg/Arg, Trp64Arg homozygotes.
[a] According to a trend test

($p = 0.02$), and higher diastolic blood pressure ($p = 0.03$) than those with lower V/S ratio. Since the relationships between the β3AR genotype and the variables relating to visceral obesity such as V and V/S are affected by BMI (data not shown), these variables were compared according to the β3AR genotype and BMI in non-diabetic subjects (Table 2). In 120 moderately obese subjects (22 ≦ BMI < 26.4 kg/m^2), the V/S ratio was increased significantly according to the number of Trp64Arg alleles ($p = 0.039$). The subjects with the mutant β3AR also tended to have higher visceral fat area (Trp64Arg homozygotes > heterozygotes > normal homozygotes), although it was not significant ($p = 0.245$) according to the trend test.

The allele frequency of the Trp64Arg mutation was significantly higher in those with lower serum triglyceride levels (triglyceride < 1.69 vs ≧ 1.69 mmol/l, 25.0 vs 16.9 %, $p = 0.02$). Consistent with this, serum triglyceride levels were lower in Trp64Arg homozygotes ($n = 9$) than those normal homozygotes ($n = 162$) (1.02 ± 0.35 vs 1.60 ± 1.11 mmol/l, $p = 0.11$) and the Trp64Arg heterozygotes ($n = 107$) (1.02 ± 0.35 vs 1.55 ± 1.28 mmol/l, $p = 0.16$). To obtain further insight into the relationship between the V/S ratio and other clinical parameters according to the presence of the Trp64Arg variant, multivariate regression analysis was performed dividing subjects with BMI ≧ 22 kg/m^2 into the two groups; one group with and another group without the Trp64Arg variant. In the subjects without this mutation, higher V/S ratio showed higher triglyceride levels ($p = 0.00$) and higher diastolic blood pressure ($p = 0.02$). On the other hand, in the subjects with this mutation, there was no association between V/S ratio and triglyceride or diastolic blood pressure. None of the other clinical parameters tested were associated with the Trp64Arg allele.

Discussion

This study is the first to show the role of the mutant β3AR in visceral obesity and the regulation of serum triglyceride levels in men. Since the β3AR is expressed at a higher level in visceral than subcutaneous fat in humans [1], the dysfunction of this receptor may have profound effects on visceral adiposity. Consistent with this, we demonstrated that those with this mutation, not only Trp64Arg homozygotes but also heterozygotes showed higher V/S ratio ($p = 0.03$) than normal homozygotes. The subjects with more Trp64Arg mutation tended to have more visceral fat area (V) and less subcutaneous fat area (S) than those without this mutation (Table 2). The association of the mutation with visceral obesity was apparent only in the moderately obese group. The reason for this is not clear, but the subjects with severe obesity may be affected by additional genetic or environmental factors, which may have masked the effect of this mutation on visceral adiposity. This study demonstrates that this mutation is associated with visceral obesity in men with Trp64Arg homozygous and heterozygous mutation. Previous studies have reported that this mutation was associated with W/H ratio only in non-diabetic women with this mutation. However, it was not significant with men. In fact, it has been pointed out that waist-to-hip ratio (W/H ratio) does not necessarily correlate with visceral obesity.

In this study, the β3AR mutation is associated with lower serum triglyceride levels. Visceral obesity is usually accompanied by hypertriglyceridaemia and other metabolic disorders, which is collectively called the insulin resistance syndrome or the visceral fat syndrome [7, 8]. In this respect, it has been reported that lipolysis from omental fat cells is accelerated in subjects with visceral obesity [9]. This causes an increase in the release of non-esterified fatty acid

(NEFA) to the portal system which could result in increased VLDL-triglyceride synthesis in liver, which may contribute to the visceral fat syndrome. In the present study, when adjusted by BMI and age, the subjects with higher V/S ratio indeed showed higher triglyceride than those without higher V/S ratio ($p = 0.02$). The β3AR mutation was associated with lower serum triglyceride levels ($p = 0.02$) despite higher V/S. Previous studies have not been able to show this point, possibly because they were not large enough to involve a certain number of subjects who are homozygous for this mutation. We speculate that this mutation causes a disturbance in lipolysis in visceral adipose tissue which results in visceral obesity; however, unlike the visceral fat syndrome, the delivery of lipolytic products from visceral adipose tissue such as glycerol or NEFA to the liver may not be increased but rather decreased in subjects with the β3AR mutation. Thus, visceral obesity associated with the β3AR mutation appears to be distinct in its pathogenesis from that described in the visceral fat syndrome which is accompanied with hypertriglyceridaemia.

We were not able to demonstrate the direct association of the β3AR mutation with hyperinsulinaemia in this study. As described above, this mutation may not necessarily cause the visceral fat syndrome or the insulin resistance syndrome by itself. However, since it is also associated with higher BMI, it is possible that in combination with other genetic or environmental factors, subjects with the Trp64Arg mutation could present with visceral fat syndrome or the insulin resistance syndrome (syndrome X).

In conclusion, the Trp64Arg homozygous and heterozygous mutation in the β3AR, which is frequent in a variety of ethnic groups, may be among genetic loci contributing to visceral obesity which now appears to be a heterogeneous entity.

Acknowledgements. This study was supported by a grant from the Ministry of Education, Science and Culture of Japan. We thank J. Kobiyama, K. Toyokuni, T. Irie, S. Nohara for their help.

References

1. Krief S, Lönnqvist F, Raimbault S et al. (1993) Tissue distribution of β3 adrenergic receptor mRNA in man. J Clin Invest 91: 344–349
2. Matsuzawa Y, Simomura I, Nakamura T et al. (1995) Pathophysiology and pathogenesis of visceral fat obesity. Obes Res [Suppl 2]: 187–194
3. Zamboni M, Armellini F, Cominacini L et al. (1994) Obesity and regional body fat distribution in men: separate and joint relationships to glucose tolerance and plasma lipoproteins. Am J Clin Nutr 60: 682–687
4. Walston J, Silver K, Bogardus C et al. (1995) Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3 adrenergic receptor gene. New Engl J Med 333: 343–347
5. Widén E, Lehto M, Kanninen T et al. (1995) Association of a polymorphism in the β3 adrenergic receptor gene with features of the insulin resistance syndrome in Finns. New Engl J Med 333: 348–351
6. Clément K, Vaisse C, Manning BSJ et al. (1995) Genetic variation in the β3 adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. New Engl J Med 333: 352–354
7. Kadowaki H, Yasuda K, Iwamoto K et al. (1995) A mutation in the β3 adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 215: 555–560
8. Diabetes mellitus (1985) Report of a WHO study group, World Health Organ Techn Rep Series 727: 7–113
9. Lönnqvist F, Thörne A, Nilsell K et al. (1995) A pathogenic role of visceral fat β3-adrenoceptors in obesity. J Clin Invest 95: 1109–1116
10. Li LS, Lönnqvist F, Luthman H et al. (1996) Pathogenic characterization of the Trp64Arg polymorphism in the β3-adrenergic receptor gene in normal weight and obese subjects. Diabetologia 39: 857–860