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The metabolic syndrome is related to β_3-adrenoceptor sensitivity in visceral adipose tissue

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Summary The metabolic syndrome is a well-known risk for the development of cardiovascular disease. In the present study the possible importance of an altered visceral adipocyte β-adrenoceptor function in this syndrome was investigated. In 65 subjects of both sexes undergoing elective surgery for non-malignant disorders, the metabolic syndrome phenotype and the lipolytic sensitivity for various β-adrenoceptor subtype agonists in omental adipocytes were determined. The study group represented a wide range of abdominal adipose tissue distribution (waist-to-hip ratios 0.76–1.13), but was otherwise apparently healthy. The subjects were divided into three subgroups according to their waist-to-hip (WHR) ratios: 1) WHR < 0.92; 2) WHR 0.92–1.04; 3) WHR > 1.04. The subgroups demonstrated significant differences regarding body mass index, sagittal diameter, systolic and diastolic blood pressures, plasma concentrations of glucose, insulin, triglycerides and HDL-cholesterol ($p = 0.005$–0.0001). Furthermore, in omental adipocytes β_3-adrenoceptor sensitivity, but not β_1- and β_2-adrenoceptor sensitivities, differed significantly between the WHR subgroups ($p = 0.0001$). β_3-adrenoceptor sensitivity was also related to the other components of the metabolic syndrome, although a strong covariation between WHR and β_3-adrenoceptor sensitivity vs blood pressure and the metabolic parameters was found. The present data provide evidence of a relationship between upper-body obesity and its associated metabolic complications and also, an increased visceral fat β_3-adrenoceptor sensitivity. We suggest that the latter finding results in an augmented release of non-esterified fatty acids from the visceral fat depot to the portal venous system. This may in turn contribute to the development of the metabolic syndrome. [Diabetologia (1996) 39: 838–844]

Keywords Adipocytes, β_3-adrenoceptors, insulin resistance, metabolic syndrome, visceral fat.

The metabolic syndrome has aroused an increasing interest in recent years as an important risk factor for cardiovascular disease, which is one of the major causes of premature death in western countries. The metabolic or insulin resistance syndrome consists of several pathological conditions [1–4], such as abdominal obesity, glucose intolerance, hypertension, hypertriglyceridaemia and low serum high density lipoprotein (HDL)-cholesterol levels. In this respect, the accumulation of visceral fat may be of particular importance. It has been postulated that an increased release of non-esterified fatty acids (NEFA) delivered to the portal venous system from the lipolytically-active visceral adipocytes may have adverse metabolic effects on the liver resulting in elevated levels of plasma insulin and triglycerides, accompanied by glucose intolerance [5–7]. However, the underlying mechanisms have not been clarified.

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Abbreviations: ANOVA, Analysis of variance; BMI, body mass index; EC_{50}, half-maximum agonist concentration; NEFA, non-esterified fatty acids; HDL-cholesterol, high density lipoprotein cholesterol; VLDL, very low density lipoproteins; WHR, waist-to-hip ratio.

Table 1. Clinical data of subjects studied

Age (years)	40 ± 10	(19–59)
Sex (male/female)	27/38	
Smoking (yes/no)	15/50	
Body mass index (kg/m^2)	34 ± 10	(20–53)
Waist-hip-ratio	0.98 ± 0.08	(0.76–1.13)
Sagittal diameter (cm)	26 ± 6	(15–37)
Glucose (mmol/l)	5.4 ± 0.7	(4.3–9.4)
Insulin (pmol/l)	91 ± 49	(15–242)
Cholesterol (mmol/l)	5.7 ± 0.98	(3.6–8.1)
HDL-cholesterol (mmol/l)	1.24 ± 0.36	(0.7–2.3)
Triglycerides (mmol/l)	1.92 ± 0.96	(0.5–6.4)
Systolic blood pressure (mm Hg)	133 ± 17	(97–170)
Diastolic blood pressure (mm Hg)	84 ± 10	(65–110)
Dobutamine sensitivity (–log mol/l)	8.44 ± 1.11	(6.28–10.78)
Terbutaline sensitivity (–log mol/l)	7.44 ± 1.01	(4.38–11.15)
CGP 12177 sensitivity (–log mol/l)	8.18 ± 1.32	(5.00–10.70)

Data are mean ± SD and (range)

In man, it has long been known that the release of NEFA and glycerol via lipolysis is mainly influenced by catecholamines and is modulated either through stimulatory β_1-, and β_2-adrenoceptors or inhibitory α_2-adrenoceptors [8]. Recent studies have related the existence of a gene coding for a third stimulatory β-adrenoceptor in man [9], which is functionally active principally in omental adipocytes [10, 11] but also present in mammary fat [12] and in subcutaneous fat in vivo [13].

Previous studies from our laboratory have shown that both the abdominal subcutaneous β_2-adrenoceptor [14] and the visceral β_3-adrenoceptor functions [15] may play a pathogenic role in upper-body obesity. The aim of the present study was therefore to examine further the possible importance of the three visceral adipose tissue β-adrenoceptor subtypes (β_1, β_2 and β_3) for the metabolic syndrome. The phenotype of the metabolic syndrome and the lipolytic sensitivity to various β-adrenoceptor agonists in visceral adipocytes were determined in subjects of both sexes representing a wide range of abdominal fat distribution.

Subjects, materials and methods

Subjects. The study group consisted of 65 randomly selected Caucasian subjects undergoing weight reduction surgery, elective cholecystectomy or operations for hiatus hernia through open or laparoscopic procedures at Huddinge University Hospital (Table 1). Before surgery, a thorough history was taken and, except for the surgery indication, all subjects were apparently healthy and not on any medication. None had a history of alcohol overconsumption. The subjects were between 19 and 60 years of age. The body mass index (BMI) ranged from 20 to 53 kg/m^2 with a span of values including non-obese, moderately obese and massively obese subjects. The waist-to-hip ratio (WHR), the sagittal diameter and the systolic and diastolic blood pressures were measured in the supine position on the day before surgery. The sagittal diameter was obtained by measuring the distance from the examination table to a horizontal cross-bar placed over the abdomen of the recumbent subject at the level of the crista. The systolic and diastolic blood pressures were determined with a mercury sphygmomanometer using phases I and V of the Korotkoff sounds. Each value is the mean of three consecutive measurements after a 10-min rest.

Following an overnight fast, the study subjects rested in bed for 15 min, whereafter venous blood samples were obtained, which were analysed by the hospital's routine chemistry laboratory, except for insulin which was measured with a radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

General anaesthesia was induced at 08.00 hours by a short-acting barbiturate and maintained by fentanyl and a mixture of oxygen and nitrous oxide. Intravenous saline was administered prior to the fat biopsies, which were taken from the major omentum, at the beginning of the operation. The study was approved by the ethics committee of Karolinska Institute, Stockholm, and all the patients gave informed consent to participate.

Lipolysis. The omental adipose tissue biopsies were immediately transported to the laboratory in saline at 37 °C and isolated fat cells from fat specimens weighing 0.3–1.0 g were prepared by collagenase treatment, as previously described [16]. The cells were kept in an albumin solution, as described below, and the cell density of the fat-cell suspension was kept constant by slow stirring with the aid of a magnet. Direct microscopic determination of the fat-cell diameter, performed according to the method of Di Girolamo and co-workers [17], was calculated by using 200 cells from each subject. The mean fat cell volume and weight were determined, taking into account the skewness in the distribution of the cell diameter and using the method described by Hirsch and Gallian [18]. The total lipid content in each incubation was determined gravimetrically after organic extraction. Assuming that lipids constitute more than 95 % of the fat-cell weight, the number of fat cells can be calculated by dividing the total lipid weight by the mean cell weight.

A detailed description of the lipolysis assay has been reported elsewhere [19]. In brief, 0.2 ml of diluted suspensions of isolated fat cells (5000–10000 cells/ml) were incubated in duplicate for 2 h with or without the selective β_1-adrenoceptor agonist dobutamine, the selective β_2-adrenoceptor agonist terbutaline or the selective partial β_3-adrenoceptor agonist CGP 12177 [20]. All incubations were performed at 37 °C in Krebs-Henseleit phosphate buffer (pH 7.4), supplemented with glucose (5.6 mmol/l), bovine serum albumin (0.3 mmol/l) and ascorbic acid (0.6 mmol/l), with air as the gas phase. The agents were added simultaneously at the start of the incubation. The concentration range for each agent ranged overall from 10^{-12} to 10^{-3} mol/l. After 2 h of incubation, a cell-free aliquot was removed for determination of the glycerol concentration, using a bioluminescence method [21].

All agonists caused a concentration-dependent stimulation of glycerol release that reached a plateau at the highest agonist concentrations. Consequently, it was always possible to determine the concentration of agonist that produced a half-maximum effect on glycerol release. These EC_{50} values (expressed as log mol/l) were determined by linear regression analysis of log-logit transformation of the ascending part of the individual concentration-response curves [22]. Lipolysis rates in the presence of maximum effective agonist concentrations were related to fat-cell number.

Drugs and chemicals. Bovine serum albumin (fraction V, lot 63F-0748), Clostridium histolyticum collagenase type I and glycerol kinase from *Escherichia coli* (G4509) were obtained

Table 2. The influence of WHR on clinical parameters and β-adrenoceptor sensitivity

	Waist-hip-rato			ANOVA	Bonferroni/Dunn		
	Low (< 0.92)	Intermediate (0.92–1.04)	High (> 1.04)		H vs I	H vs L	I vs L
n	16	33	16	*p*	*p*	*p*	*p*
Gender (male/female)	1/15	13/20	13/3				
Age (years)	36 ± 2	41 ± 2	41 ± 2	NS			
Body mass index (kg/m^2)	26 ± 1	35 ± 2	40 ± 2	0.0001	< 0.05	< 0.001	< 0.005
Sagittal diameter (cm)	20 ± 1	26 ± 1	31 ± 1	0.0001	< 0.005	0.0001	< 0.0005
Glucose (mmol/l)	5.21 ± 0.09	5.29 ± 0.09	6.01 ± 0.27	< 0.001	< 0.005	< 0.005	NS
Insulin (pmol/l)	63 ± 9	81 ± 6	137 ± 15	0.0001	0.0001	0.0001	NS
Cholesterol (mmol/l)	5.23 ± 0.27	5.81 ± 0.14	5.93 ± 0.30	NS			
HDL-cholesterol (mmol/l)	1.41 ± 0.09	1.22 ± 0.05	0.97 ± 0.06	0.0001	0.008	0.0001	< 0.05
Triglycerides (mmol/l)	1.40 ± 0.12	1.89 ± 0.15	2.53 ± 0.31	< 0.005	< 0.05	< 0.0005	NS
Systolic blood pressure (mm Hg)	118 ± 3	135 ± 3	144 ± 4	< 0.0001	NS	0.0001	< 0.005
Diastolic blood pressure (mm Hg)	75 ± 2	84 ± 2	92 ± 3	0.0001	< 0.005	0.0001	< 0.01
β_1-receptor sensitivity (–log mol/l)	8.02 ± 0.29	8.66 ± 0.17	8.46 ± 0.33	NS			
β_2-receptor sensitivity (–log mol/l)	7.17 ± 0.24	7.49 ± 0.20	7.63 ± 0.24	NS			
β_3-receptor sensitivity (–log mol/l)	6.81 ± 0.23	8.41 ± 0.20	9.07 ± 0.23	0.0001	< 0.05	0.0001	0.0001

Values, mean ± SEM were compared using analysis of variance (ANOVA) and the Bonferroni/Dunn test

from Sigma (St. Louis, Mo., USA). Terbutaline sulphate came from Draco (Lund, Sweden), dobutamine hydrochloride from Lilly (Indianapolis, Ind., USA) and CGP (±) 12177 ((–)-4-(3-t-butylamino-2-hydroxy-propoxy)benzimidazole-2-one) from Ciba Geigy (Basel, Switzerland). ATP monitoring reagent containing firefly luciferase came from LKB Wallac (Turku, Finland). All other chemicals were of the highest grade of purity commercially available. The same batches of collagenase and albumin and the same stock solutions of adrenoceptor agonists were employed throughout the study.

Statistical analysis

Values are given as the mean ± SEM or if indicated mean ± SD. EC_{50} values were logarithmically transformed to normalize the data and were defined as β-adrenoceptor subtype sensitivity. Analysis of variance (ANOVA), analysis of covariance, the Bonferroni/Dunn post-hoc test, single and multiple regression analyses and the Kolmogorov–Smirnoff one-sample test were used for statistical comparisons. All statistical calculations were performed with a software package for statistics (Abacus Concepts Inc., Berkeley, Calif., USA).

Results

The WHR in the study group showed a clear interindividual variation ranging from 0.76 to 1.13. These values were normally and symmetrically distributed around the population mean of 0.98 ± 0.08 (mean ± SD) as evaluated with the Kolmogorov–Smirnoff one-sample test. The WHR values were further subdivided into three groups with reference to the frequency distribution. The first group in the lower 25th percentile included subjects with a WHR less than 0.92 (n = 16). The second or intermediate group, between the 25th and 75th percentiles, consisted of subjects with WHR values ranging from 0.92–1.04 (n = 33). The third group of subjects with a WHR above 1.04 (n = 16) were in the higher 75th percentile.

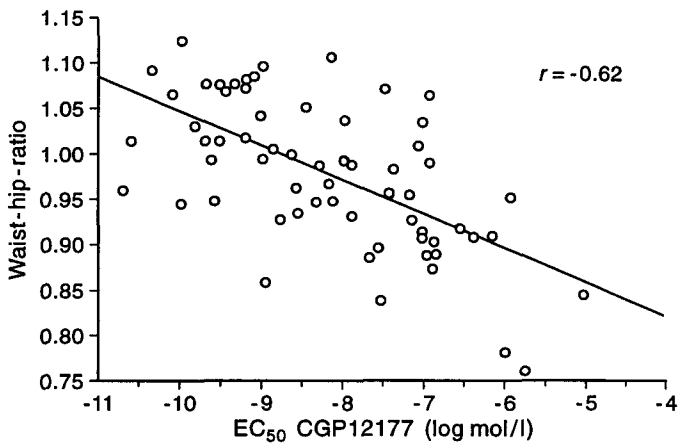

Fig. 1. Linear regression analysis showing the relationships between β_3-adrenoceptor sensitivity and WHR

To find out whether the three subgroups differed in their metabolic phenotype, a number of clinical and metabolic parameters were assessed by ANOVA with the Bonferroni/Dunn test as the post-hoc test. As shown in Table 2, the three percentile groups were considerably different from each other regarding most of the parameters, including the anthropometric data, systolic and diastolic blood pressures and metabolic variables. In general, the only parameter measured which was not influenced by the WHR was the plasma cholesterol level.

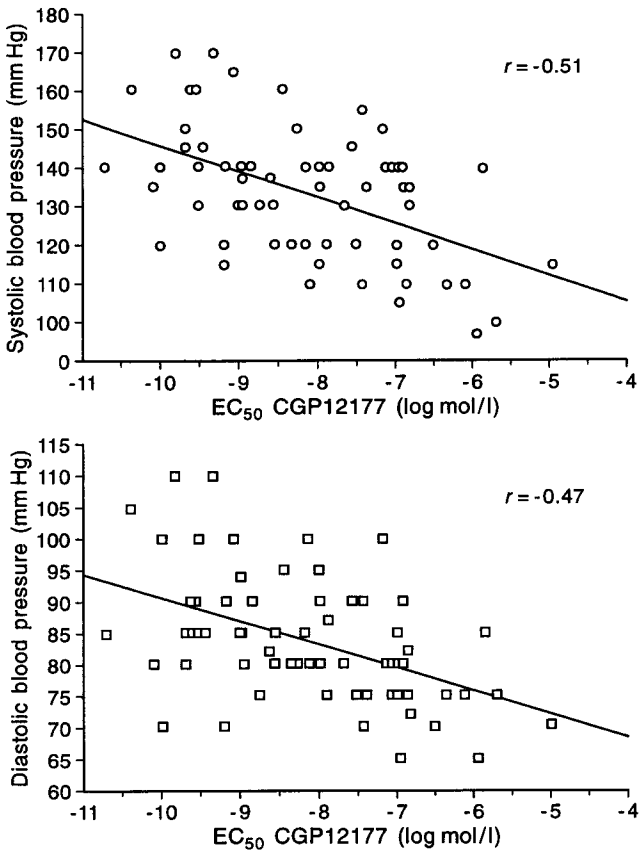

Fig. 2. Linear regression analysis showing the correlations between β_3-adrenoceptor sensitivity and the systolic and diastolic blood pressures

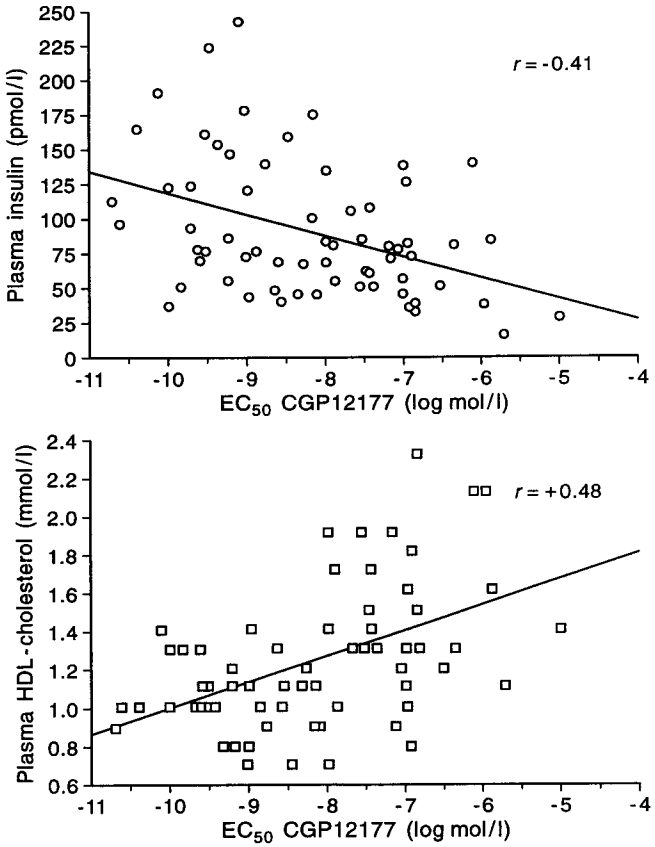

Fig. 3. Linear regression analysis showing the relationships between β_3-adrenoceptor sensitivity and the plasma concentrations of insulin and HDL-cholesterol

The contribution of the WHR values to the variations in β_1-, β_2- and β_3-adrenoceptor sensitivities was investigated using the same method as above. As demonstrated in Table 2, only the EC_{50} for CGP 12177 (β_3-adrenoceptor sensitivity), but not the EC_{50} for dobutamine (β_1-adrenoceptor sensitivity) or terbutaline (β_2-adrenoceptor sensitivity), showed a significant difference between the subjects with high, intermediate or low WHR values.

The relative importance of the β_1-, β_2-, and β_3-adrenoceptor subtypes for the various obesity measures and the clinical parameters involved in the metabolic syndrome were further investigated. In linear regression analysis, the EC_{50} for CGP 12177 was correlated to WHR, $r = -0.62$, $p = 0.0001$ (Fig. 1), systolic, $r = -0.51$, $p = 0.0001$, and diastolic, $r = -0.47$, $p = 0.0001$, blood pressures (Fig. 2) and plasma concentrations of insulin, $r = -0.41$, $p < 0.001$, and HDL-cholesterol, $r = 0.48$, $p = 0.0001$ (Fig. 3). However, the EC_{50} for CGP 12177 showed no correlation to the plasma concentrations of glucose, triglycerides and cholesterol. Since EC_{50} for CGP 12177 was also correlated to BMI ($r = -0.57$, $p = 0.0001$) and the sagittal diameter ($r = -0.60$, $p = 0.0001$), the relative importance of WHR for β_3-adrenoceptor sensitivity was investigated in a multiple regression analysis. Using EC_{50} for CGP 12177 as the dependent variable and WHR, BMI and sagittal diameter as independent variables revealed that WHR was independently correlated to β_3-adrenoceptor sensitivity ($p = 0.0001$). As previously shown [15], β_3-adrenoceptor sensitivity was also correlated to fat-cell volume ($r = 0.70$, $p = 0.0001$). Finally, no correlations were seen between EC_{50} for dobutamine or terbutaline and any of the parameters mentioned.

In order to calculate the relative importance of WHR and β_3-adrenoceptor sensitivity for metabolic complications in upper-body obesity, these parameters were entered as independent variables in a multiple regression analysis as opposed to the previously mentioned metabolic parameters and blood pressures as the dependent variables. In this analysis, only WHR was correlated to the various clinical parameters, indicating a strong covariation between WHR and β_3-adrenoceptor sensitivity.

Since the rate of lipolysis is also an important measure of adrenoceptor function, we have also measured lipolysis induced by noradrenaline, which is the most important endogenous stimulator of lipolysis. Based on the WHR subgroups, the maximum noradrenaline-induced amounts of glycerol released ($\mu mol \cdot 10^7\ cells^{-1} \cdot 2\ h^{-1}$) were 21.6 ± 2.9, 15.8 ± 1.8

and 8.8 ± 2.1 in the high, intermediate and low WHR subgroups, respectively (mean ± SEM). As calculated by ANOVA, these values were statistically different ($p < 0.01$). Furthermore, EC_{50} for CGP 12177, in contrast to dobutamine- and terbutaline-sensitivity, correlated to the maximum noradrenaline glycerol release ($r = 0.47$, $p = 0.0005$), which indicates that β_3-adrenoceptor function may also be important for lipolysis rate, in analogy with our previous findings [15].

The importance of smoking habits and gender were also examined using ANOVA. No significant differences between smokers and non-smokers were observed. However, the male subjects had significantly higher WHR values (1.03 ± 0.01 vs 0.94 ± 0.01, $p = 0.0001$) and sagittal diameter (27.8 ± 1.22 vs 24.6 ± 0.99, $p < 0.05$) than the female subjects. In order to evaluate the influence of gender on β_3-adrenoceptor sensitivity, the WHR values were again subdivided, as previously described, for the males and females separately, resulting in high (males > 1.07, $n = 7$, females > 0.99, $n = 10$), intermediate (males 0.99–1.07, $n = 14$, females 0.90–0.99, $n = 18$) and low (males < 0.99, $n = 6$, females < 0.90, $n = 10$) WHR subgroups. Regarding the males, EC_{50} for CGP 12177 were 9.38 ± 0.27, 8.88 ± 0.27 and 7.93 ± 0.47 in the high, intermediate and low WHR subgroups, respectively (mean ± SEM, $p < 0.05$, ANOVA). The female values were 8.64 ± 0.33 (high), 7.69 ± 0.30 (intermediate) and 6.91 ± 0.36 (low), respectively ($p < 0.01$, ANOVA).

The influence of gender on the clinical variables was further analysed comparing the male and female subgroups with analysis of covariance using gender as a factor, WHR and β_3-adrenoceptor sensitivity as covariates and insulin, HDL-cholesterol, triglycerides, systolic and diastolic blood pressures as dependent variables. However, no significant interaction of sex on the relationships between the covariates and the clinical parameters was demonstrated in this patient material.

Discussion

In the present study, we provide evidence of an association between the metabolic complications accompanied by abdominal obesity (the metabolic syndrome) and the β_3-adrenoceptor sensitivity in visceral fat. Subjects with high WHR, as an index for abdominal obesity, were found to have higher blood pressure levels and higher plasma concentrations of insulin, glucose and triglycerides, but lower plasma concentrations of HDL-cholesterol than non-obese subjects. Furthermore, the visceral fat cells of the upper-body obese subjects were more sensitive to lipolytic stimulation by the β_3-adrenoceptor agonist CGP 12177. However, lipolysis induced by dobutamine (a β_1-adrenoceptor agonist) or terbutaline (a β_2-adrenoceptor agonist) did not differ between upper-body obese and non-obese subjects.

Although obesity is associated with various metabolic aberrations such as glucose intolerance and dyslipoproteinaemia, not all obese subjects present these findings. As reviewed [3–6], an increase in the intra-abdominal or visceral fat mass is strongly related to a cluster of metabolic disturbances (hyperinsulinaemia, glucose intolerance, hypertriglyceridaemia, low-plasma HDL-cholesterol) and hypertension, which together constitute the insulin resistance or metabolic syndrome. Moreover, visceral obesity is a key risk factor for cardiovascular disease and non-insulin-dependent diabetes mellitus [3–6]. The regional distribution of body fat is thus of importance for the development of the metabolic syndrome.

In this regard, the role of NEFA derived from intra-abdominal adipose tissue is thought to be particularly important. It is well known that visceral fat cells have higher lipolytic activity (hydrolysis of triglycerides into NEFA and glycerol) than subcutaneous adipocytes in normal subjects [3, 4, 6]. In visceral obesity, characterized by an increase in the intra-abdominal fat mass [23], it has been shown, firstly, that the fasting plasma concentration of NEFA is elevated [24], secondly, that the lipolysis rate in hypertrophic adipocytes is higher than in normal-sized fat cells [25] and, thirdly, that the inhibitory action of insulin on NEFA release from intra-abdominal adipocytes is lower than in subcutaneous fat cells [26]. Altogether, these observations indicate an augmented release of NEFA from the visceral fat depots in upper-body obesity, although the pathophysiological mechanisms have not yet been fully elucidated.

The β_3-adrenoceptor function was found to be strongly related to visceral obesity and its various metabolic disturbances. An increased β_3-adrenoceptor sensitivity to catecholamine stimulation might thus be the underlying mechanism linking enhanced lipolytic activity in visceral fat to the aberrations observed in the metabolic syndrome. Catecholamine stimulation may lead to an increased delivery of NEFA into the portal venous system, with several possible effects on liver metabolism. NEFA are known to stimulate hepatic VLDL secretion and gluconeogenesis and to interfere with hepatic clearance of insulin [5], resulting in dyslipoproteinaemia, glucose intolerance and hyperinsulinaemia. Since hyperinsulinaemia seems to be causally related to an increase in blood pressure [27, 28], hypertension may also ensue.

As regards the β_1- and β_2-adrenoceptor function, their sensitivities were not found to be associated with visceral obesity or any of the metabolic parameters. The current findings contrast with previous studies on abdominal subcutaneous adipocytes, in which catecholamine resistance, due to a reduced expression

Table 1. Clinical and biochemical characteristics of non-obese and obese subjects according to the presence of the Trp64Arg mutation

Characteristic	Non-obese subjects		Obese subjects	
n	mutation 10	no mutation 30	mutation 10	no mutation 43
Sex (female/male)	7/3	17/3	7/3	25/18
Smokers (yes/no)	4/6	7/23	2/8	13/30
Age (years)	41 ± 8	40 ± 11	38 ± 10	38 ± 9
Body mass index (kg/m^2)	24.9 ± 1.5	23.6 ± 2.4	41.9 ± 9.9	40.9 ± 6.7
Sagittal diameter	20.9 ± 1.6	19.8 ± 2.7	29.1 ± 5.6	30.9 ± 3.9
Waist-hip ratio				
Overall	0.95 ± 0.04	0.91 ± 0.07	0.99 ± 0.07	1.00 ± 0.07
Men	0.98 ± 0.02	0.98 ± 0.05	1.06 ± 0.03	1.05 ± 0.04
Women	0.93 ± 0.04	0.87 ± 0.06[a]	0.96 ± 0.06	0.97 ± 0.06
Blood pressure (mm Hg)				
Systolic	129 ± 21	124 ± 13	135 ± 21	140 ± 17
Diastolic	78 ± 10	77 ± 6	84 ± 11	88 ± 11
Plasma insulin (mU/l)	8.6 ± 4.0	8.1 ± 4.2	20.0 ± 83	20.4 ± 12.4
Blood glucose (mmol/l)	5.0 ± 0.3	5.2 ± 0.5	5.6 ± 0.5	6.0 ± 1.6
Plasma triglycerides (mmol/l)	1.4 ± 0.6	1.4 ± 0.7	1.0 ± 0.7	2.8 ± 3.2
Plasma cholesterol (mmol/l)	5.2 ± 0.9	5.1 ± 1.0	5.5 ± 0.9	5.9 ± 1.4
Plasma HDL-cholesterol (mmol/l)	1.4 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.2 ± 0.4
Fat cell volume (pl)	280 ± 147	282 ± 175	572 ± 264	641 ± 197
Lipolysis				
Noradrenaline				
EC_{50} (log mol/l)	−8.3 ± 0.6	−8.0 ± 0.8	−8.2 ± 0.6	−8.2 ± 0.7
Responsiveness (μmol · 10^7 cells^{-1} · 2h^{-1})	9.3 ± 7.4	9.8 ± 6.7	16.4 ± 10.9	19.3 ± 9.8
CGP 12177				
EC_{50} (log mol/l)	−7.6 ± 1.3	−7.3 ± 1.3	−8.2 ± 1.3	−8.8 ± 1.2
Responsiveness (μmol · 10^7 cells^{-1} · 2 h^{-1})	3.9 ± 2.7	3.7 ± 2.7	7.0 ± 5.6	8.1 ± 5.1

Values are mean ± SD. They were compared using Student's unpaired *t*-test. [a] = $p < 0.05$

non-obese Trp64 homogygous women than in Trp64Arg heterozygous non-obese women. However, sagittal diameter and body mass index did not differ significantly between these two groups (data not shown).

Genotypes of all subjects were determined by PCR amplification and cleavage with the BstNI restriction enzyme which is specific for the C to T transition in the first codon of amino acid 64 of the beta$_3$-adrenergic receptor. All Trp64Arg heterozygotes were analysed twice to ensure that full cleavage was attained of the PCR fragments.

The lipolytic response of fat cells to stimulation with either noradrenaline or CGP 12177 is shown in Figure 1. Both agents caused a concentration-dependent stimulation of glycerol release. They were more effective in fat cells of obese as compared with non-obese subjects confirming earlier results [3]. In neither group was there a statistically significant difference between Trp64 homozygotes and Trp64Arg heterozygotes.

It is also seen in Figure 1 that CGP 12177 was less effective than noradrenaline in stimulating glycerol release. This is expected, since CGP 12177 is a partial agonist. The mean concentration-response curves for CGP 12177 in Figure 1 do not display a classical sigmoid shape. This is due to considerable interindividual variation in the sensitivity of adipocytes to this agent flattening mean curves that are obtained from different individuals [3]. However, the individual curves were always steep spanning 4–5 log units of molar concentration.

Discussion

Recent data have suggested that molecular defects in the beta$_3$-adrenergic receptor may predispose subjects to obesity, complications to obesity and changes in the regulation of the energy expenditure [4–7]. However, this study suggests that the Trp64Arg mutation in the beta$_3$-adrenergic receptor gene is not a major determinant of beta$_3$-adrenergic receptor function and obesity when present in a heterozygous form.

We observed no influence on clinical characteristics in either obese or non-obese subjects except for waist-hip ratio in a subgroup, which probably is a chance finding due to the large number of statistical comparisons that were made. In man, beta$_3$-adrenergic receptors are mainly located in the visceral adipose tissue [2]. We found that visceral fat cells with the Trp64Arg mutation displayed normal lipolytic function of the beta$_3$-adrenergic receptor (as assessed by CGP 12177) and responded in a normal fashion

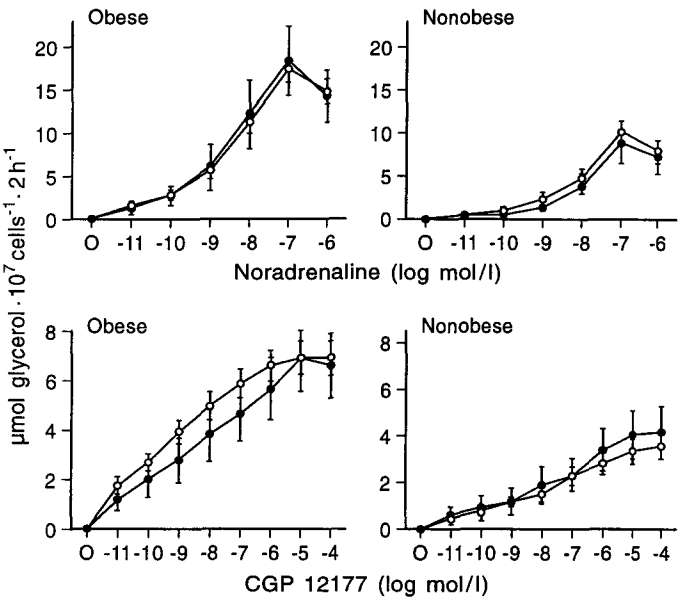

Fig. 1. Concentration response curves for noradrenaline and CGP 12177 as regards glycerol release from fat cells obtained from subjects with ■ or without □ the Trp64Arg mutation. Curves were compared using analysis of variance ($F < 1.0$). Values are mean ± SEM

to noradrenaline stimulation whether they were obtained from obese or non-obese subjects. Furthermore, the allelic frequency of the mutation was similar in obese and non-obese subjects which confirms previous findings [4–7]. It should be kept in mind that the number of subjects with the Trp64Arg mutation studied here was rather small. We cannot therefore exclude type II errors in some of the statistical calculations.

Unfortunately we have no data on subjects who are homozygous for the mutation. No homozygous subjects were found in previous investigations on 279 French and 335 Finnish subjects [5, 6]. This may suggest that homozygotes for the mutation are rare among Caucasians. However, in a study of 624 Pima Indians 9 % of subjects were found to be homozygous [4] and 5 % in an investigation of 191 Japanese subjects. Arg64 homozygote subjects had an earlier onset of non-insulin-dependent diabetes, higher serum insulin, increased body mass index and a tendency to lower resting metabolic rate than Trp64Arg heterozygotes who did not differ from normal Trp64 homozygotes. Thus, it is possible that the beta$_3$-adrenergic receptor gene mutation is of clinical and functional importance only in its homozygous form. Nevertheless, the beta$_3$-adrenergic mutation per se appears to play a relatively modest role in the pathophysiology of obesity, since the observed clinical differences between normal and mutant homozygotes were small [4, 7].

Mutations in the first cytoplasmatic lope of several other G-protein receptors such as the beta$_3$-adrenergic, rhodopsin, and vasopressin receptors are reported [8]. These mutations alter the receptor function. On the other hand, in normal weight rats the wild type beta$_3$-adrenergic gene carries Arg instead of Trp in codon 64 [9, 10]. Some caution should be exercised when comparing similar mutations between receptor subclasses or the same gene in different species. However, it is tempting to speculate that the first intracellular loop of the beta$_3$-adrenergic receptor is less important for this receptor than for other G-protein receptor subtypes. However, such a question can only be answered by site directed mutation analysis.

In summary, the present data suggest that the Trp64Arg mutation is not a major determinant of the beta$_3$-adrenergic receptor function or obesity and its complications at least not when present in the heterozygous form.

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