Diabetologia (1994) 37: 428–435

Diabetologia
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Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta$_2$-adrenoceptors

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Summary Upper-body obesity is an important risk factor for developing non-insulin dependent diabetes. To investigate the possibility that a lipolysis defect is present in this form of obesity, we examined the adrenergic regulation of lipolysis in abdominal subcutaneous fat cells from 25 women with upper-body obesity and 24 non-obese women. Lipolytic noradrenaline sensitivity (but not the maximum rate of lipolysis) was reduced by 10-fold in obese women ($p < 0.01$). The noradrenaline resistance could be ascribed to a 10-fold decrease in lipolytic beta$_2$-adrenoceptor sensitivity ($p < 0.01$). The lipolytic sensitivity of beta$_1$- and alpha$_2$-adrenergic receptors was normal in the obese women. A 70 % reduction in the cell surface density of beta$_2$-adrenoceptors was observed compared to the control subjects ($p < 0.01$). However, beta$_1$-receptor density as well as steady-state mRNA levels for beta$_1$- and beta$_2$-receptors were normal in obese women. Lipolytic noradrenaline sensitivity correlated inversely with BMI (adjusted $r^2 = 0.76$ together with fat cell volume in stepwise regression analysis). The fasting plasma level of free cortisol was 30 % lower in obese compared to non-obese women ($p < 0.05$) but obesity did not influence resting plasma catecholamine levels. Thus, lipolytic catecholamine resistance is present in abdominal obesity, due to low density of beta$_2$-adrenoceptors, which in its turn may be caused by a post-transcriptional defect in beta$_2$-receptor expression. Whether abnormalities in circulating free cortisol levels have caused the impaired lipolytic function of these receptors in upper-body obesity remains to be established. [Diabetologia (1994) 37: 428–435]

Key words Adipose tissue, beta-adrenoceptors, alpha-adrenoceptors, lipolysis, mRNA

Upper-body obesity is an important risk factor for developing non-insulin dependent diabetes mellitus. Although over-eating and physical inactivity are involved in the pathogenesis of obesity, endogenous factors, which are not well-defined, are also likely to contribute. Disturbances in the regulation of the energy balance may play a role in the development of obesity in certain individuals [1]. An attractive hypothesis is that a defect may exist in the control of mobilization of lipids through lipolysis from fat depots of obese subjects. Lipolysis is under intense hormonal control. In humans, catecholamines play a major role as they are the only hormones with a pronounced and acute lipolytic effect in adipose tissue of adult subjects [2].

Lipolysis in human fat cells can be stimulated by catecholamines through beta$_1$- and beta$_2$-adrenoceptors, and inhibited through alpha$_2$-adrenoceptors. The receptor subtypes should be considered separately. They can be regulated independently and the lipolytic effect of catecholamines depends upon the balance between stimulatory beta-receptors and inhibitory alpha$_2$-receptors [2, 3]. Little is known about adrenergic receptors in human obesity [4]. Previous investigations in vivo have shown blunted lipolytic effects of intravenous doses of catecholamines in obese subjects [5–7], which is most apparent in subjects with abdominal obesity [6]. However, a more detailed in vitro exam-

Received: 30 August 1993
and in revised form: 15 November 1993

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Abbreviations: ED50: Hormone concentration giving half maximum effect; pD$_2$: negative logarithm of ED50; amol: 10^{-18} mol.

ination of hormone-receptor interaction is necessary in order to evaluate the adrenoceptor status of fat cells in obesity. Several studies of experimental obesity, using different animal models, have shown altered function and expression of adipocyte $alpha_2$- and beta-adrenoceptors [8–12]. In this study, the lipolytic function, expression and pharmacological properties of several adrenoceptor subtypes have been investigated in abdominal subcutaneous adipocytes of non-obese women and women with upper-body obesity.

Subjects and methods

Patients and experimental protocol

The study comprised 25 women with upper-body obesity who were otherwise healthy and 24 healthy control women who never had been obese. Upper-body (abdominal) obesity was defined as BMI greater than 27.5 kg/m^2 and waist-hip ratio greater than 0.87. The latter value is mean -1 SD of the waist-hip ratio of a group of well-characterized Swedish women with abdominal obesity [13]. The subjects were not taking any medication. None had undertaken a weight reducing diet during the year preceding the study. They were either sedentary or undertook moderate exercise less than twice a week. Two women in each group were post-menopausal. The study was explained in detail to each participant and consent was obtained. It was approved by the ethics committee at the Karolinska Institute.

The women were examined at 08.00 hours after an overnight fast. For the pre-menopausal women this was done in the follicular phase of the menstruation cycle. First, after 30-min rest in the supine position, a venous plasma sample was obtained for the analysis of catecholamines [14], insulin, cortisol and the corticosteroid-binding globulin transcortin (commercial RIA assay from Kabi-Pharmacia, Uppsala, Sweden; Diagnostic Products, Calif., USA, and Medgenix Diagnostics SA, Fleurns, Belgium, respectively), as well as for glucose, cholesterol, HDL-cholesterol and triglycerides (the hospital's routine chemistry laboratory). Free plasma cortisol was calculated using total cortisol and transcortin as described by the manufacturer's instructions. Then a subcutaneous fat biopsy (2–3 g) was obtained during local anaesthesia from the abdominal area immediately to the left or right of the umbilicus.

Isolation of fat cells and determination of fat cell size and number

Isolated fat cells were prepared according to the collagenase method described by Rodbell [15]. Direct microscopic determination of the diameter of 100 fat cells was performed as described [16]. The coefficient of variation in ten experiments was 3 %. The same value was obtained using 200 cells. Mean fat cell volume, surface area and weight were determined as described [17].

The number of fat cells incubated were determined as follows. The lipid content was determined gravimetrically after extraction according to Dole and Meinertz [18]. The lipid content of the incubated fat cells was then divided with the mean fat cell weight, assuming that lipids constitute more than 95 % of the fat cell weight. This common method to determine fat cell number [19–21] was compared with a direct method [22], where all cells are counted in appropriately diluted aliquots of the cell suspension [22]. The correlation coefficient between the two methods as determined by linear regression analysis ($n = 10$) was 0.97.

Table 1. Clinical data for healthy non-obese control subjects and obese subjects

	Control subjects	Obese subjects	p
Age (years)	36 ± 3	41 ± 2	NS
BMI (kg/m^2)	22.5 ± 0.5	36.2 ± 0.8	–
Waist-hip ratio	0.86 ± 0.01	0.96 ± 0.01	–
Glucose (mmol/l)	4.7 ± 0.1	5.4 ± 0.2	< 0.001
Insulin (mU/ml)	7 ± 1	14 ± 1	< 0.001
Total cortisol (nmol/l)	493 ± 31	347 ± 31	< 0.01
Free cortisol (nmol/l)	29 ± 4	18 ± 1	< 0.05
Noradrenaline (nmol/l)	1.9 ± 0.8	1.8 ± 0.1	NS
Adrenaline (nmol/l)	0.13 ± 0.02	0.11 ± 0.01	NS
Glycerol (μmol/l)	70 ± 8	134 ± 12	< 0.05
Pulse (beats/min)	68 ± 2	72 ± 2	NS
Systolic blood pressure (mm Hg)	118 ± 3	128 ± 3	< 0.05
Diastolic blood pressure (mm Hg)	71 ± 2	78 ± 2	< 0.05
Fat cell volume (pl)	483 ± 31	903 ± 32	< 0.001
Cholesterol (mmol/l)	5.1 ± 0.2	6.0 ± 0.3	< 0.05
HDL-cholesterol (mmol/l)	1.7 ± 0.1	1.4 ± 0.1	< 0.05
Triglycerides (mmol/l)	0.9 ± 0.1	1.5 ± 0.2	< 0.01

The values are mean ± SEM. The groups were compared using the Student's t-test

Lipolysis experiments

Isolated fat cells were incubated as described in detail [23]. In brief, about 5–10,000 cells per ml were incubated in duplicate at 37°C in Krebs-Ringer phosphate (pH 7.4) containing albumin (20 g/l), glucose (1 g/l) and ascorbic acid (0.1 g/l) in the absence or presence of increasing concentrations (10^{-16}–10^{-4} mol/l) of noradrenaline, isoprenaline, terbutaline, dobutamine, clonidine, forskolin or dibutyryl cyclic AMP. In the clonidine experiments adenosine deaminase (1 mU/l) was added to the incubation medium in order to remove traces of adenosine which may interfere with the antilipolytic effect of clonidine [23]. It is, however, not necessary to add adenosine deaminase in the experiments the lipolytic agonists, since adenosine has no effect on agonist sensitivity (pD_2) or maximum lipolytic effect as discussed [23]. After 2 h, an aliquot was removed for the determination of glycerol [24]. The fat cells were viable during the whole study since the rate of glycerol release was linear for at least 4 h. The same batches of collagenase and buffer ingredients were used throughout the study. The rate of lipolysis was expressed per g lipids, per unit cell surface area (μmol of glycerol $\cdot 2\,h^{-1} \cdot mm^{-2}$) or per cell number (μmol of glycerol $\cdot 2\,h^{-1} \cdot 10^7$ cells^{-1}). In all subjects the agonists

Table 2. Basal and maximum rates of lipolysis

	Glycerol release							
	Basal		Noradrenaline		Isoprenaline		Forskolin	
	Cell	Surface	Cell	Surface	Cell	Surface	Cell	Surface
Control subjects	5 ± 1	17 ± 2	15 ± 1	53 ± 5	28 ± 2	98 ± 7	26 ± 2	85 ± 7
Obese subjects	14 ± 1	32 ± 3	27 ± 3	60 ± 6	40 ± 4	90 ± 8	36 ± 4	80 ± 8
p	< 0.001	< 0.001	< 0.001	NS	< 0.01	NS	< 0.05	NS

The glycerol release is expressed per cell ($\mu mol \cdot 2\ h^{-1} \cdot 10^7\ cells^{-1}$) or per cell surface area ($pmol \cdot 2\ h^{-1} \cdot mm^{-2}$). The values for noradrenaline, isoprenaline and forskolin are rates at the maximum effective agonist concentration. p = statistical comparison of values from control and obese subjects using Student's unpaired t-test. Values are mean ± SEM

Table 3. Correlation between fat cell volume and lipolysis

	Lipolysis			
	Basal	Noradrenaline	Isoprenaline	Forskolin
r	0.65	0.54	0.52	0.49
p	< 0.001	< 0.001	< 0.001	< 0.001

The correlation between fat cell volume and lipolysis rate was determined by linear regression analysis. Lipolysis was related to fat cell number. Noradrenaline, isoprenaline and forskolin values are the rates at the maximum effective agonist concentrations. Results with control subjects and obese subjects were pooled together

always caused a dose-dependent stimulation or inhibition of glycerol release, which in each case reached a plateau at the highest agonist concentrations. ED_{50} was determined using computerized fitting of each dose-response curve as described previously [25]. The negative logarithm of the ED_{50} value (pD_2) was defined as adrenoceptor sensitivity.

Beta-adrenoceptor binding studies

The receptor binding studies have been described in detail [26]. Isolated fat cells (20,000 cells per ml), were incubated at 37 °C in 0.5 ml of Krebs Ringer phosphate buffer (pH 7.4) containing albumin (5 g/l), glucose (1 g/l) and ascorbic acid (0.1 g/l). Saturation experiments were performed to determine the total amount of beta-adrenoceptors. The cells were incubated in duplicate for 60 min with six different concentrations of ^{125}I-cyanopindolol. Non-specific binding (in the presence of 0.1 μmol/l of propranolol) was about 30 % at low and about 45 % at high radioligand concentrations. In duplicate competition experiments, performed to determine the fraction of $beta_2$-adrenoceptors in the total beta-receptor population, 100 pmol/l of ^{125}I-cyanopindolol competed with 12 increasing concentrations of the $beta_2$-specific antagonist ICI 118,551 (10^{-11}–10^{-4} mol/l). Non-specific binding (at 10^{-4} mol/l of ICI 118,551), was about 30 %. The saturation experiments were evaluated by linear regression analysis of Scatchard plots [27]. Displacement curves were analysed by a non-linear least squares regression method [28] which provides an estimate of the relative proportions of high affinity $beta_2$-receptors and low affinity $beta_1$-receptors, as well as the affinity (K_d) of these receptors for the displacing drug, ICI 118,551 [29]. The maximum total binding capacity obtained from saturation binding was multiplied with the fraction of high- and low-affinity binding sites for ICI 118,551. This represents the maximum binding capacity of $beta_2$- and $beta_1$-receptors, since no binding for $beta_3$-receptors is detected with ^{125}I-cyanopindolol in the present type of experiments [30]. Binding capacity was expressed per unit cell surface area, which reflects the receptor density.

Measurements of mRNA

The solution hybridization assays for $beta_1$- and $beta_2$-receptor mRNA in isolated fat cells have been described in detail [31]. Briefly, complementary oligonucleotide probes corresponding to nucleotide 739–789 for $beta_1$-receptors and to nucleotide 772–822 for $beta_2$-receptors were synthesized in vivo with the plasmid pGEM-3 to produce cRNA, which was radiolabelled with (^{35}S)-UTP. About 150 μl of adipocytes were homogenized and digested with proteinase K and total nucleic acids were extracted with phenol-chloroform. The (^{35}S)-UTP cRNA was hybridized at 70 °C to total nucleic acid samples. Non-hybridized material was digested with RNase. RNase-resistant material was precipitated and collected on a glass filter. Sample hybridization was compared with a known amount of in vitro synthesized mRNA strand complementary to the radioactive probe. The amount of mRNA was related to the amount of DNA as described before [26] and to the amount of mRNA for the "house-keeping" gene beta-actin [32] which was also measured by solution hybridization in the extract [33].

Drugs and chemicals

Bovine serum albumin (fraction V, lot 63F-0748), *Clostridium histolyticum* collagenase type I, glycerol kinase from *Escherichia coli* (G4509), forskolin, dibutyryl cyclic AMP and dl-propranolol were obtained from Sigma (St Louis, Mo., USA). (–) – Isoprenaline hydrochloride came from Hässle (Mölndal, Sweden), terbutaline sulphate from Draco (Lund, Sweden), dobutamine hydrochloride from Eli Lilly (Indianapolis, Ind., USA) and ICI 118,551 from Cambridge Research Biochemicals Limited (Cheshire, UK). CGP 20712A was kindly supplied by Ciba Geigy (Basel, Switzerland). ATP monitoring reagent containing firefly luciferase was from LKB Wallac (Turku, Finland). pGEM-3 was from Promega Biotel (Madison, Wis., USA). SPG RNA polymerase and T7 RNA polymerase were obtained from Boehringer Mannheim (Mannheim, Germany). ^{35}S-UTP and ^{125}I-cyanopindolol were from New England Nuclear (Boston, Mass., USA). All other chemicals were of the highest grade of purity commerically available.

Statistical analysis

The Student's two-tailed t-test was used for comparison of data between and within groups. SEM was used as a measure of dispersion. In some cases linear or stepwise multiple regression analysis was performed. All statistics were determined by means of a software statistical package (Stat View II, 1987; Abacus Concepts, Inc, Berkeley, Calif., USA). Values for K_d, ED_{50} and pD_2 were transformed into their logarithmic form when statistically examined.

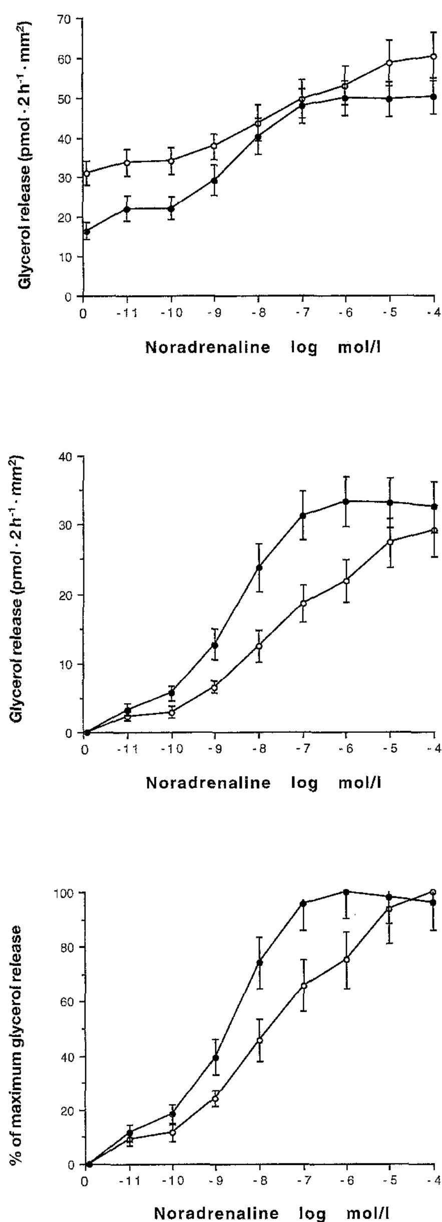

Fig. 1. Noradrenaline-induced lipolysis in control subjects (●) and obese subjects (○). Isolated fat cells were incubated with or without the indicated concentrations of noradrenaline and glycerol release to the incubation medium was determined and used as lipolysis index. The mean dose-response curves are shown per unit cell surface area, with basal lipolysis included (top), or subtracted (center) and (bottom) in percent of glycerol release at the maximum effective hormone concentration (which was set at 100 %)

Results

Clinical data are presented in Table 1. There was no statistical difference in age between the groups. By definition the obese women had a higher BMI and waist-hip ratio and, as expected, an increased fat cell volume compared to the non-obese women. Blood pressure as well as fasting plasma levels of insulin, glucose, glycerol, cholesterol, and triglycerides were increased in the obese women who also had decreased plasma HDL-cholesterol. Total and free plasma cortisol levels were unexpectedly 30 % lower in the obese group ($p < 0.05$). The plasma catecholamine level did not differ between the groups.

Data with basal and maximum stimulated glycerol release are found in Table 2. The rates of forskolin-, isoprenaline- and noradrenaline-induced lipolysis per unit cell surface area at the maximum effective agonist concentrations were similar in obese compared to non-obese women. The same was true for lipolysis induced by dibutyryl cyclic AMP (data not shown). However, the maximum agonist-induced rate of lipolysis per cell number was increased in the obese women. Basal lipolysis, on the other hand, was increased in the obese subjects regardless of the use of denominator for glycerol release. Since fat cell volume differed markedly between the groups, the observed variations in lipolysis per cell number may reflect variations in cell size. This was tested using linear regression analysis of glycerol release/cell number vs fat cell volume (Table 3). All lipolysis parameters were significantly dependent on fat cell volume (r from 0.5 to 0.7).

Figure 1 shows the mean dose response curves for noradrenaline in the two groups. Noradrenaline caused a dose-dependent stimulation of lipolysis. The obese subjects had a high basal rate of lipolysis but a reduced effect of the addition of noradrenaline which is most evident when the basal values are subtracted. To further evaluate hormone sensitivity the dose-response curves were plotted relative to the maximum lipolytic effect. In this case the results are completely independent of the denominator used or of basal lipolysis. The mean dose-response curve for the obese subjects was then markedly shifted to the right compared to that of the control subjects. At 10^{-8} mol/l of noradrenaline (which is a plasma concentration usually observed during physiological challenge) lipolysis was stimulated by 80 % of the maximum rate in control subjects and only by 40 % in the obese subjects.

Lipolytic catecholamine sensitivity was further investigated in experiments with more selective adrenergic agonists (Fig. 2). The mean dose-response curves for the non-selective beta-agonist isoprenaline and the selective beta$_2$-agonist terbutaline were shifted to the right in the obese compared to the control subjects. On the other hand, the sensitivity to the beta$_1$-agonist dobutamine and the alpha$_2$-agonist clonidine was not influenced by obesity nor was there any difference between groups as regards the maximum antilipolytic effect of clonidine (70–80 % inhibition of the basal rate).

The individual values for lipolytic sensitivity of the adrenergic agonists (pD$_2$) were statistically compared in Table 4. The sensitivity to noradrenaline and terbutaline was decreased by 10-fold ($p < 0.01$) and to isoprenaline, by almost 100-fold ($p < 0.001$) in obesity. No significant difference between the groups was observed regarding sensitivity to dobutamine or clonidine.

The intrinsic activities (maximum lipolytic action in relation to maximum lipolytic action of isoprenaline) were 0.90 ± 0.03 and 0.92 ± 0.2 for terbutaline in the

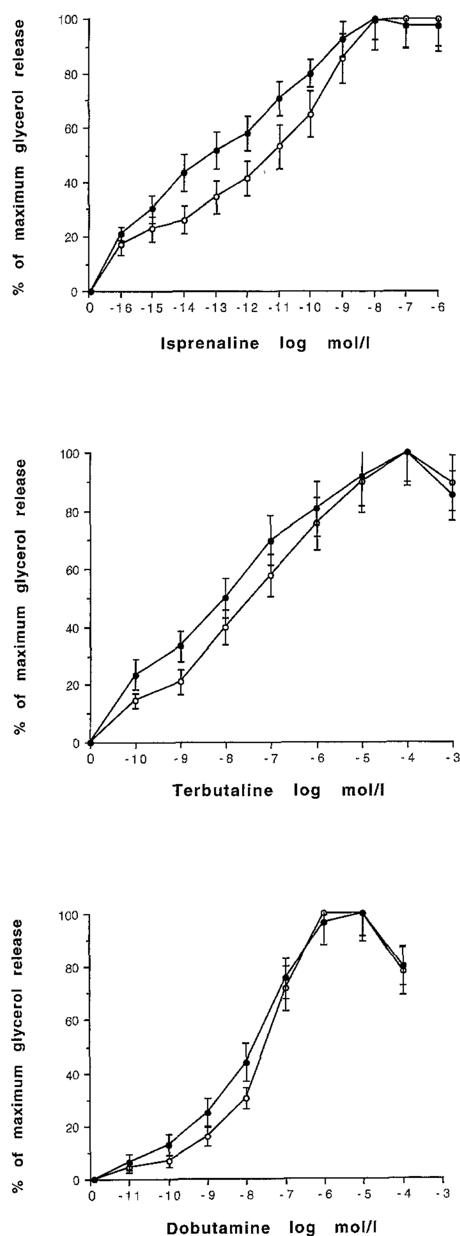

Fig. 2. Lipolysis induced by isoprenaline (non-selective beta-agonist), terbutaline (selective $beta_2$-agonist) and dobutamine (selective $beta_1$-agonist) in control subjects (●) and obese subjects (○). The mean dose response curves are shown as percent of glycerol release at the maximum effective hormone concentration (which was set at 100 %)

control and obese groups, respectively. The corresponding values for dobutamine were 0.92 ± 0.02 and 0.93 ± 0.02. These values did not differ significantly between the groups.

Data with radioligand binding are shown in Table 5. The maximum binding to $beta_2$-adrenoceptors was reduced by about 70 % in the obese compared to the non-obese women ($p < 0.01$). Neither the maximum binding to $beta_1$-receptors nor $beta_1$ or $beta_2$-receptor affinity (K_d for ICI, 118,551) were altered in obesity. The total number of beta receptor binding sites per cell did not differ significantly between the two groups. K_d for the radioligand ^{125}I-cyanopindolol in the saturation binding experiments was 65 ± 8 and 78 ± 10, respectively, in control and obese subjects. These values did not differ significantly. The Hill coefficient in the saturation binding experiments was 0.95 ± 0.03 in both groups together, which was not significantly different from 1.0.

The steady-state mRNA levels for $beta_1$- and $beta_2$-adrenoceptors were similar in obese and control subjects. The values were 929 ± 156 and 1176 ± 154 amol/μg DNA, respectively, for $beta_1$ and 1063 $\pm$ 123 and 1024 ± 124 amol/μg, for $beta_2$. Beta-adrenoceptor mRNA was also related to mRNA for the "house-keeping" gene beta-actin. These values (amol of beta-receptor subtype mRNA/amol of beta-actin mRNA) were also similar in obese and control subjects: 0.49 ± 0.14 and 0.48 ± 0.12, respectively, for $beta_1$-receptors and 0.52 ± 0.15 and 0.42 ± 0.15, for $beta_2$-receptors.

The relationship between lipolytic noradrenaline sensitivity BMI was also investigated (Table 6). Linear regression analysis showed a significant negative correlation (-0.49) between noradrenaline sensitivity and BMI. Since BMI may be influenced by several factors, a stepwise multiple regression analysis was performed using BMI as the dependent variable and age, noradrenaline-sensitivity, waist-hip ratio, fat cell volume, basal lipolysis as well as plasma insulin and total cortisol levels as independent variables. The variable with the highest partial correlation coefficient was entered at each step until no variable remained with an F-value of 4 or more. Noradrenaline sensitivity and fat cell volume were the only regressors that significantly contributes to the relationship. Fat cell volume entered as the first step ($F = 100$) and noradrenaline sensitivity as the last step ($F = 11$). Adjusted r^2 for these two variables together in the regression was 0.76. Plasma insulin, which was a strong regressor in single regression analysis ($r = 0.68$) failed to contribute to the variation in BMI using the stepwise analysis ($F = 1.1$). Comparing the total and partial correlation coefficients in Table 6 it can be noted than the r-value for noradrenaline pD_2 was almost the same in single and multiple regression, whereas for all other parameters it was much lower in multiple than in single regression analysis. Neither age nor free plasma cortisol showed any significant relation to BMI nor to any of the lipolysis or receptor binding parameters (data not shown).

Discussion

This study describes for the first time a cellular defect in lipolysis regulation which may contribute to the development of upper-body obesity in women. These women displayed many characteristics of the abdominal type of obesity; insulin resistance, increased blood pressure and dyslipidaemia. The fat cell volume differed markedly between the obese and the non-obese women. This difference does not affect evaluation of the sensitivity of the cells to hormone action (pD_2),

Table 4. Sensitivity to adrenergic agonists in control subjects and obese subjects

	pD_2				
	Noradrenaline	Isoprenaline	Terbutaline	Dobutamine	Clonidine
Control subjects	8.5 ± 0.2	12.8 ± 0.4	8.4 ± 0.3	7.6 ± 0.2	9.8 ± 0.2
Obese subjects	7.4 ± 0.2	11.2 ± 0.3	7.4 ± 0.2	7.4 ± 0.1	9.5 ± 0.2
p	< 0.001	< 0.01	< 0.01	NS	NS

pD_2 is the negative logarithm of the concentration (mol/l) of adrenergic agonist giving half-maximum effect in the lipolysis dose-response experiments. The values (mean ± SEM) were statistically compared using Student's unpaired *t*-test

Table 5. $Beta_1$- and $beta_2$-adrenoceptor binding parameters

	Binding capacity (amol/μm²)		Binding affinity (log mol/l)	
	$Beta_1$	$Beta_2$	$Beta_1$	$Beta_2$
Control subjects	1.6 ± 0.2	2.6 ± 0.3	–5.5 ± 0.1	–7.8 ± 0.1
Obese subjects	1.8 ± 0.2	1.2 ± 0.1	–5.7 ± 0.1	–8.0 ± 0.1
p	NS	< 0.001	NS	NS

Receptor binding was determined as described in Subjects and methods and is expressed per unit of cell surface area. Maximum binding capacity of ^{125}I-cyanopindolol was obtained from saturation and displacement experiments. Binding affinity is the K_d of ICI 118,551 for high- ($beta_2$) and low-affinity ($beta_1$) binding sites. *p* is statistical comparison of controls vs obese using Student's unpaired *t*-test. Values are mean ± SEM

Table 6. Correlation between BMI and clinical or adipocyte parameters

	Single regression		Stepwise regression	
Parameter	*r*	*p*	Partial *r*	*F*
Age	0.17	NS	–0.16	1.3
Fat cell volume	0.83	< 0.001	0.67	100
Noradrenaline pD_2	–0.49	< 0.001	–0.48	11
Waist-hip ratio	0.70	< 0.001	0.06	1.7
Plasma cortisol	–0.39	< 0.01	–0.22	2.4
Plasma insulin	0.68	< 0.001	0.10	1.1
Basal lipolysis per cell surface unit	0.50	< 0.001	0.13	1.8

All parameters were included together in the multiple regression analysis. Each regressor with an *F*-value of 4.0 or more was considered to contribute significantly to the relationship with BMI

which is the major aspect of lipolysis regulation examined in this study. It is, however, necessary to consider the use of a proper denominator for the maximum rates of catecholamine-induced lipolysis, since results based on cell number or unit of cell surface area usually differ when a difference in fat cell volume is found in the investigated material [34–37]. We found that lipolysis rates per cell correlated significantly with fat cell volume ($r = 0.5$–0.7), making it impossible to know to which extent the observed differences in lipolysis per cell number between the groups only reflected the differences in fat cell volume. Considering that the interaction of catecholamines with the fat cell is at the surface of the plasma membrane, we have chosen to express data per unit of cell surface area which may be more physiological in a hormone study, as pointed out previously [34–37].

A 10-fold decrease in the lipolytic sensitivity of noradrenaline was observed in obese compared to non-obese subjects. At a physiological noradrenaline concentration (10 nmol/l) the adipocytes of the obese only reached 40 % of their maximum lipolytic capacity while lipolysis was almost maximally stimulated in the cells of the control subjects. These data confirm previous in vivo findings as regards lipolytic noradrenaline resistance in upper-body obesity [6]. When the influence of cell size is accounted for, our data suggest normal maximum lipolytic capacity, increased basal lipolysis rates and a blunted response to catecholamine stimulation in fat cells of upper-body obese women. This may indicate a decreased ability of the obese to mobilize lipids during physiological challenge such as fasting or exercise in spite of increased lipolytic rate at rest.

The cellular mechanism responsible for the observed catecholamine resistance in obesity was solely localized to the $beta_2$-adrenoceptors. The antilipolytic function of $alpha_2$-receptors as well as $beta_1$-receptor density and lipolytic sensitivity were normal in obese women, while a 10-fold reduction in $beta_2$-receptor subtype sensitivity was found in adipocytes of the obese

subjects. A 70% reduction in the cell surface density of $beta_2$-receptors was found. This may explain the observed catecholamine resistance but it is possible that additional defects in the coupling of $beta_2$-receptors to the so-called G_s-protein also are present in obesity. Whether the decrease in $beta_2$-adrenoceptor density is due to changes in synthesis, degradation or internalization of the receptor subtype remains to be established. Unfortunately, it is not possible to study these processes (or coupling) in the small amounts of adipose tissue which can be obtained clinically. However, the negative findings with beta-receptor mRNA suggest alterations at a level beyond gene expression (i.e. post-transcriptionally).

The question remains whether the observed abnormality in lipolysis regulation of obese subjects is associated primarily or secondarily with the obese state. This might possibly be answered by investigating lipolysis in post-obese subjects. It is an intriguing possibility that disturbances in cortisol metabolism might contribute to the lipolysis defect. As reviewed [2], cortisol improves $beta_2$-adrenergic effects in fat cells. The fasting plasma free cortisol level in the obese women was two-thirds of that of the non-obese women. This suggests that there might be some defect in cortisol metabolism in women with upper-body obesity. As discussed recently [38] previous investigations of cortisol in obesity have demonstrated low or normal total circulating levels but increased urinary levels. Insulin is a potent antilipolytic agent which interacts with catecholamines and exerts a rapid effect on lipolysis in vitro which can at least in part be mediated by down-regulation of beta-adrenoceptors in human fat cells [39]. This acute effect of insulin on adrenoceptors is however non-selective as regards the receptor subtypes (P. Arner and Marcus, unpublished observations). On the other hand, it is possible that the mild but chronic hyperinsulinaemia found in the obese could have selective down-regulatory effects on $beta_2$-adrenoceptor function in obesity.

The recently cloned $beta_3$-adrenoceptor is also present in human fat cells at the level of mRNA [40]. However, previous data suggest that this receptor is poorly expressed on a functional level in human subcutaneous adipose tissue [30, 41]. Although, some of the present data with noradrenaline can be influenced by this receptor subtype it is not likely that the findings with terbutaline or dobutamine are influenced by interactions with the $beta_3$-receptor. Likewise, the Hill coefficient for ^{125}I-cyanopindolol was almost 1.0, suggesting almost no contribution of $beta_3$-receptors to the results with binding.

The extent to which catecholamine resistance in fat cells can contribute to the development of obesity is unknown. Stepwise regression analysis suggested that noradrenaline sensitivity, and fat cell volume together were responsible for 76% of the variations in BMI among the subjects. This figure is surprisingly high considering the large number of possible factors which were not tested and which may contribute to the development of obesity in a particular individual.

Some information about adrenoceptor sensitivity in obese men with abdominal obesity is available, showing normal beta-receptor activity but increased $alpha_2$-receptor activity in abdominal subcutaneous fat cells [42]. These data are entirely different from the present findings and suggest that the pathogenesis of obesity in men and women differs markedly regarding involvement of defects in lipolysis regulation. Sex variations are possible, considering the differences in fat distribution, lipolysis regulation and complications of obesity that exist among women and men [43].

We do not know how our data relate to adipocytes in other regions than the subcutaneous abdominal site. We studied the latter region because it is more sensitive to regulation than peripheral subcutaneous adipose tissue [42]. Studies of men, though, have revealed regional differences in the influence of obesity on lipolysis regulation [42]. Furthermore, the present study included only upper-body obese women and does therefore not address the issue of possible differences between obese women with different body composition. A recent in vivo study [6] has indeed shown that the lipolytic rates differ between upper- and lower-body obese women.

In summary, the present study shows for the first time a cellular defect in lipolysis regulation which may contribute to the development of abdominal obesity in women. Adipocytes of these subjects are resistant to catecholamines due to decreased cell surface density of $beta_2$-adrenoceptors, which in turn may be due to a post-transcriptional defect in receptor subtype expression. Whether this defect is primarily or secondarily associated with obesity or involves abnormalities in circulating free cortisol remains to be established.

Acknowledgements. This study was supported by grants from the Swedish Medical Research Council, the Swedish Diabetes Association, the Karolinska Institute, the Foundations of Osterman, Wiberg, Nordic Insulin, Old Female Servants, Golje, King Gustav V and Trygg-Hansa.

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