A pathogenic role of visceral fat beta 3-adrenoceptors in obesity.

F Lönnqvist, … , J Hoffstedt, P Arner

J Clin Invest. 1995;95(3):1109-1116. https://doi.org/10.1172/JCI117758.

Research Article

A Pathogenic Role of Visceral Fat β_3-Adrenoceptors in Obesity

Fredrik Lönnqvist, Anders Thörne,* Klas Nilsell,* Johan Hoffstedt, and Peter Arner
*Departments of Medicine and *Surgery, Huddinge University Hospital, Huddinge, Sweden; and the Research Center, Karolinska Institute, Stockholm, Sweden*

Abstract

Increased release of free fatty acids (FFA) from visceral fat cells to the portal venous system may cause several metabolic disturbances in obesity. However, this hypothesis and the underlying mechanism remain to be demonstrated. In this study catecholamine-induced lipid mobilization through lipolysis in omental adipose tissue was investigated in vitro in 25 markedly obese subjects (body mass index range 35–56 kg/m^2) undergoing weight reduction surgery and in 19 nonobese subjects (body mass index range 20–28 kg/m^2) undergoing cholecystectomy. Release of FFA and glycerol, induced by norepinephrine or adrenergic receptor subtype–specific agonists, were determined in isolated omental fat cells. The obese subjects had higher fat cell volume, blood pressure, plasma insulin levels, blood glucose, plasma triglycerides, and plasma cholesterol than the controls. There was evidence of upper-body fat distribution in the obese group. The rate of FFA and glycerol response to norepinephrine was increased twofold in the cells of obese subjects; no significant reutilization of FFA during catecholamine-induced lipolysis was observed in any of the groups (glycerol/FFA ratio near 1:3). There were no differences in the lipolytic sensitivity to β_3- or β_2-adrenoceptor specific agonists between the two groups. However, β_3-adrenoceptor sensitivity was ~ 50 times enhanced (P = 0.0001), and the coupling efficiency of these receptors was increased from 37 to 56% (P = 0.01) in obesity. Furthermore, the obese subjects demonstrated a sixfold lower alpha$_2$-adrenoceptor sensitivity (P = 0.04). β_3-Adrenoceptor sensitivity, but not alpha$_2$-, β_1-, or β_2-adrenoceptor sensitivity, correlated with norepinephrine-induced lipolysis (r = −0.67, P = 0.0001) and fat cell volume (r = −0.71, P = 0.0001).

In conclusion, catecholamine-induced rate of FFA mobilization from omental fat cells is accelerated due to elevated rate of lipolysis in obesity, mainly because of an increased β_3-adrenoceptor function, but partly also because of a decreased alpha$_2$-adrenoceptor function. This promotes an increased release of FFA to the portal system, which may contribute to the parallel metabolic disturbances observed in upper-body obesity. (*J. Clin. Invest.* 1995. 95:1109–1116.)
Key words: adrenoceptors • β_3-adrenoceptor • fat cells • free fatty acids • lipolysis

Introduction

It has long been established that obesity is associated with increased morbidity and mortality. The distribution of body fat is also of importance for the complications to obesity as reviewed (1–3), upper-body obesity has a stronger association to metabolic and cardiovascular diseases than lower-body obesity. A plausible trigger for the complications in obesity may be free fatty acids (FFA) produced by the visceral fat depots through lipolysis in fat cells in this region, as discussed in detail elsewhere (2, 3). The visceral fat mass is increased in upper-body obesity (4). Only this fat depot has direct access to the liver through the portal system. The release of excess FFA into the portal circulation may have a number of unwanted effects on the liver, such as glucose intolerance, impaired metabolism and action of insulin, and altered lipoprotein synthesis as reviewed (2, 3, 5, 6). The resulting insulin resistance may, in turn, contribute to the development of diabetes and hypertension and cause a further deterioration in lipoprotein metabolism.

Nevertheless, an increase in FFA production from the visceral fat depots in obesity remains to be established, and the possible underlying mechanisms have not been determined. However, in vivo data suggest that lipolysis regulation is altered in obesity, a finding which is less apparent in lower-body obesity than in upper-body obesity (7–9). In this respect, it is of interest to study catecholamine-induced FFA and glycerol release from visceral fat cells since, in adult humans, catecholamines are the only hormones with marked acute lipolytic effects (10). The catecholamine effects are modulated through four adrenoceptor subtypes, i.e., stimulation via β_1-, β_2-, and β_3-adrenoceptors and inhibition via alpha$_2$-adrenoceptors (11). The β_3-adrenoceptor has recently been cloned (12) and its involvement in the adipocyte lipolysis of laboratory animals is well documented (13). Although β_3-adrenoceptors are expressed in several human fat depots (14), they have little lipolytic action on the subcutaneous fat tissue but a marked lipolytic function in fat cells from the visceral region (15–18).

At present it is not possible to study the β_3-adrenoceptor with classical techniques such as radioligand binding and protein determination, in contrast to the other adrenoceptor subtypes involved in lipolysis regulation. However, one can gain insight into the function of all adrenoceptor subtypes by investigating the lipolysis concentration–response relationship for selective adrenergic agonists, as reviewed elsewhere (19). The half-maximum effective concentration (ED_{50}) can be determined and reflects specific agonist–adrenoceptor interactions, since at this concentration the selective agonist either has no or insignificant interactions with other adrenoceptor subtypes.

We have presently investigated the adrenergic regulation of

This paper won first prize in the first IASO Young Investigator Competition (Clinical Research Category) at the 7th International Congress on Obesity in Toronto, Canada on 22 August 1994.

Address correspondence to Peter Arner, M.D., Department of Medicine, Huddinge University Hospital, 14186 Huddinge, Sweden. Phone: 8-746-4113; FAX: 8-746-4133.

Received for publication 17 June 1994 and in revised form 27 October 1994.

J. Clin. Invest.

0021-9738/95/03/1109/08 $2.00
Volume 95, March 1995, 1109–1116

lipolysis in visceral fat cells from the omental region by performing concentration–response experiments with norepinephrine and various synthetic adrenergic subtype specific agonists, determining the end products in lipolysis (glycerol and FFA) in massively obese and matched nonobese subjects. The obese subjects were selected for a high waist-to-hip ratio to, above all, study subjects with an enlarged visceral fat mass. An increased rate of FFA mobilization after catecholamine stimulation was observed in omental fat cells of obese subjects. This is attributed mainly to a marked increase in the lipolytic function of β_3-adrenoceptors.

Methods

Patients. The study comprised 25 upper-body obese, otherwise healthy, drug-free subjects (body mass index 43±1 kg/m^2, range 35–56) undergoing weight reduction surgical treatment through open or laparoscopic procedures, and 19 nonobese subjects (body mass index 24±1 kg/m^2, mean±SE, range 20–28) undergoing elective laparoscopic cholecystectomy at Huddinge University Hospital. All subjects were Caucasians. The two groups were matched for sex, age, and smoking habits and were clinically characterized before surgery. In both groups about a third were men. The ages of the subjects ranged from 19 to 59 yr. The waist-to-hip ratio, the sagittal diameter, and the systolic and diastolic blood pressures were measured in the supine position on the day before surgery. All obese women had a waist-to-hip ratio > 0.95 and all obese men had a waist-to-hip ratio > 1.00. The amount of visceral fat was calculated from the sagittal trunk diameter (D) by a computer tomography-calibrated equation (0.731 × D − 11.5 for men and 0.370 × D − 4.85 for women), as described previously in detail by Sjöström (4). The sagittal diameter was obtained by measuring the distance from the examination table to a horizontal crossbar placed over the abdomen of a recumbent subject at the crista level. The blood pressure was measured with a mercury sphygmomanometer on the right arm. The systolic and diastolic pressures were determined by using phases I and V of the Korotkoff sounds, the values are the means of three consecutive measurements after a 10-min rest. After an overnight fast, the study subjects rested in bed for 15 min, whereafter venous blood samples were obtained for determinations of metabolic laboratory parameters. These were later analyzed by the hospital's routine chemistry laboratory, except for insulin, which we measured with a radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

General anesthesia was induced at 8 a.m. by a short-acting barbiturate and maintained by fentanyl and a mixture of oxygen and nitrous oxide. Intravenous saline was administered before 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 in the study.

Isolation of fat cells and determinations of cell size and number. For technical reasons, it was not possible to obtain specimens larger than 0.3–1.0 g of omental fat during the laparoscopic operations, also the weight reduction surgery was initiated with laparoscopic technique in about half of the cases. The adipose tissue was immediately transported to the laboratory in saline at 37°C, and isolated fat cells were prepared from the fat specimens by collagenase treatment, as described by Rodbell (20). 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 (21), 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 (22). The total lipid content in each incubation was determined gravimetrically after organic extraction. Assuming that lipids constitute > 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. This indirect method for determining the fat cell number was compared with a tedious direct method (23), where all cells are counted in appropriately diluted cell suspensions. The two methods gave almost identical results in 10 consecutive experiments (r = 0.97), using linear regression analysis.

Lipolysis experiments. The lipolysis assay has been described previously in detail (24). Briefly, 0.2 ml of diluted suspensions of isolated fat cells (5,000–10,000 cells/ml) were incubated in duplicate for 2 h with or without increasing concentrations of either the natural catecholamine norepinephrine, the nonselective β-adrenergic agonist isoprenaline, the selective β_1-adrenoceptor agonist dobutamine, the selective β_2-adrenoceptor agonist terbutaline, the selective partial β_3-adrenoceptor agonist CGP 12177 (18), or the selective alpha$_2$-adrenoceptor agonist UK 14304 (25). All incubations were performed at 37°C in Krebs-Henseleit phosphate buffer (pH 7.4), supplemented with glucose (1 g/liter), bovine serum albumin (20 g/liter), and ascorbic acid (0.1 g/liter), with air as the gas phase. The ligands were added simultaneously at the start of the incubation. The concentration range used for each drug depended on its lipolytic performance. Overall it ranged from 10^{-12} to 10^{-3} mol/liter. The same batches of collagenase and albumin and the same stock solutions of adrenoceptor agonists were used throughout the study. As discussed in detail previously (26), adenosine leaking out from isolated fat cells may interfere with the alpha$_2$-adrenoceptor–mediated antilipolytic effect of catecholamines. In our dilute incubation system there is minimal influence of adenosine contamination. However, in the alpha$_2$-adrenoceptor experiments, adenosine deaminase (1 mU/ml) was added to prevent antilipolytic interactions with traces of adenosine that might still be present and might induce additional inhibitory effects (26) in the diluted cell suspensions. The influence of adenosine on lipolysis in a fat cell system like ours is, on the other hand, negligible when adipocytes are stimulated with lipolytic drugs, as discussed previously (27). Therefore, we preferred not to add adenosine deaminase in the remaining concentration–response experiments. In the experiments with UK 14304, the incubation medium was also supplemented with 10^{-3} mol/liter 8-bromo cyclic AMP to increase the initial (basal) rate of lipolysis, which was too low in visceral fat cells to be inhibited by UK 14304. We have shown previously that 8-bromo cyclic AMP–induced lipolysis can be fully inhibited by antilipolytic agents in human fat cells (28). There was no difference in sensitivity to 8-bromo cyclic AMP between obese and nonobese subjects. The glycerol concentration after the 2-h incubation was determined in a cell-free aliquot by a bioluminescence method (29).

All agonists caused a concentration-dependent stimulation or inhibition of glycerol release that reached a plateau at the highest agonist concentrations. Concentration–response curves for glycerol release were used to determine the concentrations of the adrenoceptor agonists, giving half of their own maximum stimulation (β) or inhibition (alpha$_2$). These ED$_{50}$ values (expressed as log mol/liter) were determined by linear regression analysis of log-logit transformation of the ascending (β-agonists) or descending (alpha$_2$-agonists) part of the individual concentration–response curves (30). This mathematical method for determining ED$_{50}$ gave the same results as visual fitting of concentration–response curves on lin-log paper. Lipolysis rates in the absence of, or in the presence of, maximum effective agonist concentrations were related to fat cell number. The maximum lipolytic effects indicate agonist responsiveness. The isoprenaline experiments were used to determine the intrinsic activities of the selective lipolytic agents. This drug is always a full agonist since it fully activates all β-receptor subtypes at maximum effective isoprenaline concentrations (10, 11). By comparing the maximum effects of selective β-adrenoceptor agonists with isoprenaline, one can evaluate the coupling efficiency of the selective agonist to the effector system (19). Intrinsic activities were determined as the percentage of the maximal isoprenaline response for each experiment separately. Since the amount of fat tissue available was limited, we were not able to perform complete experiments in all cases. We decided on the following priorities: (a) the selective β-adrenoceptor agonists (n = 44); (b) norepinephrine (n = 38); and (c) alpha$_2$-adrenoceptor ago-

nist response (n = 24). There were no differences in age, sex, or smoking habits between the obese and nonobese subjects in any of the subgroups.

Simultaneous determination of glycerol and FFA release. Since the amount of fat tissue available was limited, we could only study the simultaneous FFA and glycerol production due to norepinephrine stimulation in 22 out of 44 subjects. The subgroups, consisting of 12 obese and 10 nonobese subjects, did not differ in regard to age, sex, and smoking habits. The isolation and incubation procedures concerning fat cells were exactly the same for these experiments as for the lipolysis experiments described above, except that fatty acid–free bovine serum albumin 0.25% (wt/vol) was used as acceptor of FFA in the incubation medium. At the end of the incubation, one aliquot of the medium was removed for glycerol determination as described above. Another aliquot was used for the measurement of FFA release. The method for determining FFA has been described in detail (31). In brief, the FFA analyses were performed as follows. The assay involved pretreatment with the detergent sodium dodecyl sulfate to liberate the FFA from the bovine serum albumin before the activation of fatty acids by acyl-CoA synthetase. This was followed by oxidation of the resulting thioesters by acyl-CoA oxidase. The H_2O_2 formed (reflecting the amount of FFA) was subsequently measured in a horseradish peroxidase–catalyzed luminol reaction, using a luminometer (LKB Wallac, Turku, Finland).

Statistical analysis. Values are given as the mean±SE. ED_{50} values were logarithmically transformed to normalize the data and were defined as β- and alpha-adrenoceptor subtype sensitivity, respectively. Analysis of covariance, analysis of variance (ANOVA), the Student's two-tailed unpaired t test, and single or stepwise regression analysis were used for statistical comparisons. All statistical calculations were performed with a statistical software package (STATVIEW II; Abacus Concepts, Inc., Berkeley, CA).

Drugs and chemicals. Bovine serum albumin (fraction V, lot 63F-0748), fatty acid–free bovine serum albumin (lot A-6003), *Clostridium histiolyticum* collagenase type I, glycerol kinase from *Escherichia coli* (G4509), acyl-CoA synthetase (EC 6.2.1.3) from *Pseudomonas specialis,* acyl-CoA oxidase from *Candida lipolytica,* horseradish peroxidase (EC 1.11.1.7., type VI; 250–330 U/mg), sodium dodecyl sulfate, ascorbate oxidase, adenosine deaminase, Triton X-100, and ATP were obtained from Sigma Immunochemicals (St. Louis, MO). (±)-Isoprenaline hydrochloride came from Hässle (Mölndal, Sweden), terbutaline sulfate from Draco (Lund, Sweden), dobutamine hydrochloride from Eli Lilly and Co. (Indianapolis, IN), UK 14304 tartrate from Pfizer (Sandwich, United Kingdom), and CGP(±)12177 ((−)-4-(3-t-butylamino-2-hydroxy-propoxy)benzimidazole-2-one) from Ciba Geigy AG (Basel, Switzerland). ATP monitoring reagent containing firefly luciferase came from LKB Wallac. All other chemicals were of the highest grade of purity commercially available.

Results

The clinical data are shown in Table I. As expected, the waist-to-hip ratios were markedly increased in both men and women in the obese group. Likewise, the total amount of visceral fat and the omental fat cell volume were increased three- and twofold, respectively, in the obese subjects, who also had elevated blood pressure, plasma insulin, plasma glucose, and plasma triglyceride levels, but a decreased HDL cholesterol level. In other words, the obese subjects showed evidence of predominantly upper-body distribution of the fat mass and signs of several classical complications to obesity, including indirect evidence of insulin resistance.

The effects of increasing concentrations of norepinephrine on the simultaneous release of glycerol and FFA from omental fat cells in obese and nonobese subjects are depicted in Fig. 1. As the fat cells were enlarged in the obese subjects, the FFA and glycerol production rates were related to the number of fat cells incubated. The norepinephrine-induced production of FFA and glycerol is given with or without basal values. The obese subjects had a twofold higher fat cell FFA response and glycerol response to norepinephrine than the nonobese subjects, both when basal values were included (repeated ANOVA, P = 0.01 and P = 0.006, respectively) or subtracted (repeated ANOVA, P = 0.03 and P = 0.02, respectively). The ratio of glycerol release to FFA release was almost 1:3 in these experiments as seen in Fig. 1. The individual ratios were calculated on net lipolysis (minus basal) at 10^{-7} mol/liter of norepinephrine, when there was a maximum rate of FFA release in both groups. The glycerol/FFA ratio was 1:2.86±0.55 in the obese and 1:2.66±0.24 in the nonobese subjects. These values did not differ statistically.

Table I. Clinical Data in Obese and Nonobese Subjects

	Obese	Nonobese	P
Age (yr)	39±2	40±2	NS
Sex (male/female)	9/16	7/12	NS
Body mass index (kg/m^2)	43±1	24±1	
Waist-to-hip ratio (men)	1.06±0.01	0.96±0.02	0.001
Waist-to-hip ratio (women)	1.00±0.01	0.90±0.02	0.0001
Visceral fat vol (liters)	8.59±0.64	2.77±0.38	0.0001
Fat cell volume (pl)	620±37	262±27	0.0001
Systolic blood pressure (mmHg)	142±3	125±3	0.0002
Diastolic blood pressure (mmHg)	89±2	76±1	0.0001
Plasma insulin (mU/liter)	17.4±1.5	8.1±0.9	0.0001
Plasma glucose (mmol/liter)	5.64±0.13	5.15±0.09	0.007
Plasma triglycerides (mmol/liter)	1.92±0.13	1.48±0.14	0.02
Total cholesterol (mmol/liter)	5.91±0.17	5.24±0.22	0.02
HDL cholesterol (mmol/liter)	1.12±0.07	1.32±0.09	NS
Plasma norepinephrine (nmol/liter)	1.91±0.12	1.79±0.09	NS
Plasma epinephrine (nmol/liter)	0.15±0.01	0.16±0.03	NS

The values are means±SE. The groups were compared using the Student's t test. Visceral fat cell number was obtained by dividing the total visceral fat volume (liter) by the visceral fat cell volume (pl).

The increased effect of catecholamines on glycerol and FFA in the obese subjects can be attributed to a change in any of the stimulatory β_1-, β_2-, and β_3-adrenoceptors or the inhibitory alpha$_2$-adrenoceptors. To investigate these four receptors, fat cells were incubated with selective β- and alpha-adrenoceptor agonists, i.e., dobutamine (β_1), terbutaline (β_2), CGP 12177 (β_3), or UK 14304 (alpha$_2$) and glycerol release was determined. The mean concentration–response curves are shown in Fig. 2 as a percentage of their maximum effect, to elucidate differences in agonist sensitivity between groups (i.e., left- or rightward shift in the concentration–response curve). There were no apparent differences in β_1- or β_2-adrenoceptor sensitivity between the two groups. However, the obese subjects were clearly much more sensitive to the β_3-adrenoceptor agonist CGP 12177. Furthermore, the obese group demonstrated a somewhat lower alpha$_2$-adrenoceptor sensitivity. The mean ED_{50} values (log mol/liter) for stimulation or inhibition, with the different agonists, as determined from the individual dose–response curves, are given in Table II. The ED_{50} values for CGP 12177 and UK 14304 were significantly different between the groups. β_3-Adrenoceptor sensitivity was 50 times increased (P = 0.0001), and alpha$_2$-adrenoceptor sensitivity was 6 times de-

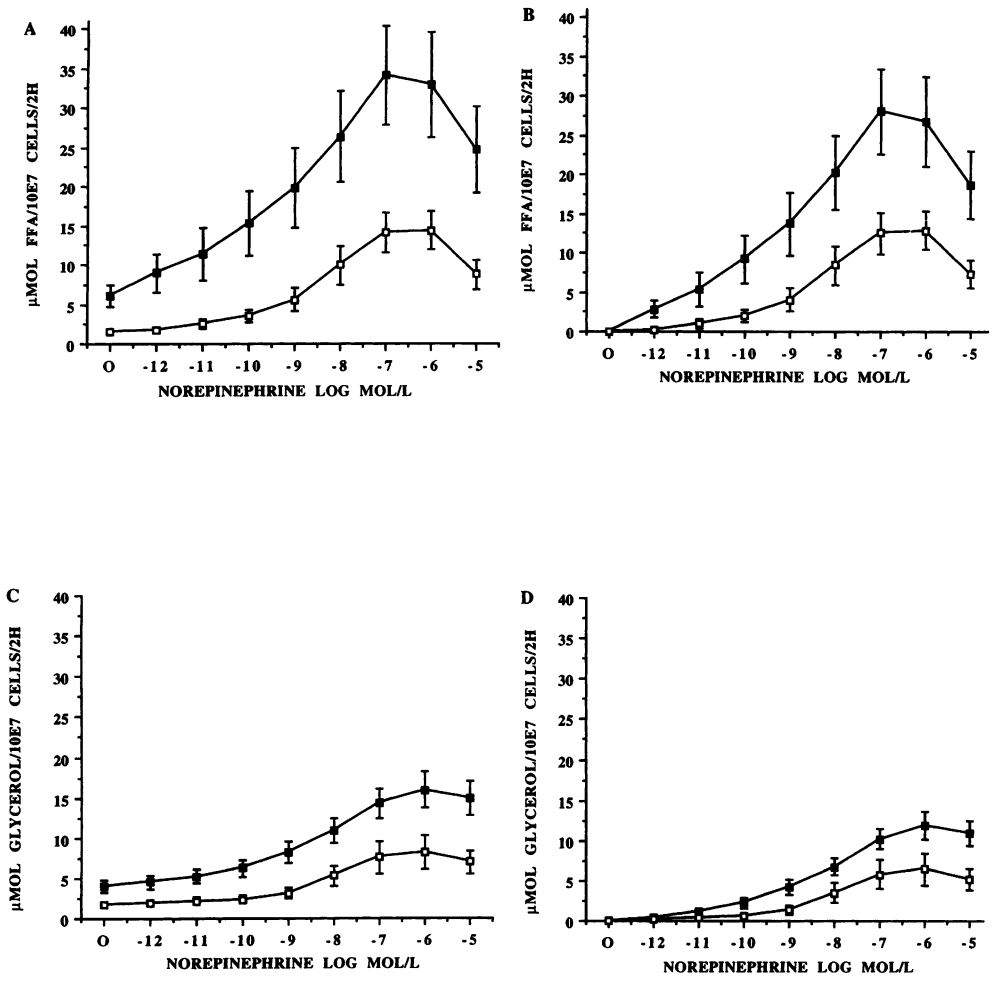

Figure 1. Simultaneous release of glycerol release and FFA from fat cells. Omental fat cells from obese (*filled boxes*) and nonobese (*open boxes*) subjects were incubated with increasing concentrations of norepinephrine. The figures for production of FFA and glycerol are given with (*A* and *C*) or without basal lipolytic activity (*B* and *D*). The results (means±SE) are expressed as micromoles of glycerol or FFA per 10^7 cells per 2 h.

creased ($P = 0.04$) in obesity. In addition, the sensitivity to norepinephrine was also significantly enhanced by five times in the obese group (Table II).

The intrinsic activity, i.e., the maximum effect of a selective agent in relation to the maximum effect of isoprenaline, was also determined for the selective β-adrenoceptor agonists. The mean values for the maximal lipolytic response induced by isoprenaline were 16.4±1.3 and 15.3±1.9 in the obese and nonobese group, respectively. The difference was not significant. Dobutamine and terbutaline were both full agonists relative to isoprenaline in the visceral fat of both obese and nonobese subjects in this study, while CGP 12177 was a partial agonist. However, the intrinsic activity of CGP 12177 was significantly higher in the obese subjects (56±5 vs 37±5%, $P = 0.01$). The absolute concentration–response curves (μmol glycerol/10^7 cells/2 h) for the three β-adrenoceptor agonists are given in Fig. 3. The lipolytic response to CGP 12177 was clearly more pronounced in the obese subjects than in the nonobese subjects (repeated ANOVA, $P = 0.01$). On the other hand, no significant differences were seen in regard to the dobutamine or terbutaline responses. Neither did the maximum alpha$_2$-adrenoceptor–mediated antilipolytic response induced by UK 14304 differ between obese and nonobese subjects. It was defined as glycerol release in the absence of UK 14304 minus glycerol release in the presence of the maximum effective concentration of UK 14304. The responses to UK 14304 were 5.9±1.5 and 5.7±1.0 μmol glycerol/10^7 cells/2 h in the obese and the nonobese subjects, respectively.

Finally, the importance of adrenoceptor subtype sensitivity for the variations in lipolytic function of catecholamines was investigated in the whole study material. The relationship between β_1-, β_2-, and β_3- and alpha$_2$-adrenoceptor agonist sensitivity (log ED_{50} for dobutamine, terbutaline, and CGP 12177) on the one hand, and norepinephrine-induced lipolysis, on the other hand, was first investigated by single regression analysis. β_3-Adrenoceptor sensitivity correlated with all concentrations of norepinephrine-induced lipolysis, with r values ranging from 0.46 to 0.67 and P values ranging from 0.0017 to 0.0001. The relationship between β_3-adrenoceptor sensitivity and lipolysis induced by 1 nmol/liter of norepinephrine ($r = -0.67$, $P = 0.0001$) is given in Fig. 4. Neither the β_1- or the β_2- nor the alpha$_2$-adrenoceptor sensitivities correlated significantly with norepinephrine-induced lipolysis. The r values for β_1- and β_2-adrenoceptors ranged from 0.10 to 0.36 and 0.01 to 0.34, respectively, while the r values for the alpha$_2$-adrenoceptor ranged from 0.01 to 0.30. By a stepwise regression analysis, the relative contribution of β_1-, β_2, or β_3-adrenoceptor sensitivity was compared with norepinephrine-induced lipolysis. The adrenoceptor with the highest partial correlation coefficient was entered as the first step and the lowest F value (i.e., variance ratio) for entry was set to 4. β_3-Adrenoceptor sensitivity was entered as the first and only step (adjusted $r^2 = 0.44$, F = 29.9).

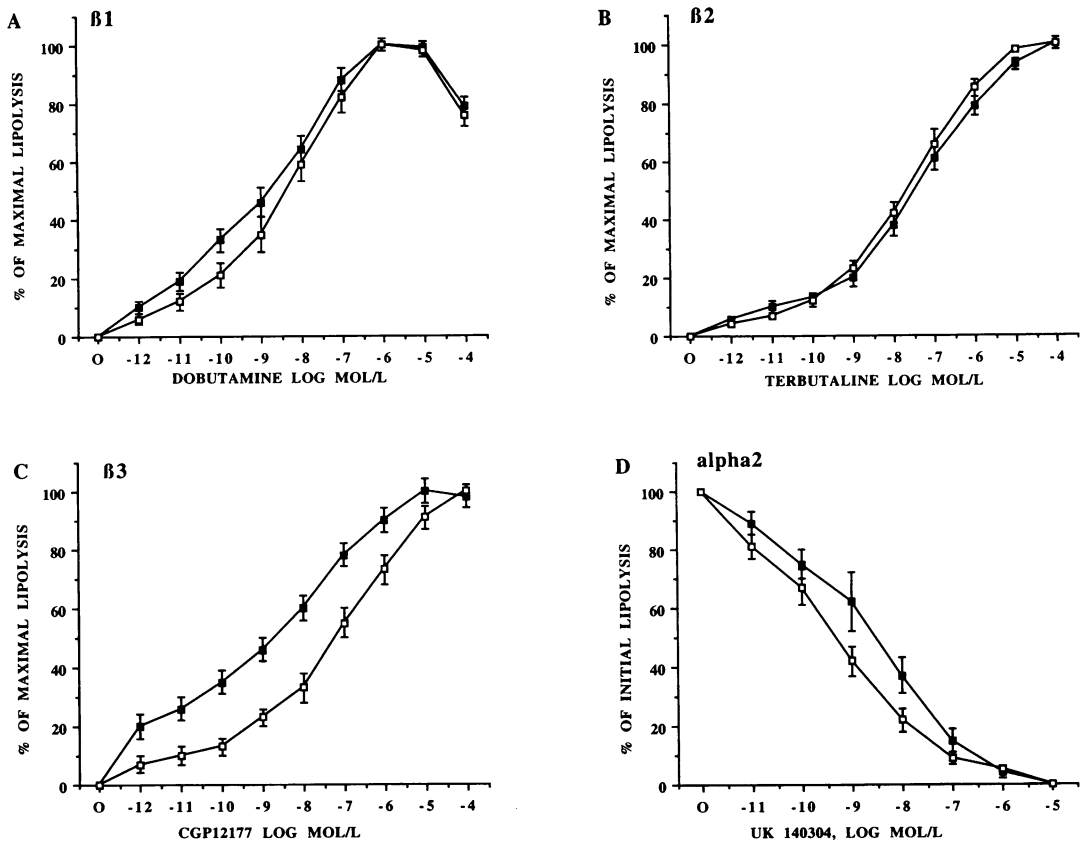

Figure 2. Mean concentration–response curves for selective adrenergic agonists. Omental fat cells from obese (*filled boxes*) and nonobese (*open boxes*) subjects were incubated with increasing concentrations of selective adrenoceptor agonists, i.e., dobutamine (β_1) (*A*), terbutaline (β_2) (*B*), CGP 12177 (β_3) (*C*), or UK 14304 (alpha$_2$) (*D*). The adrenoceptor effect is expressed as a percentage of the maximal stimulatory or inhibitory effect. Values are means±SE. The mean ED_{50} values (expressed as log mol/liter) derived from these experiments are given in Table II.

We also investigated the possible correlation between fat cell volume and β-adrenoceptor sensitivity (i.e., ED_{50}) using linear regression analysis (figure not shown). There was a strong association between fat cell volume and β_3-adrenoceptor sensitivity ($r = -0.71$, $P = 0.0001$). However, no significant relationship was observed between fat cell volume and the sensitivities of the β_1- and the β_2-adrenoceptor ($r = -0.08$ and 0.16, respectively).

Table II. Lipolytic Sensitivity to Adrenergic Agonists in Obese and Nonobese Subjects

	ED_{50} nonobese	*n*	ED_{50} obese	*n*	*P*
	log mol/liter		*log mol/liter*		
Norepinephrine (glycerol)	−8.11±0.12	18	−8.76±0.14	20	0.001
Dobutamine (β_1)	−8.43±0.24	19	−8.75±0.21	25	NS
Terbutaline (β_2)	−7.36±0.18	19	−7.46±0.18	25	NS
CGP 12177 (β_3)	−7.11±0.20	19	−8.81±0.20	25	0.0001
UK 14304 (alpha$_2$)	−9.40±0.21	14	−8.69±0.27	10	0.04

The values are mean±SE. ED_{50}, the concentration that induces 50% of the maximal lipolytic or antilipolytic response.

Discussion

This demonstrates for the first time that the rate of FFA mobilization from the visceral fat cells is increased in obesity, at least when investigated in vitro in omental fat cells from massively obese subjects. Norepinephrine stimulation of visceral fat cells in obese subjects resulted in a simultaneous doubling of the average FFA and glycerol responses, compared to nonobese subjects. Furthermore, glycerol to FFA ratio was near 1:3 in both groups, which indicates a minimal level of reesterification of FFA during lipolysis. This clearly indicates that the increased catecholamine-induced FFA mobilization in obesity is due mainly to an altered lipolytic response and to a lesser extent secondary to an alteration in the reutilization of FFA.

The present effects of norepinephrine on FFA and glycerol release in obese subjects are to some extent in opposition with previous reports of in vivo studies showing decreased lipolytic responses to catecholamine infusions in obese as compared to lean subjects (7, 32, 33). However, in these studies the overall lipolysis rate was measured in vivo. This mainly reflects the lipolytic effects on the subcutaneous fat mass which, due to its high proportion of the total fat mass (~ 80%), dominates the response. It has recently been demonstrated in vitro that the lipolytic sensitivity to epinephrine and norepinephrine is impaired in the subcutaneous region in upper-body obese subjects (34, 35) indicating regional differences in the impact of abdom-

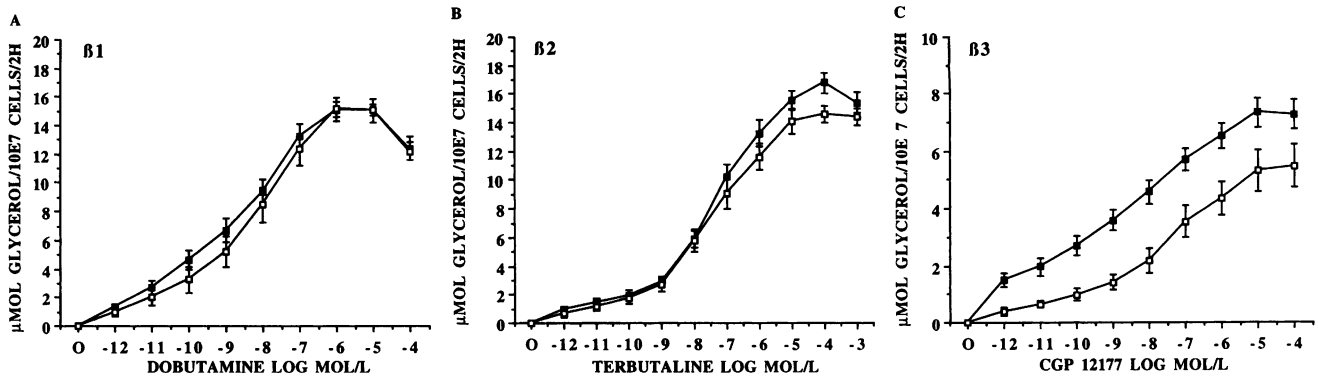

Figure 3. Rate of lipolysis induced by selective β-adrenergic agonists. Values for glycerol release (means±SE) are derived from the experiments depicted in Fig. 2. The responses were expressed as micromoles of glycerol/10^7 cells/2 h. Omental fat cells from obese (*filled boxes*) and nonobese (*open boxes*) subjects were incubated with increasing concentrations of selective adrenoceptor agonists, i.e., dobutamine (β_1) (*A*), terbutaline (β_2) (*B*), and CGP 12177 (β_3) (*C*).

inal obesity on lipolysis, i.e., increased catecholamine action in the visceral fat depot but decreased action in the subcutaneous fat depot. Furthermore, the overall rate of FFA release after an overnight fast is higher in upper-body obese subjects than in normal or lower-body obese subjects in vivo (36). It is likely that this increase in FFA release in vivo in upper-body obesity is due to the presently observed enhancement of the lipolytic rates in fat cells from the visceral depot which may in quantitative terms overcome the decreased action in subcutaneous adipose tissue. On the other hand, it has been proposed on the basis of in vivo studies of splanchnic and femoral palmitate fluxes that the increased total palmitate release in upper-body obese subjects may originate from the abdominal subcutaneous fat depots (9). However, it is important to note that the splanchnic palmitate release measured in the hepatic vein is not a measure of splanchnic lipolysis. The splanchnic palmitate uptake is considerably greater than the release. Furthermore, FFA uptake occurs from both the intestine and the liver (37) whereas release of FFA into the portal circulation occurs from the omental and mesenteric adipose tissues. Since the proportion of nonhepatic splanchnic FFA uptake in humans is unknown, splanchnic lipolysis cannot be predicted accurately without direct access to the portal vein. Finally, some caution should be exercised in extrapolating from in vitro to the in vivo situation. For example, there may be other hormonal (primarily insulin) or blood flow issues that could affect FFA release in vivo.

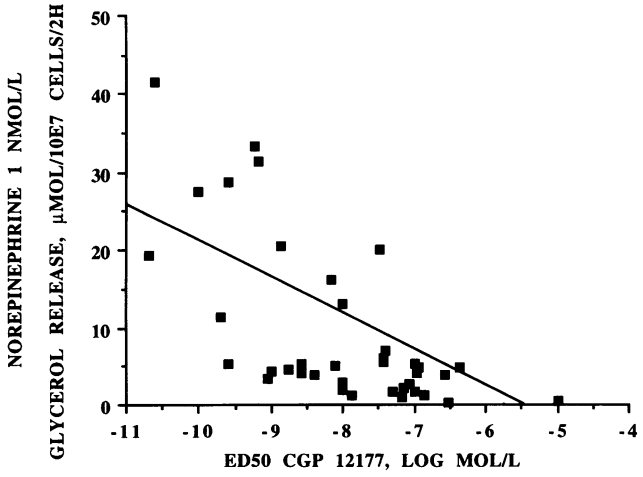

Figure 4. Relationship between β_3-adrenoceptor sensitivity and catecholamine-induced lipolysis. Scatterplot showing the linear relationship between lipolytic sensitivity (ED_{50}) for CGP 12177 and the glycerol response to a physiological norepinephrine concentration (1 nmol/liter) in the entire study group (n = 38). r = −0.67, P = 0.0001.

The mechanisms underlying the increase in FFA release from the omental fat cells in upper-body obesity were further investigated and partly clarified. The lipolytic sensitivity (i.e., ED_{50}) to norepinephrine was increased in obesity which indicates an alteration in the function of one or several adrenoceptor subtypes. The increased lipolytic activity of catecholamines in omental fat cells of obese subjects could mainly be explained by the β_3-adrenoceptor, but not by the β_1- or β_2-adrenoceptors. The obese subjects were 50 times more sensitive to CGP 12177, which is at present the only known selective β_3-adrenoceptor agonist in humans (18). In addition, both the β_3-adrenoceptor responsiveness and the β_3-adrenoceptor agonist intrinsic activity were enhanced in the obese individuals' omental fat cells. The last finding indicates an increased coupling efficiency of the receptor subtype. The potential importance of the β_3-adrenoceptor in the omental fat cell is further stressed by the fact that the sensitivity of this receptor correlated strongly with norepinephrine-induced lipolysis and fat cell volume. Neither the β_1- or β_2-adrenoceptor sensitivity nor the $alpha_2$-adrenoceptor sensitivity correlated with these variables.

The molecular mechanism behind the increased β_3-adrenoceptor function in obesity remains to be established. However, as the absolute lipolytic responses to both isoprenaline, dobutamine, and terbutaline were equal in both groups, a major postreceptor difference is highly unlikely. This is further supported by the finding that the maximum absolute antilipolytic effect of UK 14304 did not differ between the groups. The increased β_3-adrenoceptor might rather relate to the structure, number, or coupling of the receptor subtype to the lipolytic system. We suggest primarily that the increased β_3-adrenoceptor activity could be due to an enhanced receptor number in the obese subjects since there was a marked increase in the sensitivity (i.e., lower ED_{50}) of CGP 12177 in the obese subjects. As discussed in detail (19) changes in agonist sensitivity usually reflect changes in receptor number. Unfortunately, this hypothe-

sis was not possible to investigate as no methods to quantify the β_3 receptor protein or the mRNA in human fat are available at present. There are yet no radioligands suitable for this purpose because of methodological problems with nonspecific binding, as reported previously (15, 18). Furthermore, the low level of β_3-adrenoceptor mRNA expression makes quantitative methods based on both Northern blot or RNAse protection unsuitable because their detection limit is too high (38). It has been possible to perform a quantification of the β_3-adrenoceptor mRNA signal in fat cells with the polymerase chain reaction technique in cells with a high expression level (39), but it is not useful when the expression level is low. At present, there are no antibody techniques available to measure the amount of human β_3-adrenoceptor protein. A recently constructed specific human β_3-adrenergic antibody is, however, not suitable for a quantitative assay (Haasemann, M., personal communication).

The findings with β_3-adrenergic subtypes are representative for the whole material because the three selective β-adrenoceptor agonists were used in all subjects. Furthermore, a sixfold decrease in alpha$_2$-adrenoceptor sensitivity was observed in the obese subjects' omental fat cells. However, this finding was based on a limited number of subjects, and the alpha$_2$-adrenoceptor was not the major focus in the present investigation. This receptor has complex interactions (located at the level of the so-called G-proteins) with the β-adrenoceptors in human fat cells, which can be investigated in detail only in very large amounts of tissue (26). However, it is thought likely that a decrease in the alpha$_2$-adrenoceptor function contributes to the increase in lipolytic action of catecholamines in omental fat cells in obesity.

The increased β_3-adrenoceptor function and decreased alpha$_2$-adrenoceptor function in the obese subjects were highly unexpected findings. It has been reported that the β_3-adrenoceptor mRNA levels in white fat of obese Zucker rats was reduced by $\sim$ 70%, as compared with lean control animals (40). Furthermore, it is has been shown that the alpha$_2$-adrenoceptor agonist sensitivity is higher in obese than in lean dogs (41). These data indicate that animal models are not always valid for conclusions concerning the mechanisms underlying obesity in humans.

The clinical relevance of the present findings should also be considered. None of the obese patients included in this study had any overt disease apart from obesity. However, these patients showed as a group several signs of classical complications, which together form the so-called insulin resistance or metabolic syndrome. The results of anthropometric measurements strongly indicated an upper-body type of fat distribution, although such measures may be less accurate in massively obese subjects as discussed in detail recently (42). Furthermore, the obese subjects had hyperinsulinemia, glucose intolerance, dyslipidemia, and elevated blood pressure, which are all factors resulting in an increased risk for cardiovascular disease (1–3, 5, 6). We suggest that these abnormalities may, to some extent, be induced by an increased FFA release from the visceral fat depots in upper-body obesity secondary to an increased β_3-adrenoceptor function in the omental fat cells. Whether these conclusions are true also for moderately obese individuals remains to be investigated.

Our data concerning the β_3-adrenoceptor may also be useful for future pharmacotherapeutic research in the fields of obesity and metabolic diseases. Until now, pharmacological β_3-adrenoceptor research has focused on the development of potential β_3-adrenoceptor agonists that may activate brown fat cells and possibly result in thermogenic effects (13, 43). Our data indicate that it could be of an equally high interest to develop β_3-adrenoceptor antagonists that would inhibit the metabolic effects of a β_3-adrenoceptor–induced increase in the FFA release from visceral white fat tissue in certain obese subjects.

We presently used very diluted fat cell suspensions to study lipolysis. There are several advantages to this method. A large number of experiments can be performed on a small amount of tissue. The problems with adenosine contamination in the medium are minimized. The cells appear to be very sensitive to physiological stimulation since a clear lipolytic effect is obtained with norepinephrine concentrations in the same range as those in the circulation (1–10 nmol/liter).

In conclusion, we show for the first time that the FFA mobilization from visceral (i.e., omental) fat cells is increased in obesity. This increment is due to an enhanced lipolytic action of catecholamines in omental fat cells, which in turn is secondary to a markedly increased β_3-adrenoceptor action and, to a minor extent, to a slightly decreased alpha$_2$-adrenoceptor action in the adipocytes. These alterations may increase the rate of FFA mobilization to the portal system during lipolysis and contribute to the metabolic disturbances in obesity.

Acknowledgments

The excellent technical assistance by Britt-Marie Leijonhufvud, Catharina Sjöberg, Eva Sjölin, and Kerstin Wåhlin is greatly appreciated.

This study was supported by grants from the Swedish Medical Research Council (B91-19F-9390-01), Karolinska Institute, the Swedish Society of Medicine, the Swedish Diabetes Association, the Nordic Insulin Foundation, Sandoz, Parke-Davis, and the Foundations of Åke Wiberg, Tore Nilsson, Fredrik and Ingrid Thuring, Osterman and Golje.

References

1. Kaplan, N. M. 1989. The deadly quartet: upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch. Intern. Med.* 149:1514–1520.

2. Björntorp, P. 1991. Metabolic implications of body fat distribution. *Diabetes Care.* 14:1132–1143.

3. Frayn, K. N., and S. W. Coppack. 1992. Insulin resistance, adipose tissue and coronary heart disease. *Clin. Sci. (Lond.).* 82:1–8.

4. Sjöström, L. 1991. A computer-tomography based multicompartment body composition technique and anthropometric predictions of lean body mass, total and subcutaneous adipose tissue. *Int. J. Obes.* 15:19–30.

5. Ferranini, E., E. J. Barrett, S. Bevilaquas, and R. A. DeFronzo. 1983. Effects of fatty acid on glucose production and utilization in man. *J. Clin. Invest.* 72:1737–1744.

6. Felber, M. G., and R. Jequier. 1982. Glucose storage deficiency as a cause of insulin resistance in obese hyperinsulinaemic diabetes. *Int. J. Obes.* 6(Suppl. 1):131–135.

7. Jensen, M. D., M. W. Haymond, R. A. Rizza, P. E. Cryer, and J. M. Miles. 1989. Influence of body fat distribution on free fatty acid metabolism in obesity. *J. Clin. Invest.* 83:1168–1173.

8. Jensen, M. D. 1991. Regulation of forearm lipolysis in different types of obesity. *J. Clin. Invest.* 87:187–193.

9. Martin, M. L., and M. D. Jensen. 1991. Effects of body fat distribution on regional lipolysis in obesity. *J. Clin. Invest.* 88:609–613.

10. Arner, P. 1992. Adrenergic receptor function in fat cells. *Am. J. Clin. Nutr.* 55:228S–236S.

11. Lafontan, M., and M. Berlan. 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* 34:1057–1092.

12. Emorine, L. J., S. Marullo, M. M. Briend-Sutren, G. Patey, K. Tate, C. Delavier-Klutchko, and A. D. Strosberg. 1989. Molecular characterization of the human beta$_3$-adrenergic receptor. *Science (Wash. DC).* 245:1118–1121.

13. Arch, J. R. S., and A. J. Kauman. 1993. β_3 and atypical β-adrenoceptors. *Med. Res. Rev.* 13:663–729.

14. Krief, S., F. Lönnqvist, S. Raimbault, B. Baude, A. van Sprousen, P.

Arner, A. D. Strosberg, D. Ricquier, and L. J. Emorine. 1993. Tissue distribution of β_3-adrenergic receptor mRNA in man. *J. Clin. Invest.* 91:344–349.

15. Langin, D., M. Portillo, J. S. Saulnier-Blanche, and M. Lafontan. 1991. Coexistence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.* 199:291–301.

16. Rosenbaum, M., C. C. Malbon, J. Hirsch, and R. L. Leibel. 1993. Lack of β_3-adrenergic effect on lipolysis in human subcutaneous adipose tissue. *J. Clin. Endocrinol. & Metab.* 77:352–355.

17. Hollenga, C., M. Haas, J. T. Deinum, and J. Zaagsma. 1990. Discrepancies in lipolytic activities induced by β-adrenoceptor agonists in human and rat adipocytes. *Horm. Metab. Res.* 22:17–21.

18. Lönnqvist, F., S. Krief, A. D. Strosberg, B. Nyberg, L. J. Emorine, and P. Arner. 1993. Evidence for a functional β_3-adrenergic receptor in man. *Br. J. Pharmacol.* 110:929–936.

19. Kenakin, T. 1984. The classification of drugs and drug receptors in isolated tissue. *Pharmacological Reviews.* 36:165–214.

20. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* 239:375–380.

21. Di Girolamo, M., S. Mendlinger, and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Am. J. Physiol.* 221:850–858.

22. Hirsch, J., and E. Gallian. 1968. Methods for determination of adipose cell size and number in man and animals. *J. Lipid Res.* 9:110–119.

23. Kather, H., E. Wieland, A. Scheurer, G. Vogel, U. Widenberg, and C. Joose. 1987. Influences of variation in total energy intake and dietary composition. *J. Clin. Invest.* 80:566–572.

24. Arner, P., J. Bolinder, J. Hellmér, and P. Engfeldt. 1986. Studies on human fat cell metabolism in small adipose tissue samples. *In* Methods in Diabetes Research. Vol. 2. W. L. Clarke, J. Larner, and S. L. Pohl, editors. John Wiley and Sons Inc., New York. 234–258.

25. Cambridge, D. 1981. UK-14,304, a potent and selective alpha$_2$-agonist for the characterization of alpha-adrenoceptor subtypes. *Eur. J. Pharmacol.* 72:413–415.

26. Lönqvist, F., and P. Arner. 1989. Interaction beween adenylate cyclase inhibitors and beta-adrenoceptors in isolated human fat cells. *Biochem. Biophys. Res. Commun.* 161:654–660.

27. Marcus, C., B. Karpe, P. Bolme, T. Sonnenfeldt, and P. Arner. 1987. Changes in catecholamine-induced lipolysis in isolated human fat cells during the first year of life. *J. Clin. Invest.* 79:1812–1818.

28. Lönqvist, F., A. Wennlund, and P. Arner. 1989. Antilipolytic effects of insulin and adenylate cyclase inhibitors on isolated human fat cells. *Int. J. Obes.* 13:137–146.

29. Hellmér, J., P. Arner, and A. Lundin. 1989. Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal. Biochem.* 177:132–137.

30. Östman, J., P. Arner, H. Kimura, H. Wahrenberg, and P. Engfeldt. 1984. Influence of fasting on lipolytic response to adrenergic agonists and on adrenergic receptors in subcutaneous adipocytes. *Eur. J. Clin. Invest.* 13:383–391.

31. Näslund, B., K. Bernström, A. Lundin, and P. Arner. 1993. Release of small amounts of free fatty acids from human adipocytes as determined by chemiluminescence. *J. Lipid Res.* 34:633–642.

32. Wolfe, R. R., E. J. Peters, S. Klein, O. B. Holland, J. Rosenblatt, and H. Gary. 1987. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am. J. Physiol.* 2523:E189–E196.

33. Connacher, A. A., W. M. Bennet, R. T. Jung, D. M. Bier, C. T. Smith, C. M. Scrimgeour, and M. J. Rennie. 1991. Effect of adrenaline infusion on fatty acid and glucose turnover in lean and obese human subjects in post-absorptive and fed states. *Clin. Sci. (Lond.).* 81:635–644.

34. Mauriege, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. 1991. Regional variation in adipose tissue lipolysis in lean and obese men. *J. Lipid Res.* 32:1625–1633.

35. Reynisdottir, S., H. Wahrhenberg, K. Carlström, S. Rössner, and P. Arner. 1994. Catecholamine resistence in fat cells of upper-body obese women due to decreased expression of beta$_2$-adrenoceptors. *Diabetologia.* 37:428–435.

36. Roust, L. R., and M. D. Jensen. 1993. Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes.* 42:1567–1573.

37. Basso, L. V., and R. J. Havel. 1970. Hepatic metabolism of free fatty acids in normal and diabetic dogs. *J. Clin. Invest.* 49:537–547.

38. Granneman, J. G., K. N. Lahners, and D. D. Rao. 1992. Rodent and human β_3-adrenergic receptor genes contain an intron within the protein coding block. *Mol. Pharmacol.* 42:964–970.

39. Krief, S., B. Feve, B. Baud, V. Zilberfarb, and A. D. Strosberg. 1994. Transcriptional modulation by *n*-btyric acid of β_1-, β_2- and β_3-adrenergic receptor balance in 3T3-F442A adipocytes. *J. Biol. Chem.* 269:6664–6670.

40. Muzzin, P., J. P. Revelli, F. Kuhne, J. D. Gocayne, W. R. Mccombie, J. C. Venter, J. P. Giacobino, and C. M. Fraser. 1991. An adipose tissue-specific β-adrenergic receptor. Molecular cloning and down-regulation in obesity. *J. Biol. Chem.* 266:24053–24058.

41. Taouis, M., P. Valet, L. Estan, M. Lafontan, P. Monastruc, and M. Berlan. 1989. Obesity modifies the adrenergic status of dog adipose tissue. *J. Pharmacol. Exp. Ther.* 250:1061–1066.

42. van der Kooy, K., and J. C. Seidell. 1993. Techniques for the measurement of visceral fat: a practical guide. *Int. J. Obes.* 17:187–196.

43. Howe, R. 1993. β3-adrenergic agonists. *Drugs Future.* 18:529–549.