Regional variation in adipose tissue metabolism of severely obese premenopausal women

P. Mauriège,*·§ A. Marette,§·** C. Atgié,** C. Bouchard,* G. Thériault,* L. K. Bukowiecki,** P. Marceau,†† S. Biron,†† A. Nadeau,† and J. P. Després[1]·§

Physical Activity Sciences Laboratory,* Laval University; Diabetes Research Unit,† Lipid Research Center,§ CHUL Medical Research Center; Department of Physiology,** Faculty of Medicine, Laval University; and Department of Surgery,†† Laval Hospital, Ste-Foy, Québec, Canada G1V 4G2

Abstract Lipolytic and lipoprotein lipase (LPL) activities were studied in isolated human adipocytes obtained from two intraabdominal depots (round ligament and omental) and from the subcutaneous abdominal region of nine severely obese premenopausal women (with body mass indices ranging from 37 to 51 kg/m^2), aged 36 ± 3 yr, undergoing gastrointestinal surgery. Both fat cell weight and LPL activity were significantly greater in round ligament adipose cells than in subcutaneous abdominal or in omental adipocytes ($P < 0.05$). The antilipolytic effect of insulin and the sensitivity to this hormone were also higher in round ligament adipose cells than in omental adipocytes ($P < 0.05$). Although epinephrine initiated a similar biphasic profile of response in all cell types, the catecholamine promoted a weaker inhibition of lipolysis in omental adipocytes than in subcutaneous abdominal adipose cells ($P < 0.05$). In addition, a lack of regional variation was found in the maximal antilipolysis initiated by UK 14304 and the α2-adrenergic sensitivity, although the maximum number of α2-adrenoceptors was higher in both subcutaneous abdominal and round ligament fat cells than in omental adipocytes. Moreover, the maximal lipolytic response to isoproterenol or to agents acting at post-receptor levels was not different among fat depots. Finally, a lower β-adrenergic lipolytic sensitivity associated with a reduced β-adrenoceptor density was observed in round ligament as compared to omental adipose cells. These data suggest that in massively obese premenopausal women, omental and round ligament adipose tissues show distinct metabolic properties that may contribute to limit the impact of intraabdominal obesity.—**Mauriège, P., A. Marette, C. Atgié, C. Bouchard, G. Thériault, L. J. Bukowiecki, P. Marceau, S. Biron, A. Nadeau, and J. P. Després.** Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J. Lipid Res.* 1995. **36:** 672–684.

Supplementary key words lipolysis • human adipocytes • α2-/β-adrenoceptor balance • insulin • catecholamines • massive obesity • regional variation

It has become increasingly evident that abdominal obesity is closely associated with cardiovascular complications, non-insulin-dependent diabetes mellitus, and premature death (1–3). Because of its anatomical location, which provides direct access to the hepatic portal circulation, excess visceral fat accumulation appears to be a greater health hazard than subcutaneous adipose tissue deposition, as the delivery of free fatty acids to the liver from intraabdominal adipocytes may contribute to hyperinsulinemia, hypertriglyceridemia, and glucose intolerance (4, 5). It is also now well established that the differential regulation of lipolysis in various adipose depots could play an important role in the pathophysiology of obesity (see refs. 6 and 7 for recent reviews). Furthermore, catecholamines and insulin are the main regulatory hormones acting on fat cell lipolysis in adult humans (1). In this regard, a higher lipolytic response to catecholamines and a lower inhibition of lipolysis by insulin in omental than in subcutaneous abdominal adipocytes have already been documented (8–14). Although several mechanisms may be responsible for these regional differences, variation in either the β- (13, 15) and/or the α2-adrenoceptor density (9, 16) as well as changes in insulin receptor affinity (8) have been proposed to explain the heterogeneity of response among various fat depots. It has also been suggested that variation in lipoprotein lipase activity could be of importance for explaining the existence of site differences in adipose tissue metabolism (10, 11, 17, 18). Furthermore, only a few experiments have attempted to define the mechanisms underlying regional differences in adipose tissue metabolism observed in massive obesity (11, 14, 16–19).

Therefore, the aims of the present study were *1)* to determine the metabolic characteristics (and more particularly, lipolytic and lipoprotein lipase activities) of adipocytes obtained from different adipose depots of severely obese women undergoing gastrointestinal surgery for

Abbreviations: LPL, lipoprotein lipase; HDL, high density lipoprotein; KRB, Krebs-Ringer-bicarbonate buffer; YOH, yohimbine; CGP, CGP 12177; CYP, cyanopindolol; ADA, adenosine deaminase.

[1]To whom correspondence should be addressed.

obesity; *2)* to examine regional variation in these responses; and *3)* to identify the cellular mechanisms (at receptor or post-receptor levels) underlying such site differences.

MATERIAL AND METHODS

Selection of patients

The study group included nine massively obese premenopausal women undergoing biliopancreatic diversion (or bypass) at the Laval Hospital of Quebec. This surgical procedure, which consisted in bypassing the small intestine and diverting the bile and pancreatic juice to the distal ileum, generally produced maldigestion and selective malabsorption essentially for fat and starch (20). None of the patients had any identified chronic disease (diabetes mellitus, cardiomyopathy, obstructive sleep apnea, endocrine disorders such as hypogonadism and hirsutism). Their body weight was stable at the time of the study, i.e., no subject had been on a diet or involved in a slimming program during the last 6 months. All women were also non-smokers and moderate alcohol consumers. They signed an informed consent document, as required by the Medical Ethics Committee, and had a complete examination by a physician that included a medical history. Subjects had fasted overnight before adipose tissue removal. General balanced anesthesia was induced by a short-acting barbiturate and maintained by fentanyl and a mixture of oxygen and nitrous oxide. The patients did not receive drugs active on the autonomic nervous system or modifying catecholamine levels. After surgical excision, the different adipose tissue samples (of about 10 g) were obtained within less than 30 min from the following sites. Subcutaneous abdominal fat (close to the umbilicus) was removed at the beginning of the surgery whereas the round ligament surrounding the liver and part of the major omentum were taken 15 min later. The two latter adipose tissues are classified as intraabdominal depots and both are drained by the portal vein (21). In addition, the round ligament of the liver is the *ligamentum teres hepatis* which represents the remains of the umbilical vein of the fetus. The hepatic end of the round ligament may still contain a lumen in the adult and the left branch of the portal vein receives this "ligament" (21).

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique (22) and percent body fat was derived from body density (23). Pulmonary residual volume was measured using the helium dilution method (24). Fat mass was obtained by multiplying percent body fat by body weight. Waist and hip girths were measured according to the procedures recommended at the Airlie Conference (25) and the ratio of the waist-to-hip circumferences was calculated.

Metabolic determinations

Blood samples were obtained in the morning after a 12-h fast from an antecubital vein. Plasma glucose was measured enzymatically (26) whereas plasma insulin was measured by radioimmunoassay using precipitation with polyethylene glycol (27). Cholesterol and triglyceride levels in both plasma and HDL obtained by precipitation of apoB lipoproteins, were measured enzymatically using an automated technique, as previously described (28).

Adipose tissue lipoprotein lipase (LPL) activity

Samples of approximately 250 mg of adipose tissue from each depot were immediately frozen for later measurement of heparin-releasable LPL activity, according to Savard et al. (29). Due to the well-known associations between fat cell size and adipose tissue LPL activity, the latter was expressed per unit of adipocyte surface area.

Adipocyte isolation

After collection, adipose tissue was quickly transferred to the laboratory, in saline (0.9% NaCl)–HEPES (5 mM) (pH 7.4) and used within a 15-min period. Adipocytes were isolated according to the method of Rodbell (30) in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin (KRBA) and 5 mM glucose, plus 1 mg/ml collagenase as already described (31). Digestion took place in a shaking water bath under a gas phase of 95% O_2 and 5% CO_2, for 40 min at 37°C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally resuspended in KRBA, to obtain a final concentration of approximately 500 cells per 50 μl.

Measurement of adipocyte lipolysis

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. Fifty-μl aliquots of the continuously stirred cell suspension were placed in 1.5-ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 μl KRB were immediately placed on ice and provided an evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation or inhibition were added just before the beginning of the assay in 10-μl portions in order to obtain the desired final concentration. After a 2-h incubation at 37°C in a shaking water bath, under a gas phase of 95% O_2 and 5% CO_2, 50 μl HCl (1 N) was added to all tubes to stop the reaction; then 50 μl NaOH (1 N) was added to neutralize the medium. All tubes were stored at −20°C until glycerol determination according to Kather, Schroder, and Simon (32). In this regard, NADH concentration was measured by bioluminescence with a luciferase solution, using a 1251 LKB Wallac luminometer (32, 33). For each concentration of stimulator or inhibitor, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Fat cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ). Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein (0.915 g/ml) was used to transform adipose cell volume into fat cell weight (31).

The lipolytic activity of the isolated fat cells was tested with isoproterenol (β-agonist), UK 14304 (α2-agonist) (34), and epinephrine which is a mixed agonist ($\alpha 2/\beta$) with a higher affinity for α2-adrenoceptor sites (9). Ascorbic acid (0.1 mmol/l) was included in the incubation medium in order to prevent catecholamine degradation. Some experiments were also conducted with procaterol (β2-agonist) (35), BRL 37344 (β3-agonist) (36), forskolin (direct activator of adenylate cyclase) (37), theophylline (mainly an inhibitor of phosphodiesterase), and dibutyryl-cAMP (stimulator of the protein kinase/hormone-sensitive lipase complex) (38). When antilipolytic effects were investigated, the incubation buffer was supplemented with 5 μg/ml adenosine deaminase (ADA) to remove adenosine released in the incubation medium by isolated fat cells; this procedure allows for more accurate investigations of the α2-mediated antilipolytic effects (9). Glycerol release was expressed per cell surface area in order to adjust for regional and individual differences in fat cell size. In cases where complete dose-response curves were obtained, they were compared for responsiveness and sensitivity. The responsiveness or maximal lipolytic effect was calculated as the difference between basal glycerol release and glycerol release at the maximum effective concentration of the lipolytic or antilipolytic agent. Sensitivity considered as the drug concentration giving half-maximal response (EC_{50}) was evaluated by logarithmic conversion from each dose-response curve.

Preparation of adipocyte membranes

Isolated fat cells were resuspended in a hypotonic lysing medium to elicit total cell breakage and recover fat cell ghosts. The lysing medium used was composed of 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, 2 mM Tris-HCl, pH 7.5, and of the following protease inhibitors: leupeptine (1 μg/ml) and phenylmethylsulfonyl fluoride (PMSF) (0.1 mM). [Ethylenebis (oxyethylenenitrilo)] tetraacetic acid (EGTA) (3 mM) was also added to prevent any tight-binding of endogenous catecholamines on adrenoceptors (9, 39). Cell lysis was performed at 20-22°C under vigorous shaking to minimize trapping of plasma membranes in the coalescing fat cake. Crude adipocyte ghosts were pelleted by centrifugation (40,000 *g*, 20 min) at 15°C, washed twice in the same buffer, and repelleted at 4°C. At the end of the washing procedure, the pellet was resuspended in 1 ml of lysing buffer and immediately frozen. The membrane preparations were stored at −80°C and generally used within 1 month for binding analysis. The protein content was determined according to the method of Lowry et al. (40), using bovine serum albumin as standard.

Radioligand binding studies

Two series of binding assays were carried out, one on intact adipocytes, and the second on fat cell membranes.

Assays on intact adipocytes. (−) [^{3}H]CGP 12177 (CGP) (β-antagonist) and (−) [^{3}H]yohimbine (YOH) (α2-antagonist) were used, respectively, for the identification of β- and α2-adrenoceptor sites on intact adipocytes (41, 42). Briefly, 0.5-2 × 10^5 fat cells were incubated in duplicate in a water bath for 15 min, under gentle shaking at around 80 cycles/min, at 37°C in 0.2 ml of KRBA buffer supplemented with chloroquine (0.03 mg/ml) to prevent ligand uptake and internalization, and ascorbic acid (0.1 mM) to inhibit catecholamine degradation. Specific binding was taken as the amount of radioactivity bound to intact adipocytes and defined as the difference between total and nonspecific binding determined in the presence of 10 μM unlabeled (−)propranolol (non-selective β-adrenergic antagonist) or (−)phentolamine (non-selective α-adrenergic antagonist). Adipocytes were incubated either with 5 nM of [^{3}H]CGP, or with 10 nM of [^{3}H]YOH, for the β- or the α2-adrenoceptor tracer experiments, respectively. As both radioligands bound to single classes of homogeneous noninteracting binding sites that gave straight lines on Scatchard analysis leading to Hill coefficients close to 1 (41, 42), we believe that the use of only one ligand concentration to determine maximal antagonist binding was justified under such conditions. It must be noted that, at the concentration used in our experiments, [^{3}H]CGP labeled high affinity binding sites that correspond to β1- and β2-adrenoceptors rather than low affinity binding sites that can be ascribed to β3-adrenergic receptors (36). Both incubations were stopped by a 10-fold dilution of the samples with ice-cold saline followed by rapid vacuum filtration under reduced pressure through Whatman GF/C glass fiber filters placed on a Millipore manifold. Filters were rinsed twice with 10 ml ice-cold saline and put into vials containing 4 ml of scintillation liquid, then counted in an LKB scintillation counter, at an efficiency of 35%.

Assays on fat cell membranes. (−)-^{125}I-labeled cyanopindolol (CYP) (β-antagonist) and (−)[^{3}H]YOH were used, respectively, for the quantification of β- and α2-adrenoceptor sites on fat cell membranes, as previously described (9, 39). Thawed crude adipocyte membranes were homogenized further with four pestle strokes in a Potter apparatus and washed once in 50 mM Tris-HCl, 0.5 mM $MgCl_2$, pH 7.5 (Tris-Mg buffer). The pellet was then adjusted to a final concentration of 0.5 to 1 mg protein/ml. Total binding was determined by incubating 50-μl aliquots of the resuspended membrane preparation with increasing concentrations of ^{125}I-labeled CYP

(10–300 pM) in a total volume of 200 μl Tris-Mg buffer. Nonspecific binding was evaluated, in parallel assays, in the presence of 10 μM (–)propranolol. Incubations were carried out in a water bath for 45 min at 37°C, under constant shaking at around 120 cycles/min. Under these conditions, we believe that ^{125}I-labeled CYP binds mainly to the high affinity binding sites that correspond to β1-/β2-adrenoceptors rather than to the low affinity binding sites that can be ascribed to β3-adrenergic receptors (36, 39). Specific binding was defined as the difference between total binding and binding in the presence of 10 μM (–) propranolol. A similar radioligand binding technique was used to identify α2-adrenoceptors with increasing concentrations of [^{3}H]YOH (1–15 nM). Nonspecific binding was determined with 10 μM (–) phentolamine and incubations were carried out in a water bath for 25 min at 25°C, under constant shaking at around 120 cycles/min. For all binding assays, the reaction was stopped by the addition of 4 ml ice-cold binding buffer followed by rapid filtration, using a 12-sample Skatron Cell Harvester. The tubes and filters were then washed twice with 10 ml portions of ice-cold binding buffer. For ^{125}I-labeled CYP binding, the radioactivity retained on the filters was directly counted in an LKB gamma counter (at an efficiency of 75%), whereas for YOH binding, filters were placed in minivials containing 2 ml of liquid scintillation cocktail and counted in an LKB scintillation counter (at an efficiency of 35%). Both radioligands, [^{3}H]YOH and ^{125}I-labeled CYP, displayed saturable specific binding to crude fat cell membranes prepared from the different tissues, and nonspecific binding did not exceed 20–30% of total binding. Saturation curves were analyzed according to the method of Scatchard, using a non-linear curve-fitting program (43) to define the maximum number of binding sites (B_{max}) and the radioligand dissociation constant or affinity (K_D).

In competition studies, a fixed concentration of either ^{125}I-labeled CYP (200 pM) or [^{3}H]YOH (6 nM) corresponding to twice the K_D was used, and the effect of 14 different concentrations of epinephrine (ranging from 10^{-9} to 10^{-4} M) was investigated. Nonspecific binding evaluated at the highest concentration of 10^{-4} M epinephrine was not different from that determined with 10^{-5} M propranolol or phentolamine. The catecholamine was diluted and added to the assay (50 μl) just prior to the experiments, along with a mixture containing ascorbic acid (750 μM), pargylin (25 μM), and pyrocatechol (0.3 mM) to prevent hormone degradation (9, 39, 42). Displacement of radiolabeled antagonists by unlabeled agonist revealed shallow biphasic curves, because of binding to both coupled (high affinity) and uncoupled (low affinity) receptors identified by the hormone. The inhibition data were analyzed by the computer program TWOSITEINHIB (43), according to the Hill coefficient values (ranging from 0.40 to 0.60), which allowed iterative curve-fitting to a model for two classes of non-interacting sites giving the high and low affinity states of β- and α2-adrenoceptors for epinephrine as well as the proportion of receptors in each affinity state.

Drugs and chemicals

Collagenase, bovine serum albumin, adenosine deaminase, and enzymes for glycerol assays were obtained from Boehringer (Mannheim, Canada). Insulin (Iletin II) (500 U/ml) came from Eli Lilly (Indianapolis, IN). Ascorbic acid, pargylin, pyrocatechol, PMSF, EGTA, leupeptine, chloroquine, (–) isoproterenol bitartrate, (–) epinephrine bitartrate, (–) propranolol hydrochloride, theophylline, forskolin, and dibutyryl-cAMP were purchased from Sigma Chemical Co. (St. Louis, MO). UK 14304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) was generously provided by Dr. D. A. Faulkner (Pfizer, Sandwich, England) and phentolamine mesylate came from Ciba Geigy (Canada). Procaterol (OPC-2009) (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate) was a generous gift from Otsuka Pharmaceuticals (Tokushima, Japan) and BRL 37344 (4-[-[(2-hydroxy-(3-chloro-phenyl)ethyl)-amino]propyl] phenoxyacetate) was kindly provided by Dr. M. A. Cawthorne (Smithkline-Beecham Pharmaceuticals, Epsom, England). (–)(O-methyl-[^{3}H])yohimbine (YOH) (sp act, 85 Ci/mmol) and (–) ^{125}I-labeled cyanopindolol (CYP) (sp act, 2200 Ci/mmol) were obtained from DuPont/New England Nuclear (Boston, MA). (–)[^{3}H]CGP-12177 (4-(3-butylamino-2-hydroxypropoxy)-(5,7-^{3}H)-benzimidazole-2-1-hydrochloride (sp act, 41 Ci/mmol) was purchased from Amersham International (Canada). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of hormones, pharmacological agents, collagenase, and albumin were used in all experiments.

Statistical methods

All experiments were performed in duplicate. Overall regional differences were first analyzed by a three-way analysis of variance (ANOVA) and multiple comparisons among the three fat depots were handled with the Duncan Multiple Range test.

RESULTS

The physical and metabolic characteristics of the subjects are summarized in **Table 1** and **Table 2,** respectively. Body fatness variables such as the percentage of body fat (ranging from 50 to 66%) and the mean body mass index (reaching 45 kg/m^2) clearly indicate that patients were severely obese (Table 1). In addition, the mean waist-to-hip ratio (WHR = 0.84) indicated that these women tended to show a high proportion of abdominal fat. However, despite the subjects' massive obesity, the

TABLE 1. Physical characteristics of the subjects

	Mean ± SD	Range
Age (yr)	37 ± 6	27-48
Body weight (kg)	112 ± 17	83-140
Body mass index (kg/m²)	45 ± 5	37-51
Body fat (%)	58 ± 5	50-66
Fat mass (kg)	65 ± 14	41-89
Waist circumference (cm)	113 ± 9	106-132
Hip circumference (cm)	136 ± 14	117-158
Waist-to-hip ratio	0.84 ± 0.06	0.74-0.93

metabolic profile was not markedly deteriorated, with the exception of fasting insulin levels (Table 2) which were quite elevated as compared to those from moderately obese premenopausal women of similar age (2).

Figure 1 shows regional variation in fat cell weight and lipoprotein lipase (LPL) activity. Round ligament adipose cells were slightly but significantly larger ($P < 0.05$) than subcutaneous abdominal or omental adipocytes. Similarly, LPL activity, corrected for variation in fat cell surface area, was higher in round ligament than in the two other cell types ($P < 0.05$). Moreover, there was a trend in both fat cell weight and LPL activity to be lower in omental than in subcutaneous abdominal adipose cells but the difference did not reach statistical significance ($P = 0.06$).

As illustrated in **Fig. 2,** basal lipolysis was significantly higher in subcutaneous abdominal than in both round ligament and omental adipocytes ($P < 0.05$). Addition of adenosine deaminase (ADA) at 5 μg/ml in the incubation medium increased the basal lipolytic rate by about 2- to 3-times as compared to that observed in standard conditions (in the absence of this enzyme) in all fat depots. However, the level of glycerol release reached with ADA was still significantly higher in subcutaneous abdominal than in intraabdominal adipose cells ($P < 0.05$). Results were essentially the same when lipolysis or LPL activity was expressed per cell number (not shown).

In order to control for variation in fat cell weight, lipolysis measurements have been expressed per cell surface area. **Figure 3** shows the lipolytic responses of adipocytes

TABLE 2. Metabolic profile of the subjects

	Mean ± SD	Range
Fasting glucose (mmol/l)	5.54 ± 0.83	4.80-7.40
Fasting insulin (pmol/l)	141 ± 75.4	64-285
Triglycerides (mmol/l)	1.61 ± 0.76	1.11-3.57
Cholesterol (mmol/l)	5.00 ± 1.23	3.25-6.95
LDL-CHOL (mmol/l)	3.32 ± 0.77	2.17-4.76
HDL-CHOL (mmol/l)	1.23 ± 0.23	0.86-1.68
CHOL/HDL-CHOL	4.03 ± 1.08	2.41-5.57

CHOL, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein.

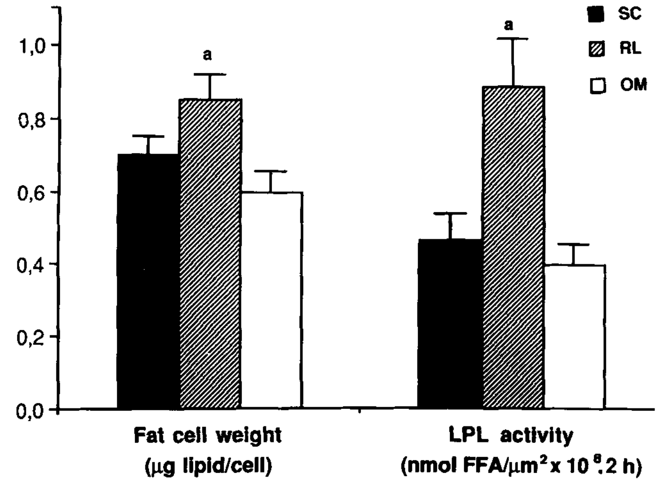

Fig. 1. Site differences in fat cell weight and in lipoprotein lipase (LPL) activity. Values are means ± standard error (SE) of 9 experiments run in duplicate;[a], indicates regional variation at $P < 0.05$.

from different depots to the physiological hormones: epinephrine (panel A) and insulin (panel B). In the presence of ADA, epinephrine, known for its mixed agonist ($\alpha 2/\beta$) adrenergic properties, initiated a biphasic response profile in all adipose cells (Fig. 3A). An inhibition of lipolysis was observed at the lowest concentrations (10^{-9} to 10^{-7} M), this effect being completely reversed at higher doses (10^{-6} to 10^{-5} M), indicating a preferential recruitment of $\alpha 2$- followed by β-adrenoceptor sites. However, the maximal antilipolytic response to epinephrine (at 10^{-7} M) was weaker in omental than in subcutaneous abdominal adipocytes ($P < 0.05$). Significant differences were also observed in epinephrine-induced antilipolysis between these two latter cells at concentrations (10^{-9} and

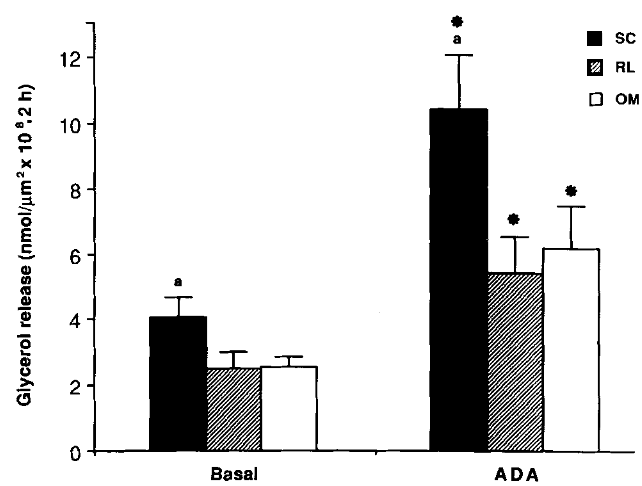

Fig. 2. Regional variation in basal lipolytic rate and ADA-stimulated lipolysis. Values are means ± standard error (SE) of 9 experiments performed in duplicate. The symbol * indicates a significant difference from the corresponding basal lipolysis value at $P < 0.05$.[a], Indicates regional variation at $P < 0.05$.

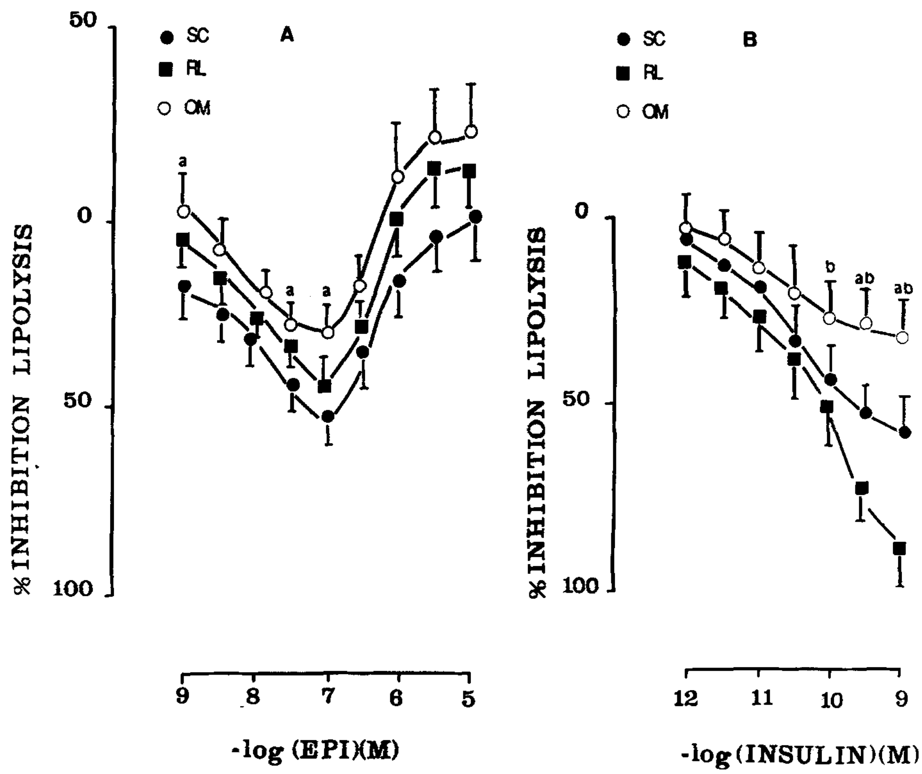

Fig. 3. Regional variation in the effects of epinephrine (EPI) (panel A) and insulin (panel B) on glycerol release. Fat cells were incubated in the presence of 5 μg/ml of adenosine deaminase (ADA). Glycerol release was calculated as the difference between stimulated (with EPI) and basal values, and then expressed on a percentage basis (panel A). The antilipolytic effect of insulin is given as percent inhibition of ADA-stimulated lipolysis, i.e., (ADA minus insulin/ADA)× 100 (panel B). Values are means ± SE (bars) of 7, 6, and 6 experiments run in duplicate for subcutaneous abdominal, round ligament, and omental adipocytes, respectively. Means not sharing a common superscript are significantly different at $P < 0.05$.

5 × 10^{-8} M) at which the α2-adrenoceptor component is expressed ($P < 0.05$). On the other hand, maximal lipolysis promoted by the hormone (at 10^{-5} M) was not different among the three depots, although the subcutaneous abdominal region displayed a slight tendency for a reduced lipolytic activity (when expressed on an absolute basis), as compared to the other sites.

The ability of insulin to inhibit ADA-stimulated glycerol release was also investigated (Fig. 3B). The maximal antilipolytic effect (at 10^{-9} M of the hormone) was higher in round ligament adipocytes where it reached 92 ± 15% of ADA-stimulated lipolysis ($P < 0.005$), compared to the other adipose cells. However, in subcutaneous abdominal fat cells, maximal insulin inhibition of lipolysis reached only 51 ± 9% but was still significantly higher than that observed in omental adipocytes (29 ± 7%) ($P < 0.005$). In addition, insulin sensitivity, defined as the concentration of the hormone required for half-maximal inhibition of lipolysis, was greater in round ligament adipocytes (95 ± 25 pmol/l) than either in subcutaneous abdominal (264 ± 102 pmol/l) or in omental fat cells (608 ± 221 pmol/l) ($P < 0.01$).

As epinephrine responsiveness results from both α2- and β-adrenoceptor stimulation, selective adrenergic agonists were used to discriminate between these two antagonistic effects (**Fig. 4**). To characterize the α2-adrenoceptor component, the selective α2-agonist UK 14304 was tested on ADA-induced lipolysis. In order to control for variations in the level of stimulated lipolysis achieved, results were expressed as the percent inhibition of a maximal response (Fig. 4A). UK 14304 inhibited lipolysis in a dose-dependent manner in all adipocytes, and the maximal antilipolytic effect noted at 10^{-6} M of the α2-agonist was similar, irrespective of the anatomic location of fat (values clustering 95% inhibition of ADA-induced lipolysis). A lack of regional variation was also observed in the α2-adrenergic sensitivity (values ranging from 10 to 20 nM). To study the influence of the β-adrenoceptor component, the lipolytic effect of isoproterenol was examined (Fig. 4B). The relative stimulation of lipolysis initiated by the β-agonist was not strikingly different among the three adipose regions. In addition, maximal lipolytic responses to isoproterenol (when expressed on absolute rates) were similar whatever the anatomic location of fat. However, the dose–response curve of isoproterenol shifted to the left in omental adipocytes indicated a higher β-adrenergic

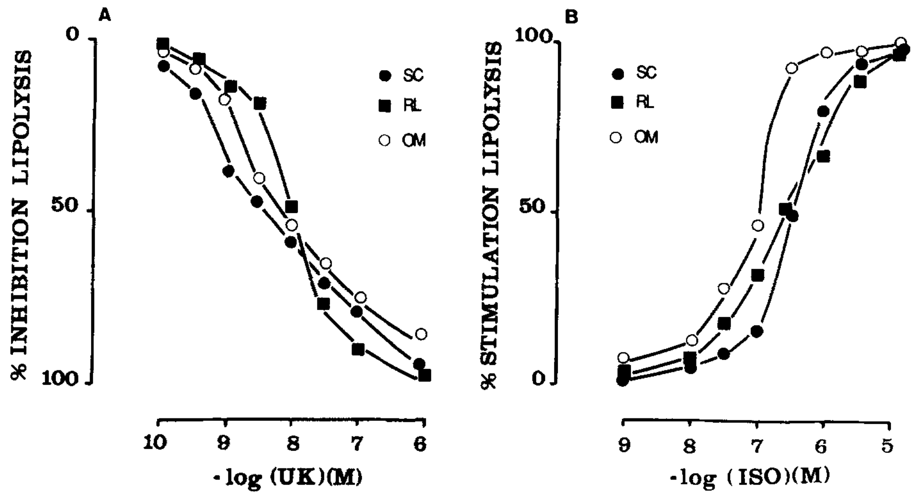

Fig. 4. Comparison of UK 14304 (UK)-induced inhibition of ADA-stimulated lipolysis (panel A) and isoproterenol (ISO)-induced lipolysis (panel B) in adipocytes from different sites. As the three curves were superimposed, SE were excluded for clarity. The number of experiments is similar to Fig. 3. Fat cells were incubated in the presence of 5 μg/ml of ADA and the antilipolytic effect promoted by UK is given as percent inhibition of ADA-stimulated lipolysis, i.e., (ADA minus UK/ADA) × 100 (panel A). Adipocytes were incubated under basal conditions and the lipolytic effect initiated by ISO was expressed on a percent value of maximal response (panel B). The agonist concentrations required for half-maximal inhibition (UK) or stimulation (ISO) of lipolysis were determined from these dose–response curves. The maximal lipolytic response to ISO (expressed in nmol glycerol/$\mu m^2 \times 10^8 \cdot 2$ h) was defined as the glycerol release at 10^{-5} M of the β-agonist minus basal lipolysis. Values obtained were 5.5 ± 0.9, 5.5 ± 1.5, and 5.1 ± 1.3 in subcutaneous abdominal, round ligament, and omental fat cells, respectively.

sensitivity in these cells, as compared to subcutaneous abdominal and round ligament adipocytes (**Table 3**).

As site differences in catecholamine responsiveness seem to be partly explained by the β-adrenoceptor function, additional experiments were performed using procaterol (β2-agonist) and BRL 37344 (β3-agonist). The fact that the dose–response curve for procaterol was shifted to the left in the omental depot as compared to the other regions, suggested an increase in the β2-adrenoceptor sensitivity. In addition, the β3-adrenoceptor sensitivity was slightly but significantly higher in omental than in round ligament fat cells (Table 3). Despite the lack of regional variation in the maximal lipolytic response to the β-agonists tested, neither procaterol nor BRL 37344 was as potent as isoproterenol in stimulating lipolysis. The β2-agonist (at 10^{-5} M) partially activated lipolysis in adipose cells from all depots (Table 3). Although the β3-agonist (at 10^{-5} M) exerted a very weak lipolytic effect in subcutaneous abdominal and round ligament fat cells, it appeared slightly more efficient in omental adipocytes. However, this difference did not reach statistical significance, probably because of the few experiments performed (Table 3). EPI-induced maximal lipolysis (10^{-5} M) was also lower than that initiated by isoproterenol (values clustering at 50 to 60% of the maximal effect promoted by the β-agonist) ($P < 0.05$). Therefore, the relative order of potency in the initiation of the maximal lipolytic activity was isoproterenol > procaterol ≥ epinephrine > > BRL 37344, irrespective of the anatomic site investigated.

The mechanisms underlying the site differences in epinephrine responsiveness may be located at any step of the lipolytic cascade. Therefore, the effects of agents that stimulate lipolysis at post-receptor levels were also investigated (**Table 4**). The rates of glycerol release were not different among the various adipose cells when lipolysis was stimulated at maximum concentrations of forskolin

TABLE 3. Sensitivity and intrinsic activity for different β-adrenoceptor agonists estimated from in vitro lipolysis studies on intact fat cells of various depots

	Subc Abdo	Round Ligament	Omental
Sensitivity or EC_{50} (nM)			
Isoproterenol (7)	356 ± 86[a]	481 ± 173[a]	174 ± 48[b]
Procaterol (4)	520 ± 115[a]	704 ± 206[a]	82 ± 29[b]
BRL 37344 (4)	> 10.000	2420 ± 783	631 ± 228
Intrinsic activity (%)			
Procaterol (4)	75 ± 8	63 ± 7	57 ± 7
BRL 37344 (4)	< 5	19 ± 8	32 ± 10

Subc Abdo, subcutaneous abdominal adipose tissue. Values are means ± SE of (n) separate experiments performed in duplicate. Sensitivity estimated by the agonist concentration required for half-maximal stimulation of lipolysis (EC_{50}) was calculated from each dose–response curve (from 10^{-9} to 10^{-5} M of the β-agonists tested). Intrinsic activity for each agonist was expressed as the percentage of maximal lipolysis initiated by isoproterenol at 10^{-5} M, which was taken as a reference (100%). All the agents tested behaved as partial agonists.

[a,b]Means not sharing a common superscript are significantly different ($P < 0.05$).

TABLE 4. Lipolytic responsiveness of agents acting at post-receptor levels in adipocytes from different depots

	Subc Abdo	Round Ligament	Omental
DcAMP (8)	3.9 ± 0.7	4.9 ± 1.0	2.6 ± 0.9
Forskolin (5)	2.5 ± 0.5	3.9 ± 0.6	2.9 ± 0.9
Theophylline (6)	3.4 ± 0.9	4.4 ± 1.2	2.5 ± 0.7

DcAMP, dibutyryl-cyclic AMP. Subc Abdo, subcutaneous abdominal adipose tissue. Values are means ± SE of (n) separate experiments performed in duplicate. Fat cells were incubated with maximum effective concentrations of DcAMP (10^{-3} M), forskolin (10^{-5} M), or theophylline (10^{-3} M). Lipolytic responsiveness (expressed in nmol glycerol/$\mu m^2 \times 10^8 \cdot 2$ h) was calculated as the difference between the glycerol release at maximum concentration of each agent minus basal lipolysis.

(10^{-5} M), theophylline (10^{-3} M), or dibutyryl-cyclic AMP (10^{-3} M) which selectively act at the level of adenylate cyclase, phosphodiesterase, and protein kinase, respectively. In addition, there was no significant difference between the compounds tested for a given adipose depot (Table 4).

To verify whether site differences in catecholamine-induced lipolysis can be explained at the receptor level, β- and $\alpha 2$-adrenoceptor sites were also studied in both intact adipocytes and fat cell membranes from the three depots (**Fig. 5**). The radioligands [^{3}H]YOH and ^{125}I-labeled CYP displayed specific binding to the different adipose cell membranes (not shown). The dissociation constants K_{DS} (nmol/l) for [^{3}H]YOH were 2.2 ± 0.2, 1.4 ± 0.2, and 1.3 ± 0.2 whereas the K_{DS} (nmol/l) for ^{125}I-labeled CYP were 0.21 ± 0.04, 0.10 ± 0.02, and 0.11 ± 0.02 in subcutaneous abdominal, round ligament, and omental fat cell membranes, respectively. These results indicate that $\alpha 2$- and β-adrenoceptor binding sites exhibited a decreased affinity (i.e., a higher K_D) for their respective radioligands in subcutaneous abdominal, compared to intraabdominal adipocyte membranes ($P < 0.05$). However, the density of [^{3}H]YOH binding sites was significantly higher in both subcutaneous abdominal and round ligament than in omental adipose cell membranes ($P < 0.01$) while the lowest number of ^{125}I-labeled CYP binding sites was observed in the former preparations ($P < 0.05$) (Fig. 5A). Because the evaluation of the functional balance between $\alpha 2$- and β-adrenoceptors seemed to be of major interest, data were expressed as the ratio of [^{3}H]YOH to ^{125}I-labeled CYP binding sites which was calculated for each adipose cell membrane and averaged for the different sites. The mean ratio of $\alpha 2$- to β-adrenoceptors found in the subcutaneous abdominal region (5.4 ± 1.3) was higher than that obtained in the round ligament adipose tissue (2.5 ± 0.7) ($P < 0.05$), whereas the ratio in the omental fat depot (1.2 ± 0.5) was significantly lower than in the other adipose regions ($P < 0.05$). As it was not possible to perform complete saturation experiments because of the limitations in the amount of tissue available, only one maximal concentration of each radioligand (i.e., [^{3}H]CGP and [^{3}H]YOH) was used to evaluate the number of β- or $\alpha 2$-adrenergic binding sites on intact fat cells (Fig. 5B). As round ligament adipocytes were larger than subcutaneous abdominal and omental adipose cells, binding results were corrected for adipocyte size. At 10 nM, [^{3}H]YOH binding was almost twice as high in subcutaneous abdominal and round ligament than in omental fat cells ($P < 0.01$), whereas at 5 nM, [^{3}H]CGP binding was 1.5 to 2 times lower in the two other adipose cells com-

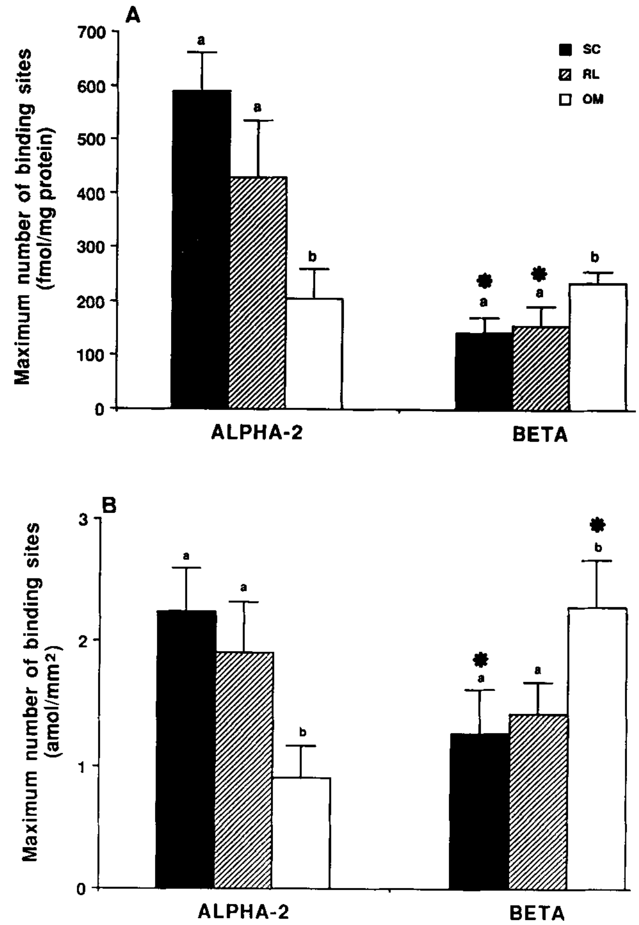

Fig. 5. Comparative study of $\alpha 2$- and β-adrenoceptors on adipose cell membranes (panel A) and intact adipocytes (panel B) from the different depots. Adipose cell membranes were incubated with increasing concentrations of [^{3}H]yohimbine ($\alpha 2$-antagonist) or ^{125}I-labeled cyanopindolol (β-antagonist), as described in Material and Methods. Values are means ± SE (bars) of 7, 6, and 6 determinations performed in duplicate, for subcutaneous abdominal, round ligament, and omental fat cell membranes, respectively. The maximum number of binding sites was defined from Scatchard analysis of the saturation data as previously described (9, 39) (panel A). Adipocytes were incubated with 10 nM of [^{3}H]yohimbine or 5 nM of [^{3}H]CGP12177 (β-antagonist), as described in Material and Methods. Values are means ± SE (bars) of 6, 5, and 5 determinations run in duplicate for subcutaneous abdominal, round ligament, and omental adipose cells, respectively. The maximum number of binding sites was corrected for variation in cell surface area (panel B). Means not sharing a common superscript are significantly different at $P < 0.05$. The symbol * indicates a significant difference from the corresponding site at $P < 0.05$.

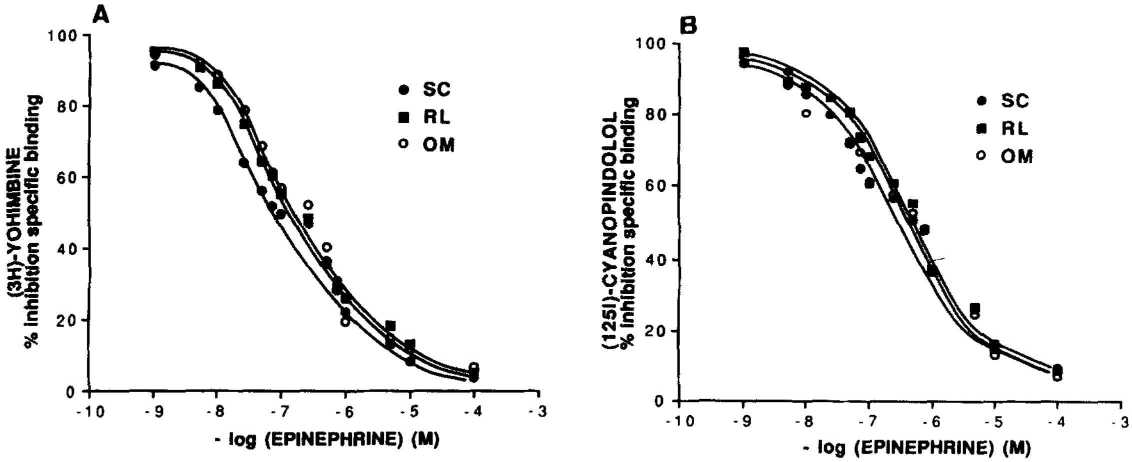

Fig. 6. Epinephrine inhibition of [^{3}H]yohimbine (YOH) (α2-antagonist) (panel A) and ^{125}I-labeled cyanopindolol (CYP) (β-antagonist) (panel B) binding in adipose cell membranes from the various depots. Competition assays were carried out against 6 nM [^{3}H]YOH and 200 pM ^{125}I-labeled CYP. Points shown are the means of 6, 5, and 5 experiments performed in duplicate for subcutaneous abdominal (●), round ligament (■), and omental (○) fat cell membranes, respectively. Standard errors were around 15% and deleted for clarity as the curves were superimposed on each other. Results are expressed as the percentage of inhibition of specific binding for each radioligand. Data given by computer-assisted analysis of the curves (43), obtained in each tissue of the different patients, are provided in Table 5.

pared to omental adipocytes ($P < 0.05$).

Finally, among the receptor factors modulating fat cell epinephrine responsiveness, the apparent affinities of the physiological agonist for both β- and α2-adrenoceptors in fat cell membranes from various depots were also determined under identical conditions. As shown in **Fig. 6,** epinephrine competition curves yielded shallow binding isotherms; the reduced slope factors between 0.40 and 0.60 indicated that a portion of the adrenoceptor population for each kind of site bound the catecholamine with high affinity while the remaining receptors were in a low affinity state. Binding parameters defined by computer analysis of these competition-inhibition experiments are presented in **Table 5.** Epinephrine always exhibited a higher affinity for α2- than for β-adrenoceptors, whatever the anatomic location of fat. However, there was no regional variation in the percentage of β-adrenoceptors in a high affinity state, nor in the dissociation constants for the corresponding high and low affinity states. A larger proportion of α2-adrenoceptors (40%) in a high affinity state was observed in the subcutaneous abdominal than in the intraabdominal depots (30%) ($P < 0.05$). Despite similar K_H values, K_L was significantly lower in subcutaneous abdominal than in intraabdominal depots ($P < 0.05$). An increased proportion of α2-adrenoceptors (40%) was also in a high affinity state, as compared to β-adrenergic receptors (30%) in the subcutaneous abdominal adipose tissue ($P < 0.05$).

TABLE 5. Comparison of β- and α2-adrenoceptor affinities for (−)epinephrine in fat cell membranes of various depots

	Subc Abdo (6)	Round Ligament (5)	Omental (5)
β-Adrenoceptor sites (^{125}I-labeled CYP)			
K_H (nmol/l)	19 ± 5	24 ± 5	24 ± 6
K_L (nmol/l)	2601 ± 613	3442 ± 830	3778 ± 979
% R_H	29 ± 4	35 ± 5	·37 ± 6
α2-Adrenoceptor sites ([^{3}H]YOH)			
K_H (nmol/l)	8 ± 3	11 ± 4	14 ± 2
K_L (nmol/l)	396 ± 72^a	589 ± 87^b	601 ± 169^b
% R_H	42 ± 4^{a*}	30 ± 5^b	29 ± 3^b

Subc Abdo, subcutaneous abdominal adipose tissue. Values are means ± SE of (n) separate experiments performed in duplicate. The dissociation constants for high (K_H) and low (K_L) affinity states of both β- and α2-adrenoceptors and the percentage of these receptors in a high affinity state (% R_H) were calculated from inhibition data (43).

^{a,b}Means not sharing a common superscript are significantly different ($P < 0.05$). The symbol * indicates a significant difference between % R_H of α2- and β-adrenoceptors for the subcutaneous abdominal fat depot ($P < 0.01$).

DISCUSSION

Until recently, few studies have attempted to elucidate the mechanisms responsible for the site differences observed in adipose tissue metabolism from morbidly obese humans (11, 14, 17, 19). Therefore, the present study was conducted to examine the cellular mechanisms underlying regional variation of adipose cell metabolism in massively obese premenopausal women undergoing gastrointestinal surgery for obesity. For this purpose, lipolysis and lipoprotein lipase assays combined with radioligand binding experiments were performed.

From their physical characteristics, it was clear that our patients were severely obese (44). However, although our subjects were hyperinsulinemic, they did not show a major deterioration of their lipid-lipoprotein profile as compared to moderately obese premenopausal women of similar age (2). This finding may contribute to explain the fact that our massively obese women were not characterized by the cardiovascular complications that are frequently related to such a morbidly obese state (44).

It has already been proposed that a high lipolytic activity of visceral fat depots could be an important factor linking intraabdominal adiposity to the development of obesity-related metabolic complications (3-5). The increased lipolysis of omental adipocytes from obese men and women has also been widely described (10-15, 19). However, to the best of our knowledge, it is the first time that, as an intraabdominal fat depot, the adipose tissue of the round ligament has been used for adrenoceptor characterization and for the measurement of both lipolysis and lipoprotein lipase.

Subcutaneous abdominal and omental adipose cells did not differ in size despite a slight tendency for a lower fat cell weight in deep than in subcutaneous adipocytes. This result may appear at variance with some previous findings (11, 14, 17), although it is concordant with other observations (45). However, both fat cell weight and LPL activity were greater in adipose tissue from the round ligament as compared to the omentum. In this regard, functional heterogeneity among intraabdominal fat depots has already been documented by Fried and Kral (17) who found a higher LPL activity in the mesenteric region than in the omental site, although other investigators did not observe any significant regional variation either in lean (10) or in severely obese men and women (11).

Our data also show the existence of site differences in the regulation of lipolysis among intraabdominal adipose tissues. The higher basal lipolytic rate of subcutaneous abdominal than visceral adipocytes noted in our study is in agreement with previous observations (8, 12-14). Similarly, the greater ADA-stimulated lipolysis in subcutaneous abdominal than in intraabdominal adipose cells suggests that adenylate cyclase was more sensitive to inhibition by adenosine in the former than in the latter adipocytes, a finding consistent with previous observations (46). Insulin-induced inhibition of lipolysis also exhibited regional variation, the highest effect being observed in round ligament adipose cells, followed by subcutaneous abdominal adipocytes; the weakest antilipolytic effect is found in omental fat cells. In this regard, the more pronounced antilipolytic effect of insulin in subcutaneous abdominal than in omental adipocytes could be explained by a higher responsiveness to endogenous adenosine in subcutaneous than in deep abdominal adipose cells (46). Although elevated cAMP levels have been shown to modulate insulin sensitivity and action (47), the blunted response to this hormone in the omentum could not be due to the addition of adenosine deaminase in our lipolysis assays as the antilipolytic effect of insulin (measured in standard conditions) lower in omental than in subcutaneous abdominal adipocytes, has been partly attributed to a decreased insulin-receptor affinity in omental cells (8). Regional differences in the insulin response have also been observed by other investigators who reported similar rates of lipolysis, when stimulated by ADA, among subcutaneous and intraabdominal fat depots (12). Moreover, the greater antilipolytic effect of insulin found in adipose tissue from the round ligament than in the omentum questions the importance of high cAMP levels for impairing the insulin signal transduction mechanisms, as there was no variation in ADA-induced lipolysis among the two intrabdominal fat depots (as shown in Fig. 2). Because both insulin and LPL favor triglyceride (TG) storage in adipocytes (18), the highest LPL activity as well as the greatest insulin-induced antilipolysis and sensitivity to this hormone in round ligament adipose cells may therefore suggest that this depot was quite efficient in TG storage. In accordance with this hypothesis, round ligament adipocytes were more sensitive to the stimulatory effect of insulin on glucose transport, as compared to omental or subcutaneous abdominal fat cells (A. Marette, P. Mauriège, C. Atgié, C. Bouchard, G. Thériault, L.K. Bukowiecki, P. Marceau, S. Biron, A. Nadeau, and J.P. Després, unpublished data). However, the fact that antilipolysis was still observed in our massively and hyperinsulinemic obese women, suggests that this process may play a significant role in the excessive abdominal fat accumulation noted in these patients. Hyperinsulinemia could potentially counteract the resistance to insulin-induced glucose metabolism in obese individuals, but could also reduce lipolysis because of the antilipolytic effect of the hormone, leading to both enlargement of adipocytes (as shown in Fig. 1) and to body fat accretion (1). In accordance with previous studies performed in nonobese (10) as well as in moderately (9, 12, 13) and in severely obese women (11, 14), our results demonstrated the presence of regional variations in epinephrine responsiveness and more particularly between omental and subcutaneous abdominal adipose depots. Indeed, the functional balance between α2- and β-adrenoceptors seems to be of importance in explaining these site differences. The lack of regional difference in lipolysis stimulated at post-receptor steps re-emphasizes the fact that variation in hormone responsiveness was probably localized at the adrenoceptor level. As subcutaneous abdominal adipocytes possess the highest α2-adrenoceptor density and the greatest ratio of α2- to β-sites, an increased number of both α2-adrenergic receptors and receptors in high affinity states could explain the more pronounced α2-antilipolytic effect observed in this depot at the lowest concentrations of epinephrine. Although the ratio of α2- to β-sites was the weakest in omental fat cells, the α2-adrenergic component of the epinephrine responsiveness was still predominant (probably because of the higher affinity of the catecholamine for α2- than for β-adrenoceptors shown in Table 5) and could not be entirely compensated by the β-adrenergic activity of the hormone, despite a significantly higher β-adrenoceptor density as compared to round ligament adipocytes. Such data support the fact that, among the factors controlling epinephrine efficiency, the α2-adrenoceptor component plays an important role in explaining site differences in catecholamine-induced lipid mobilization (9, 16, 33). A clear antilipolytic effect of epinephrine was also observed in the two intraabdominal adipose tissues investigated. It is noteworthy that in contrast to our previous study performed on lean women (9), omental fat cells displayed an antilipolytic response to the catecholamine. In this regard, it must be kept in mind that our subjects were massively obese and that a significant α2-adrenergic component may exist in the omentum of such patients, although it may be less pronounced than in the subcutaneous abdominal adipose region. Moreover, the presence of such a potent α2-antilipolytic effect assessed by both lipolysis and radioligand binding studies has recently been found in the pericolonic visceral adipose tissue (16).

The "in vivo" implication of the antilipolytic responsiveness to epinephrine of both omental and round ligament adipose cells from our massively obese women is not well understood. As lipolysis is generally increased in intraabdominal adipose tissues, their venous drainage directly into the portal circulation may lead to a high flux of free fatty acids (FFA) through the liver which may contribute to hypertriglyceridemia, hyperinsulinemia and glucose intolerance (3–5). In this regard, FFA reesterification (and therefore, TG synthesis) may be as important as lipolysis in determining the net release of FFA from adipose tissue. This possibility has been strengthened by the fact that upper body obese women have higher rates of FFA turnover compared to non-obese or lower body obese women (48). In addition, low rates of TG synthesis in omental and mesenteric depots that may prevent accumulation of large intraabdominal fat stores have already been reported in severely obese women (49). From the latter observations, it could be hypothesized that the α2-adrenergic component found in round ligament and omental adipocytes may represent a protective mechanism against the metabolic complications associated with obesity, by impairing FFA release from these two visceral cell types.

Finally, both the higher β- and β2-adrenoceptor sensitivities (as shown in Table 3) in omental than in subcutaneous abdominal or round ligament adipose cells further support the concept of a non-negligible role of the β-adrenergic component (10, 11, 13, 15). The higher lipolytic sensitivity of omental adipocytes to catecholamines could be attributed to the higher amount of β1- and β2-adrenergic receptors (15, 17), but also to an atypical β-adrenoceptor contribution (50, 51). From the very weak stimulation of lipolysis by BRL 37344 in omental adipocytes, the possibility that such adipocytes may contain a population of atypical β-adrenoceptors on which this agent acts as a partial agonist cannot be excluded. Indeed, recent evidence at both functional and molecular levels has pointed out the existence of putative β3-adrenergic receptors in omental adipocytes and to a minor extent in subcutaneous abdominal adipose cells (50, 51). However, further investigations addressing the potential role of β3-adrenoceptors in the regulation of human fat cell lipolysis are required as the physiological function of this β-adrenoceptor subtype is still controversied (36, 52, 53).

In conclusion, the present study underscores the particular interest of the round ligament adipose tissue. This intraabdominal depot displayed properties that are distinct from the omentum: a higher number of α2-adrenoceptors, a lower β-adrenoceptor sensitivity associated with a reduced β-adrenoceptor density, a higher lipoprotein lipase activity as well as both a greater antilipolytic response to insulin, and an increased sensitivity to this hormone. These data are concordant with previous reports suggesting that intraabdominal adipose tissues are not functionally similar (14, 17).

The authors want to express their gratitude to Martine Marcotte, France Levasseur, Judith Maheux, Jacinthe Hovington, Henri Bessette, and Claude Leblanc of the Physical Activity Sciences Laboratory for their excellent collaboration at various stages of the study. The subjects and the Physical Activity Sciences Laboratory staff are also gratefully acknowledged. We also thank Marie Martin and Rachel Duchesne of the Diabetes Research Unit for their valuable technical assistance. Thanks are also expressed to the Surgical staff of the Laval Hospital, for providing us with adipose tissue specimens. Supported by the Fonds FCAR-Québec, the Fonds de la Recherche en Santé du Québec (FRSQ), and the Medical Research Council of Canada.

Manuscript received 31 May 1994 and in revised form 13 October 1994.

REFERENCES

1. Arner, P. 1988. Control of lipolysis and its relevance to development of obesity in man. *Diabetes Metab. Rev.* **4:** 507–515.
2. Després, J. P., S. Moorjani, P. J. Lupien, A. Tremblay, A. Nadeau, and C. Bouchard. 1990. Regional distribution of body fat, plasma lipoproteins and cardiovascular disease. *Arteriosclerosis.* **10:** 497–511.
3. Björntorp, P. 1991. Metabolic implications of body fat distribution. *Diabetes Care.* **14:** 1132–1143.
4. Kissebah, A. H. 1991. Insulin resistance in visceral obesity. *Int. J. Obes.* **15:** 109–115.
5. Björntorp, P. 1993. Visceral obesity: a "civilization syndrome." *Obes. Res.* **1:** 206–222.
6. Leibel, R. L., N. K. Edens, and S. K. Fried. 1989. Physiologic basis for the control of body fat distribution in humans. *Annu. Rev. Nutr.* **9:** 417–443.
7. Bouchard, C., J. P. Després, and P. Mauriège. 1993. Genetic and nongenetic determinants of regional fat distribution. *Endocrine Rev.* **14:** 72–93.
8. Bolinder, J., L. Kager, J. Ostman, and P. Arner. 1983. Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes.* **32:** 117–123.
9. Mauriège, P., J. Galitzky, M. Berlan, and M. Lafontan. 1987. Heterogeneous distribution of beta- and alpha2-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. *Eur. J. Clin. Invest.* **17:** 156–165.
10. Rebuffé-Scrive, M., B. Andersson, L. Olbe, and P. Björntorp. 1989. Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metabolism.* **38:** 453–458.
11. Rebuffé-Scrive, M., B. Andersson, L. Olbe, and P. Björntorp. 1990. Metabolism of adipose tissue in intraabdominal depots of severely obese men and women. *Metabolism.* **39:** 1021–1025.
12. Richelsen, B., S. B. Pedersen, T. Moller-Pedersen, and J. F. Bak. 1991. Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin and prostaglandin E_2. *Metabolism.* **40:** 990–996.
13. Hellmér, J., C. Marcus, T. Sonnenfeld, and P. Arner. 1992. Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J. Clin. Endocrinol. Metab.* **75:** 15–20.
14. Fried, S. K., R. L. Leibel, N. K. Edens, and J. G. Kral. 1993. Lipolysis in intraabdominal adipose tissues of obese women and men. *Obes. Res.* **1:** 443–448.
15. Arner, P., L. Hellström, H. Wahrenberg, and M. Brönnegard. 1990. Beta-adrenoceptor expression in human fat cells from different regions. *J. Clin. Invest.* **86:** 1595–1600.
16. Castan, I., P. Valet, D. Larrouy, T. Voisin, A. Remaury, D. Daviaud, M. Laburthe, and M. Lafontan. 1993. Distribution of PYY-receptors in human fat cells: an antilipolytic system alongside the alpha2-adrenergic system. *Am. J. Physiol.* **265:** E74–E80.
17. Fried, S. K., and J. G. Kral. 1987. Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. *Int. J. Obes.* **11:** 129–140.
18. Fried, S. K., C. D. Russell, N. L. Grauso, and R. E. Brolin. 1993. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J. Clin. Invest.* **92:** 2191–2198.
19. Martin, L. F., C. M. Klim, S. J. Vannucci, L. B. Dixon, J. R. Landis, and K. F. LaNoue. 1990. Alterations in adipocyte adenylate cyclase activity in morbidly obese and formerly morbidly obese humans. *Surgery.* **108:** 228–235.
20. Biron, S., H. Plamondon, R. A. Bourque, P. Marceau, M. Potvin, and P. Piché. 1986. Clinical experience with biliopancreatic bypass and gastrectomy or selective vagotomy for morbid obesity. *Can. J. Surg.* **29:** 408–410.
21. Gray, H. 1973. The liver. *In* Anatomy of Human Body. C. M. Goss, editor. Lea & Febiger, Philadelphia, 1244–1253.
22. Behnke, A. R., and J. H. Wilmore. 1974. Evaluation and Regulation of Body Build and Composition. Prentice-Hall, Englewood Cliffs, NJ. 20–37.
23. Siri, W. E. 1956. The gross composition of body fat. *Adv. Biol. Med. Phys.* **4:** 239–280.
24. Meneely, G. R., and N. L. Kaltreider. 1949. Volume of the lung determined by helium dilution. *J. Clin. Invest.* **28:** 129–139.
25. Lohman, T. G., A. F. Roche, and R. Martorell. 1988. The Airlie (VA) consensus conference. Anthropometric Standardization Reference Manual. Human Kinetics Publishers Inc., Champaign, IL. 39–80.
26. Richterich, R., and H. Dauwalder. 1971. Zur bestimmung der plasmaglukose-konzentration mit der hexokinase-glucose-6-phosphatase-dehydrogenase-methode. *Schweiz. Med. Wochenschr.* **101:** 615–618.
27. Desbuquois, B., and G. D. Aurbach. 1971. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassay. *J. Clin. Endocrinol. Metab.* **37:** 732–738.
28. Després, J. P., S. Moorjani, M. Ferland, A. Tremblay, P. J. Lupien, A. Nadeau, S. Pinault, G. Thériault, and C. Bouchard. 1989. Adipose tissue distribution and plasma lipoprotein levels in obese women: importance of intra-abdominal fat. *Arteriosclerosis.* **9:** 203–210.
29. Savard, R., Y. Deshaies, J. P. Després, M. Marcotte, L. Bukowiecki, C. Allard, and C. Bouchard. 1984. Lipogenesis and lipoprotein lipase in human adipose tissue. Reproducibility of measurements and relationships with fat cell size. *Can. J. Physiol. Pharmacol.* **62:** 1448–1452.
30. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
31. Després, J. P., C. Bouchard, L. Bukowiecki, R. Savard, and J. Lupien. 1983. Morphology and metabolism of human fat cells: a reliability study. *Int. J. Obes.* **7:** 231–240.
32. Kather, H., F. Schroder, and B. Simon. 1982. Microdetermination of glycerol using bacterial NADH-linked luciferase. *Clin. Chim. Acta.* **120:** 295–300.
33. Mauriège, 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.
34. Galitzky, J., P. Mauriège, M. Berlan, and M. Lafontan. 1989. Human fat cell alpha2-adrenoceptors. I. Functional exploration and pharmacological definition with selected alpha2-agonists and antagonists. *J. Pharmacol. Exp. Ther.* **249:** 583–591.
35. Yabuuchi, Y., S. Yamashita, and S. S. Tei. 1977. Pharmacological studies of OPC-2009, a newly synthesized selective beta-adrenoceptor stimulant, in the bronchomotor and cardiovascular system of the anesthetized dog. *J. Pharmacol. Exp. Ther.* **202:** 326–336.
36. Langin, D., M. P. Portillo, J. S. Saulnier-Blache, and M. Lafontan. 1991. Coexistence of three beta-adrenoceptor subtypes in white fat cells from various mammalian species. *Eur. J. Pharmacol.* **199:** 291–301.
37. Daly, J. W. 1984. Forskolin, adenylate cyclase and cell physiology: an overview. *Adv. Cyclic Nucl. Res.* **17:** 81–90.
38. Weishaar, R. E., M. M. H. Cain, and J. A. Bristol. 1985. A new generation of phosphodiesterase inhibitors:

multiple molecular forms of phosphodiesterase-inhibitors and the potential for drug selectivity. *J. Med. Chem.* **28:** 537–545.
39. Mauriège, P., G. de Pergola, M. Berlan, and M. Lafontan. 1988. Human fat cell beta-adrenergic receptors: beta-agonist dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J. Lipid Res.* **29:** 587–601.
40. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and N. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
41. Richelsen, B., and O. Pedersen. 1985. Alpha2-adrenergic binding and action in human adipocytes: comparison between binding to plasma membrane preparations and to intact adipocytes. *Eur. J. Pharmacol.* **119:** 101–112.
42. Lacasa, D., P. Mauriège, M. Lafontan, M. Berlan, and Y. Giudicelli. 1986. A reliable assay for beta-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [^{3}H]CGP-12177. *J. Lipid Res.* **27:** 368–376.
43. Munson, P. J., and D. Rodbard. 1980. LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* **107:** 220–239.
44. Bray, G. A. 1992. Pathophysiology of obesity. *Am. J. Clin. Nutr.* **55:** 488S–494S.
45. Maslowska, M. H., A. D. Sniderman, L. D. McLean, and K. Cianflone. 1993. Regional differences in triacylglycerol synthesis in adipose tissue and in cultured preadipocytes. *J. Lipid Res.* **34:** 219–228.
46. Vikman, H. L., and J. J. Ohisalo. 1993. Regulation of adenylate cyclse in plasma membranes of human intraabdominal and subcutaneous adipocytes. *Metabolism.* **42:** 739–742.
47. Wesslau, C., J. W. Eriksson, and U. Smith. 1993. Cellular cyclic AMP levels modulate insulin sensitivity and responsiveness. Evidence against a significant role of Gi in insulin signal transduction. *Biochem. Biophys. Res. Commun.* **196:** 287–293.
48. 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.
49. Edens, N. K., S. K. Fried, J. G. Kral, J. Hirsch, and R. L. Leibel. 1993. In vitro lipid synthesis in human adipose tissue from three abdominal sites. *Am. J. Physiol.* **265:** E374–E379.
50. Lönnqvist, F., S. Krief, A. D. Strosberg, B. Nyberg, L. J. Emorine, and P. Arner. 1993. Evidence of a functional β3-adrenoceptor in man. *Br. J. Pharmacol.* **110:** 929–936.
51. Krief, S., F. Lönnqvist, S. Raimbault, B. Baude, A. Van Spronsen, P. Arner, A. D. Strosberg, D. Ricquier, and L. Emorine. 1993. Tissue distribution of beta3-adrenergic receptor mRNA in man. *J. Clin. Invest.* **91:** 344–349.
52. Rosenbaum, M., C. C. Malbon, J. Hirsch, and R. L. Leibel. 1993. Lack of β3-adrenergic effect on lipolysis in human subcutaneous adipose tissue. *J. Clin. Endocrinol. Metab.* **77:** 352–355.
53. Van Liefde, I., A. Van Ermen, and G. Vauquelin. 1994. No functional atypical β-adrenergic receptors in human omental adipocytes. *Life Sci.* **54:** PL 209–PL 214.