Regional and gender variations in adipose tissue lipolysis in response to weight loss

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Abstract Catecholamine-induced lipolysis was investigated in 32 obese subjects (14 men and 18 premenopausal women), aged 36–50 years, whose body mass index ranged from 30 to 42 kg/m². Isolated subcutaneous (subc) abdominal and femoral adipocytes were studied before and after a 15-week weight reducing program, during which mean body weight loss averaged 9 vs. 10 kg in women and men, respectively ($P <$ 0.0001). Participants were re-examined when they were weight-stable. Fat cell weight decreased by about 15–20% in both depots (P values ranging from 0.01 to 0.05). Epinephrine (mixed α2-/β-adrenoceptor (AR) agonist) induced antilipolysis at low concentrations and a net lipolytic response at higher doses, irrespective of subjects' fatness and anatomic location of fat. Basal lipolysis, maximal lipolytic responses to isoprenaline (β-AR agonist), dobutamine and procaterol (β1- and β2-AR agonists, respectively) as well as maximal antilipolytic effects of epinephrine or UK-14304 (α2-AR agonist) were similar before and after weight reduction. However, both β- and β2-AR lipolytic sensitivities and the β-AR density were increased in both genders after weight reduction, this effect being more marked in subc abdominal than in femoral adipocytes (P values ranging from 0.001 to 0.05). The α2-AR antilipolytic sensitivity was reduced in adipose cells from both regions in women, but only in subc abdominal adipocytes in men ($P <$ 0.05), although the α2-AR density remained unchanged after weight reduction. In conclusion, a moderate weight loss leads to a higher adipose cell lipolytic efficiency which is associated with changes at receptor levels (mainly an increased β2- and a decreased α2-AR sensitivities), in both genders.—Mauriège, P., P. Imbeault, D. Langin, M. Lacaille, N. Alméras, A. Tremblay, and J. P. Després. **Regional and gender variations in adipose tissue lipolysis in response to weight loss.** *J. Lipid Res.* 1999. **40:** 1559–1571.

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Since the pioneering clinical observations of Vague (1), numerous studies have re-emphasized the notion that premenopausal women who tend to accumulate fat preferentially in the gluteal and femoral regions, are at lower risk of complications than men who are generally character-

ized by a high relative accumulation of abdominal adipose tissue (2–4). Human adipose tissue is also well established as being heterogeneous in its metabolic activity, and regional variations in rates of lipid storage and/or mobilization in adipose cells have already been suggested as contributing to local differences in adiposity (5–9). Among the various hormones that control lipid mobilization, catecholamines and insulin appear to be powerful regulators of in vitro adipose cell lipolysis in adult humans (5, 6, 10, 11). The final response of subcutaneous adipocytes to catecholamines (antilipolysis or lipolysis) depends upon the functional balance between inhibitory α2- and stimulatory β-adrenoceptors (6, 9–11) whose activation regulates adenylyl cyclase activity and thereby cAMP production, which in turn modulates hormone-sensitive lipase (12, 13). Moreover, marked sex and site differences for the in vitro adipose cell lipolytic response to catecholamines have been extensively described in both normal and obese individuals (14–19). The in vitro lipolytic resistance of subcutaneous abdominal adipose cells to catecholamines in obese patients could be mainly attributed to a reduced β2- and/or an increased α2-adrenoceptor component and to an impaired activation of hormone-sensitive lipase, compared to nonobese controls (12, 18–21).

Among the various forms of treatment of obesity, dietary interventions such as fasting, low or very low calorie diets (LCD or VLCD) remain widely used therapies to promote weight reduction in obese patients (22). As body weight loss and the related decrease in adipose tissue mass are generally accompanied by a concomitant reduction in adipose cell size, changes in the adipocyte lipolytic potencies could contribute to explain variation in lipid mobilization (5, 7–9). However, to the best of our knowledge, with the exception of two studies that examined the effect

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Abbreviations: CT, computed tomography; HSL, hormone-sensitive lipase; ADA, adenosine deaminase; AT, adipose tissue; PDE, phosphodiesterase.

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of caloric restriction on both subcutaneous abdominal and gluteal adipose tissues of obese women (23, 24), there has been no attempt to clarify the mechanisms underlying the changes in regional adipose cell lipolysis occurring during weight reduction in both genders. Indeed, prior experiments addressing this issue have been generally conducted on women who underwent LCD or VLCD therapy (25–31). Furthermore, these studies generally did not consider the well known regional differences in fat cell lipolysis, as subcutaneous abdominal adipose tissue was the only depot investigated.

Therefore, the aims of the present study were *1*) to examine the lipolysis regulation of subcutaneous abdominal and femoral adipocytes in both obese men and premenopausal women who were subjected to a weight loss therapy through dietary energy restriction, participants being re-examined after weight-stabilization, *2*) to verify the putative existence of regional and gender variations in these responses, and *3*) to identify the cellular mechanisms (located at receptor and/or post-receptor levels) underlying such differences.

<center>MATERIAL AND METHODS</center>

Subjects and experimental design

Thirty-two healthy obese subjects (14 men and 18 premenopausal women) (all Caucasian), aged 43 ± 5 years (mean $\pm$ SD) (range: 36–50 yr) were recruited through the media and gave their written informed consent to participate in this intervention protocol aimed at weight reduction. All individuals were subjected to a physical examination by a physician, which included a medical history. Subjects with metabolic (cardiovascular disease or non-insulin-dependent diabetes mellitus) or endocrine disorders such as hypogonadism or hirsutism, or those on medication potentially affecting lipid metabolism (β-blockers, anti-hypertensive drugs, etc.) were excluded from the study. All participants were sedentary, non-smokers, and moderate alcohol consumers. None had recently been on a diet or involved in a weight reducing program, and their body weight had been stable for at least 6 months prior to the study. Women had regular menstrual cycles and none was using oral contraceptives or lactating at the time of the study. All measurements were performed while they were in the early follicular phase of their menstrual cycle.

Subjects were first examined 2–4 weeks before entering the study which was approved by the Laval University Medical Ethics Committee. They were then subjected to a 15-week dietary restriction which took into account their individual macronutrient composition evaluated by a 3-day dietary record prior to the experiment (15–18% protein, 38–39% fat, 41–46% carbohydrate, and 1–2% alcohol). With the exception of carbohydrate being higher in women than in men ($P < 0.05$), the macronutrient composition did not differ between genders. The energy deficit which was established according to measurements of daily energy expenditure estimated by indirect calorimetry and from the estimated daily energy intake obtained by a 3-day dietary record, corresponded approximately to 500–800 kcal/day. This dietary restriction was accompanied by the daily oral intake of 60 mg fenfluramine, a serotonin reuptake inhibitor which has been shown to facilitate body weight loss (32). Participants were then re-examined 4–6 wk after the end of the treatment when they were weight-stable. As managing treatment of obesity is highly dependent on the subject's attendance to the weight-reducing program, participants underwent weight control and a 24-h dietary recall interview with a dietician, twice a month, to verify compliance to the experimental procedure. Subjects whose body weight varied $\pm$ 2 kg from the end of the protocol to the day of the adipose tissue biopsy were excluded from the study.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique (33) and percent body fat was derived from body density (34). Pulmonary residual volume was measured using the helium dilution method (35). Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference (36).

Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, West Germany), according to the methodology previously described (37). Briefly, the subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) and at the femoral (mid-distance between the knee joint and the iliac crest) levels with a radiograph of the skeleton as a reference to establish the position of the scan to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing the AT surfaces using an attenuation range of -190 to -30 HU (37). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Adipocyte isolation and lipolysis

After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the midthigh level (femoral site). A small cutaneous incision (1 cm) was performed in both sites and about 400–500 mg of subcutaneous adipose tissue was surgically removed from the two fat depots. Samples of approximately 100 mg of adipose tissue from each region were immediately frozen in liquid nitrogen and stored at $-80°C$ for later measurement of the hormone-sensitive lipase (HSL) activity as well as of the α2- and β-adrenoceptor density.

Samples of 250 mg of adipose tissue from each site were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (38) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described (18). Digestion took place in a shaking water bath under an air 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 of KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 μl.

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 starting the incubation 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 95% O_2 and 5% CO_2 gas phase, 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 stoppered and stored at $-20°C$ until glycerol determination and NADH concentration was measured by bioluminescence with a luciferase solution, using an automated 2250 Dynatech lu-

minometer (18,19). 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. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available (18, 19). Briefly, a 50-μl aliquot of adipose cell suspension was taken and dropped into 25 μl of saline (NaCl 0.9%) containing 0.4% of trypan blue. A 14-μl aliquot of this final suspension was taken and the average fat cell diameter was assessed using a Leitz microscope equipped with a graduated ocular, at a magnification of 100$\times$ (Rockleigh, NJ). Adipocyte size was measured with a precision of 1 μm and fat cell diameters were individually computer recorded in 5-μm classes from 0 to 260 μm. Mean adipose cell diameter was assessed from the measurement of at least 500 cells per site and per subject. Because of the spherical shape and high lipid content of the adipocytes (95%), both the adipose cell volume and surface area can be calculated from the mean adipocyte diameter and the density of triolein (0.915 g/ml) was used to transform adipose cell volume into fat cell weight, as previously described (18, 19, 39, 40).

The lipolytic activity of the isolated fat cells was tested with epinephrine which is a mixed agonist (α2/β) with a higher affinity for α2- than for β-adrenoceptor (AR) sites (17), UK-14304 (selective α2-AR agonist), isoproterenol (non selective β-AR agonist) (19), procaterol (β2-AR agonist), and dobutamine (β1-AR agonist) (39, 41). Ascorbic acid (0.1 mmol/l) was included in the incubation medium in order to prevent catecholamine degradation. Some experiments were conducted with forskolin (direct activator of adenylate cyclase), dibutyryl-cyclicAMP (stimulator of the protein kinase hormone-sensitive lipase complex and phosphodiesterase-resistant cyclic AMP analogue), theophylline (mainly inhibitor of cyclicGMP-inhibited phosphodiesterase, cGI-PDE) (19), and cilostamide (selective inhibitor of cGI-PDE) (42). When antilipolytic effects were investigated, the incubation buffer was supplemented with 5 μg/ml adenosine deaminase (ADA) to remove adenosine released into the incubation medium by the isolated fat cells, this procedure allowing better investigations of α2-AR-mediated antilipolytic effects (17–19). Lipolysis was expressed either per cell number (i.e., μmol of glycerol/10^6 cells $\times$ 2 h) or per unit of cell surface area (i.e., nmol of glycerol/μm^2 $\times$ 10^8 $\times$ 2 h) in order to compensate for regional and gender variations in fat cell size (18, 19) as well as for the putative differences due to weight reduction. In cases where complete concentration–response curves were obtained, they were compared for both responsiveness and sensitivity. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} m various β-adrenoceptor agonists, forskolin, or cilostamide, 10^{-3} m dibutyryl-cyclicAMP or theophylline). Maximal inhibition of lipolysis noted either at 10^{-7} m epinephrine or at 10^{-6} m UK-14304 was calculated as the following ratio: (ADA $-$ epinephrine or UK-14304)/(ADA $-$ basal) where ADA represents ADA-stimulated lipolysis. The β-adrenergic sensitivity was considered as the β-AR agonist concentration giving half-maximal stimulation of lipolysis (EC$_{50}$), whereas the α2-adrenergic sensitivity was calculated as the concentration of UK-14304 which produced half-maximal inhibition of lipolysis (IC$_{50}$). Both were evaluated by logarithmic conversion of each concentration–response curve. The higher was the EC$_{50}$ (various β-AR agonists) or the IC$_{50}$ (UK-14304) value, the lower was the β- or the α2-adrenergic sensitivity, respectively.

Hormone-sensitive lipase (HSL) assays

This assay was performed as previously described by Fredriksson et al. (43) with some modifications for the handling of small samples (44). Briefly, small pieces of adipose tissue (about 100

mg) were homogenized at 4°C in 0.8 ml of a buffer containing 0.25 m sucrose, 1 mm EDTA, 1 mm dithiothreitol, and the protease inhibitors leupeptin and antipain, both at 20 μg/ml (pH 7.4). Samples were then centrifuged at 100,000 g for 45 min at 4°C in a Beckman ultracentrifuge, and the fat cake was removed. Pellets containing crude adipose tissue membranes obtained after centrifugation were used for radioligand binding studies. The fat-free infranatant was recovered for analysis of maximal enzymatic activity, using 1(3)-mono-[^{3}H]oleoyl-2-oleylglycerol as substrate (43, 44). All samples were incubated in duplicate for 30 min at 37°C and were analyzed on the same occasion. As this substrate only has one hydrolyzable ester bond at the 1(3)-position, neither the substrate itself nor its hydrolysis products can be hydrolyzed by monoacyl-glycerol lipase which is abundant in adipose tissue. Furthermore, under our incubation conditions (pH 7.0 and no apoC-II present), lipoprotein lipase activity is negligible (13, 43). As the phosphorylated and dephosphorylated forms of the enzyme have similar activities towards the substrate, the total amount of activable enzyme in the sample is measured. Moreover, the sensitivity of the assay is enhanced by the use of a diacylglycerol analogue as substrate, as HSL has a 10-fold higher activity towards diacylglycerol than triacylglycerol (13, 43). One unit of enzyme activity is defined as 1 μmol of fatty acid released per minute at 37°C. Lipase activity was related to both fat cell number and adipocyte surface area which were estimated from in vitro lipolysis assays.

Radioligand binding studies

α2- as well as β-ARs were quantified with radioligands selective for each adrenergic receptor subtype, i.e., [^{3}H]RX 821002 (a more selective α2-AR antagonist than [^{3}H]yohimbine currently used) and ^{125}I-labeled cyanopindolol (CYP), a non-selective β-AR antagonist (39, 41, 45). Previous experiments have shown that radioligand binding assays were unaffected by the composition of the buffer (0.25 m sucrose, 1 mm EDTA, 1 mm dithiothreitol) used for adipose tissue membrane preparations (P. Mauriège, unpublished observations). Briefly, membranes were incubated either with 5 nm of [^{3}H]-RX 821002 or with 300 pm of ^{125}I-labeled CYP for α2- or the β-AR tracer experiments, respectively. As both radioligands bind to a single class of homogenous noninteracting binding sites that give straight lines on Scatchard analysis leading to Hill coefficients close to 1, the use of only one ligand concentration to determine maximal antagonist binding is therefore justified under such conditions (16, 17, 39, 41). In addition, the latter concentrations corresponding to almost twice the affinity of each radioligand label the totality of both ARs (39, 45). Thawed crude adipose tissue membranes were homogenized further with four pestle strokes in a Potter apparatus and washed once in 50 mm Tris-HCl, 1 mm MgCl$_2$, pH 7.5 (Tris-Mg buffer). The pellet was then adjusted to a final concentration of approximately 0.5 mg protein/ml. The protein content was determined according to the method of Lowry et al. (46), using bovine serum albumin as standard.

Total binding was determined by incubating 50-μl aliquots of the resuspended membrane adipose tissue preparation with a fixed concentration of ^{125}I-labeled CYP (300 pm) in a total volume of 200 μl Tris-Mg buffer. Under these conditions, it is believed that ^{125}I-labeled CYP binds mainly to the high affinity binding sites which correspond to β1-/β2-ARs rather than to the low affinity binding sites which can be ascribed to β3-ARs (10). Specific binding was defined as the difference between total binding and binding in the presence of 10 μm unlabeled ($-$) propranolol (non-selective β-adrenergic antagonist). A similar radioligand binding technique was used to identify α2-ARs with a fixed concentration of [^{3}H]-RX 821002 (5 nm) in a total volume of 200 μl Tris-Mg buffer. Specific binding was defined as the difference between total and nonspecific binding determined in the presence

of 10 μm unlabeled (−) phentolamine (nonselective α-adrenergic antagonist). Incubations were carried out in a water bath for 25–30 min at 37°C, under constant shaking at around 120 cycles/min and the reaction was stopped by the addition of 4 ml of ice-cold binding buffer followed by rapid filtration, using a Cell Harvester Micromate C-96 (Packard, Canada). 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 a Clini Gamma counter (at an efficiency of 85%), whereas for RX binding, filters were placed in minivials containing 2 ml of liquid scintillation cocktail and counted in a LKB scintillation counter (at an efficiency of 35%). Both radioligands, [^{3}H]RX 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 (39, 45). The maximum number of α2- and β-AR binding sites was expressed per cell number or corrected for variation in adipocyte surface area which were both estimated from in vitro lipolysis assays.

Drugs and chemicals

Collagenase, bovine serum albumin, adenosine deaminase, and enzymes for glycerol assays were obtained from Boehringer (Mannheim, Canada). Ascorbic acid, leupeptin, antipain (−) isoproterenol bitartrate (−) epinephrine bitartrate (−) propranolol hydrochloride, theophylline, forskolin, and dibutyryl-cyclic AMP 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) whereas phentolamine mesylate came from Ciba Geigy (Canada). Procaterol (OPC-2009) (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate) and cilostamide were generous gifts from Otsuka Pharmaceuticals (Tokushima, Japan), whereas dobutamine (Dobutrex) came from Eli Lilly (Indianapolis, IN). (−)[^{3}H]-RX821002 (RX) (1,4-[6,7(n)-^{3}H]benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride) (specific activity, 53 Ci/mmol) and (−) ^{125}I-labeled cyanopindolol (CYP) (specific activity, 2200 Ci/mmol) were obtained from Amersham International (Canada) and Mandel Scientific (Canada), respectively. 1(3)-Mono-[^{3}H]oleoyl-2-oleylglycerol was generously provided by Sevicon AB (Lund, Sweden). 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 analysis

Values presented in figures are means ± standard error (SE). Lipolysis and HSL experiments were performed on all subjects (i.e., 14 men and 18 women). However, as radioligand binding assays required large amounts of adipose tissue, these assays could therefore only be performed on 12 men and 16 women. A two-way analysis of variance was also performed to verify whether significant sex and site differences for lipolysis, HSL activity, or α2 versus β-adrenoceptor density existed before and after weight loss and post hoc comparisons were tested with a paired t-test. All statistical analyses were performed using the Jump Program (SAS Institute Inc., Cary, NC) adapted for MacIntosh computers.

RESULTS

Subjects' characteristics

Subjects' physical characteristics are presented in **Table 1**. Visceral abdominal fat accumulation, estimated by CT, was more pronounced in men, whereas higher levels of subcutaneous abdominal adipose tissue were observed in women (P values ranging from 0.001 to 0.05). Women also showed a greater femoral adipose depot and larger femoral adipocytes than men (P values ranging from 0.0001 to 0.001), although no sex difference was found for subcutaneous abdominal fat cell weight. Both men and women achieved a moderate weight loss (9–10 kg) in response to the 15-week weight reducing program ($P <$ 0.0001). However, all subjects were weight-stable when re-examined 4–6 weeks after the end of the treatment. Changes in selected body fatness indices were significantly more pronounced in men than in women (P values ranging from 0.001 to 0.05). In both genders, subcutaneous and visceral abdominal adipose tissue areas as well as the cross-sectional area of femoral fat determined by CT were reduced to a similar extent in response to weight loss (P values ranging from 0.0001 to 0.001). Fat cell weight

TABLE 1. Physical characteristics of men and women before and after weight loss

	Men (n = 14)		Women (n = 18)	
	Before	After	Before	After
Weight (kg)	104 ± 11^c	$94 \pm 10^*$	92 ± 14	$83 \pm 14^*$
BMI (kg/m^2)	34 ± 3	$30 \pm 3^*$	36 ± 4	$33 \pm 5^*$
Body fat (%)	38 ± 4^a	$32 \pm 5^{*,b}$	49 ± 5	$47 \pm 7^*$
Fat mass (kg)	40 ± 6	$30 \pm 5^{*,c}$	45 ± 10	$40 \pm 11^{**}$
Fat-free mass (kg)	64 ± 7^a	64 ± 8^b	47 ± 6	44 ± 5
Waist girth (cm)	110 ± 8	$101 \pm 6^{*,d}$	99 ± 11	$93 \pm 11^*$
Adipose tissue areas measured by CT (cm^2)				
Abdomen (L4-L5)				
Subcutaneous	404 ± 71^d	$328 \pm 70^*$	559 ± 144	$498 \pm 152^{**}$
Visceral	192 ± 55^b	$140 \pm 52^*$	153 ± 47	$121 \pm 42^{**}$
Midthigh	76 ± 11^a	$60 \pm 12^{*,b}$	181 ± 41	$159 \pm 40^{**}$
Regional fat cell weight (μg lipid/cell)				
Abdominal	0.64 ± 0.12	$0.54 \pm 0.14^{\&}$	0.71 ± 0.15	$0.61 \pm 0.13^{\&}$
Femoral	0.58 ± 0.10^b	$0.48 \pm 0.10^{***}$	0.81 ± 0.18	$0.62 \pm 0.16^{***}$

Values are means ± standard deviation (SD).
BMI, body mass index; CT, computed tomography.
Significant difference before and after weight loss at * $P < 0.0001$, ** $P < 0.001$, *** $P < 0.01$, $^{\&}P < 0.05$.
Significant gender variation at ^{a} P < 0.0001, ^{b} P < 0.001, ^{c} P < 0.01, ^{d} P < 0.05.

TABLE 2. Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in subcutaneous abdominal and femoral adipocytes of men and women before and after weight loss

	Abdominal		Femoral	
	Before	After	Before	After
Men (n = 14)				
Basal (/cell)	0.7 ± 0.1	1.2 ± 0.3	0.7 ± 0.2	0.9 ± 0.2
ADA (/cell)	1.3 ± 0.3	2.1 ± 0.5	1.0 ± 0.2	1.8 ± 0.4
Basal (/surface area)	1.7 ± 0.4	3.5 ± 0.9[&]	1.8 ± 0.4	3.1 ± 0.6
ADA (/surface area)	3.7 ± 0.7	6.8 ± 1.8[&]	3.1 ± 0.7	5.3 ± 1.2
Women (n = 18)				
Basal (/cell)	1.0 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
ADA (/cell)	1.9 ± 0.3	1.9 ± 0.4	1.1 ± 0.2	1.1 ± 0.2
Basal (/surface area)	2.0 ± 0.4	2.7 ± 0.5	1.4 ± 0.3	1.6 ± 0.3
ADA (/surface area)	4.9 ± 1.0	5.8 ± 1.6	2.9 ± 0.6	3.3 ± 0.6

Values are means ± SE of (n) separate experiments performed in duplicate.

[&] P = 0.065. Lipolysis is expressed per cell number (μmol glycerol/10^6 cells × 2 h) or corrected for variation in cell surface area (nmol glycerol/μm^2 × 10^8 × 2 h).

also decreased by about 15–20%, regardless of the depot and the sex (P values ranging from 0.01 to 0.05).

Adipocyte function

Basal lipolytic rate and ADA-stimulated lipolysis. Basal lipolysis measured in subcutaneous abdominal or femoral adi-

pocytes did not differ among genders in response to weight reduction, whatever the mode of expression of data (**Table 2**). On the other hand, when the incubation buffer was supplemented with adenosine deaminase (ADA) at 5 μg/ml, the basal lipolytic rate was increased by about 1.5- to 2.5-times in all cell types. Neither sex nor site differences were observed in the glycerol release achieved in the presence of this enzyme, results being expressed per cell number or per unit of cell surface area (Table 2). Indeed, the difference noted in basal and ADA-stimulated lipolysis (when corrected for the adipocyte surface area) of male subcutaneous abdominal fat cells, after weight loss, was below the level of statistical significance (P = 0.065).

In order to control for the variation in fat cell weight observed during the weight-reducing program, all lipolysis measurements were further expressed per unit of cell surface area which may be physiologically more relevant, as previously suggested (5, 19, 21, 30, 40). Such a mode of expression of the data allows an adequate comparison of lipolysis in large versus small adipocytes, as differences in adipose cell size were found in both genders, in response to weight loss (Table 1).

Epinephrine responsiveness. In the presence of ADA, epinephrine (EPI) which is known for its mixed (α2/β-ad-
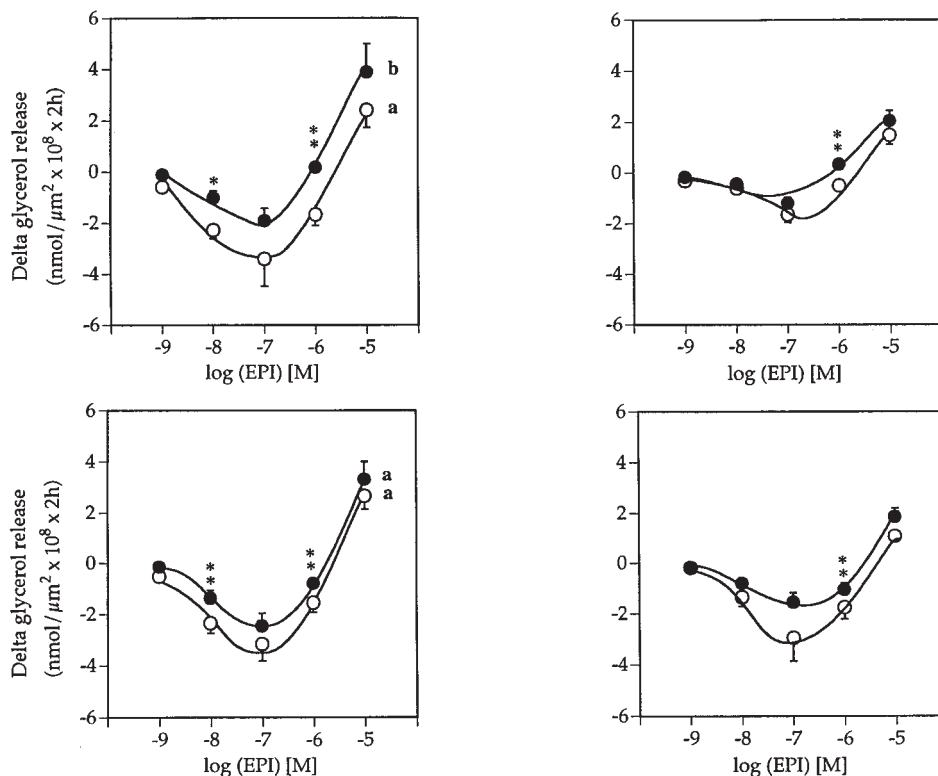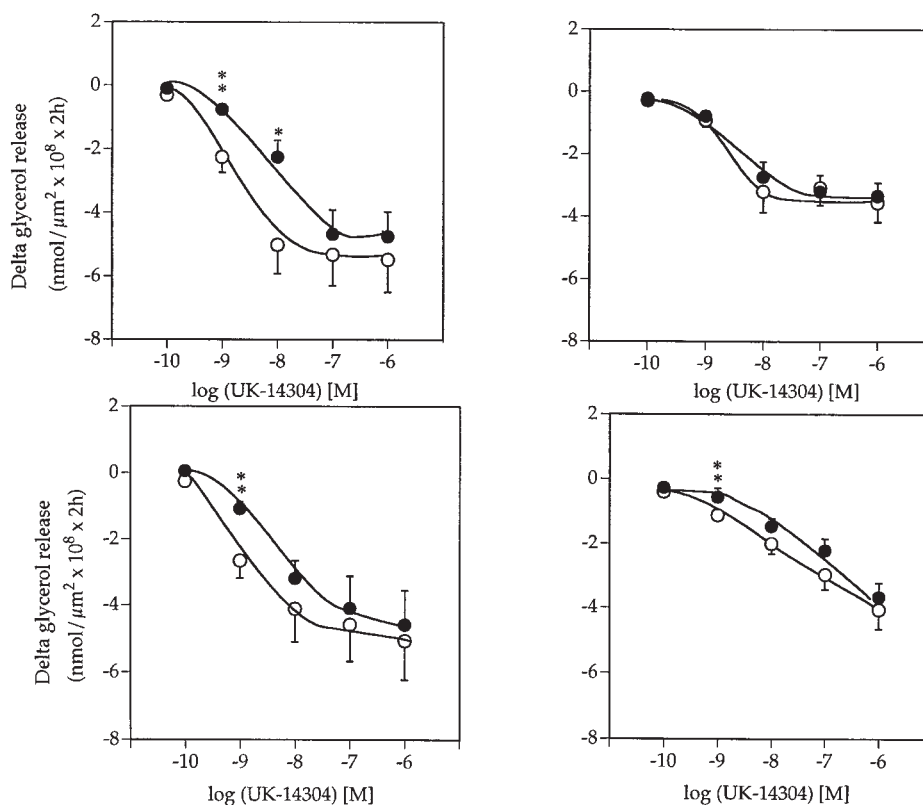

Fig. 1. Effect of epinephrine (EPI) on adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Values are means ± SE of (n) experiments performed in duplicate. Glycerol release was expressed as the difference between stimulated (with epinephrine) and basal values determined in the presence of 5 μg/ml of adenosine deaminase (ADA). Negative values reflect inhibition of lipolysis. Significant effect of treatment at * P < 0.01 and ** P < 0.05. [a,b] indicate regional variation at P < 0.05 and P < 0.01.

renergic) agonist properties on lipolysis, initiated a biphasic responsiveness in subcutaneous abdominal and femoral adipocytes (**Fig. 1**). The catecholamine promoted an inhibition of lipolysis at low concentrations (from 10^{-9} to 10^{-7} m), but exerted a net lipolytic response at higher doses (10^{-6}–10^{-5} m), indicating thus a preferential recruitment of $\alpha 2$-, followed by β-adrenoceptor sites. Significant differences were observed after weight reduction in epinephrine-induced antilipolysis at 10^{-8} m (concentration at which the $\alpha 2$-adrenoceptor component is predominant) in subcutaneous abdominal adipose cells of men ($P < 0.01$) and women ($P < 0.05$). However, maximal antilipolysis promoted by the hormone at 10^{-7} m, did not show any site or sex difference, subjects being examined at both occasions. On the other hand, the maximal adipose cell lipolytic response to the catecholamine (at 10^{-5} m) did not differ in response to weight reduction, although subcutaneous abdominal adipocytes appeared to be more responsive to hormonal stimulation than femoral adipose cells, regardless of the gender or of the treatment period (P values ranging from 0.01 to 0.05).

Selective $\alpha 2$- and β-adrenergic effects. As epinephrine responsiveness results from both $\alpha 2$- and β-adrenoceptor stimulation, selective adrenergic agonists were used to discriminate between these two antagonistic effects.

To study the influence of the $\alpha 2$-adrenoceptor component, the effect of the selective $\alpha 2$-agonist UK-14304 was tested on ADA-stimulated lipolysis (**Fig. 2**). UK-14304 inhibited lipolysis in a dose-dependent manner in all adipocytes, and the maximal antilipolytic response noted at 10^{-6} m did not differ strikingly among genders and adipose sites. However, at 10^{-8} m, UK-14304 promoted a less potent antilipolysis in subcutaneous abdominal adipocytes of men and in adipose cells of women, after weight reduction (P values ranging from 0.01 to 0.05). In addition, the $\alpha 2$-adrenergic sensitivity estimated as the half-maximal antilipolysis induced by UK-14304 was almost 2 times lower in subcutaneous abdominal adipocytes of men, after weight loss compared to baseline ($P < 0.05$) (**Table 3**). The concentration–response curves slightly shifted to the right for UK-14304 after weight reduction and also revealed a 1.8- to 2-times decrease in adipose cell $\alpha 2$-adrenergic sensitivity in women, irrespective of the anatomic site investigated ($P < 0.05$) (Table 3).

In order to characterize the β-adrenoceptor component, the effect of the β-agonist isoproterenol on basal lipolysis was examined (**Fig. 3**). Maximal lipolytic responses to isoproterenol at 10^{-5} m, when expressed as absolute rates, were similar regardless of the anatomical location of fat or of the gender (Fig. 3). Isoproterenol-stimulated

Fig. 2. UK-14304-induced inhibition of ADA-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Values are means $\pm$ SE of (n) experiments performed in duplicate. Fat cells were incubated in the presence of ADA (5 μg/ml). Antilipolysis is given as the difference between values in the presence of UK and basal values (with ADA alone). Significant effect of treatment * $P < 0.01$ and ** $P < 0.05$. Agonist concentrations required for half-maximal inhibition of lipolysis (IC$_{50}$) were determined from these concentration–response curves.

TABLE 3. Sensitivity for α2- and β-adrenoceptor agonists estimated from in vitro lipolysis studies on subcutaneous abdominal and femoral adipocytes of men and women before and after weight loss

	Subcutaneous adipose cell sensitivity			
	Abdominal		Femoral	
	Before	After	Before	After
Men				
EC$_{50}$ (isoproterenol) (14)	27 ± 4[a]	5 ± 1*,[a]	44 ± 7	13 ± 3**
EC$_{50}$ (procaterol) (12)	12 ± 3[b]	6 ± 1[&],[a]	62 ± 17	18 ± 5[&]
EC$_{50}$ (dobutamine) (10)	97 ± 30[$]	61 ± 13[$$]	140 ± 26	131 ± 46
IC$_{50}$ (UK-14304) (14)	2.2 ± 0.4	4.3 ± 1.0[&]	3.0 ± 0.9	4.9 ± 1.1
Women				
EC$_{50}$ (isoproterenol) (18)	26 ± 3[b]	6 ± 1*,[c]	56 ± 7	17 ± 3*
EC$_{50}$ (procaterol) (14)	26 ± 7[b]	9 ± 2***,[a]	56 ± 11	16 ± 3***
EC$_{50}$ (dobutamine) (12)	306 ± 62	169 ± 40[&]	218 ± 46	114 ± 29[&]
IC$_{50}$ (UK-14304) (18)	2.8 ± 0.5	5.8 ± 1.6[&]	3.3 ± 0.7	6.1 ± 1.6[&]

Values are means ± SE of (n) separate experiments performed in duplicate. β-adrenergic sensitivity estimated by the concentration of either isoproterenol, procaterol or dobutamine required for half-maximal stimulation of lipolysis (EC$_{50}$) (nm) was calculated from each concentration–response curve (10^{-9} to 10^{-5} m of the β-AR agonists tested). α2-adrenergic sensitivity estimated by the concentration of UK-14304 required for half-maximal inhibition of lipolysis (IC$_{50}$) (nm) was calculated from each concentration–response curve (10^{-10} to 10^{-6} m).
Significant difference before and after weight loss at * $P < 0.0001$, ** $P < 0.005$, *** $P < 0.01$, [&] $P < 0.05$.
Significant regional variation at [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.
Significant gender difference at [$] $P < 0.01$, [$$] $P < 0.05$.

maximal lipolysis, when expressed on a relative basis (i.e., after subtraction of basal lipolytic rate) also did not differ in response to weight loss, whatever the adipose site or the gender considered [values clustering 9–11 ± 2 vs. 10–12 ± 3 nmol glycerol/(μm^2 × 10^8 × 2 h), before vs. after weight loss]. However, the shift to the left of the concentration–response curves in all adipose cells after weight reduction attested for a 3- to 5-times increased β-adrenergic sensitivity in both genders (P values ranging from 0.005 to 0.05). The concentration of β-agonist required for half-maximal lipolysis was also higher in subcutaneous abdominal than in femoral adipocytes of men ($P < 0.05$) and women (P values ranging from 0.005 to 0.01), before and after weight loss (Table 3).

Selective β-adrenergic receptor subtype effects. As site differences in the catecholamine response profile appeared to be partly explained by the β-adrenoceptor function, additional experiments were conducted using procaterol (β2-agonist) and dobutamine (β1-agonist) (**Fig. 4** and **Fig. 5**, respectively). Concentration–response curves for these agonists were examined to evaluate the relative importance of each adrenoceptor subtype for explaining the changes observed in isoproterenol sensitivity. In men, the concentration–response curves for procaterol shifted to the left in adipocytes from both regions, attested for a 2- to 3.5-times increased β2-adrenoceptor sensitivity after weight loss (P values ranging from 0.01 to 0.05) (Table 3). However, the fact that the curves for dobutamine were practically superimposed suggested an unchanged β1-adrenoceptor sensitivity (Table 3). In contrast, the shift to the left of concentration–response curves for both agonists in response to weight reduction indicated an increase in the adipose cell sensitivity to the β1- and β2-adrenergic agonists used (1.8- to 1.9- and 3- to 3.5-times, respectively; P values ranging from 0.001 to 0.05), in women (Table 3). The β2-adrenergic sensitivity was also higher in subcuta-

neous abdominal than in femoral adipocytes (P values ranging from 0.01 to 0.05), from both genders, before and after weight loss. However, sensitivity of subcutaneous abdominal adipocytes to dobutamine was greater in men than in women, irrespective of the treatment period ($P < 0.05$) (Table 3). Despite the lack of site or sex difference in the maximal lipolytic response to the selective β-agonist tested (Figs. 4 and 5), neither procaterol nor dobutamine was as potent as isoproterenol in stimulating lipolysis, before and after weight loss. Indeed, both agents (used at 10^{-5} m) only induced a partial activation of lipolysis (values clustering at 65–80% of the maximal effect promoted by the nonselective β-agonist). When expressed on a relative basis, responsiveness to either the β1- or β2-agonist was also not significantly different, irrespective of the gender or of the adipose depot [values clustering 5 to 7 ± 2 vs. 6 to 7 ± 2 nmol glycerol/(μm^2 × 10^8 × 2 h), before vs. after weight loss]. On the other hand, similar results were obtained for epinephrine-, UK14304-, isoproterenol-, procaterol-, and dobutamine-stimulated lipolysis when expressed on a per cell basis (not shown).

Radioligand binding assays. To verify whether differences observed in catecholamine-induced lipolysis could be explained at the receptor level, β- and α2-adrenoceptor sites were also studied in adipose tissue membranes, in response to the weight-reducing program. 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 (5 nm of [^{3}H]-RX 821002 or 300 pm of ^{125}I-labeled CYP) was used to evaluate the number of α2- or β-adrenergic binding sites on crude adipose tissue membranes (**Table 4**). As both subcutaneous abdominal and femoral adipocytes were larger before than after weight loss (Table 1), binding results were corrected for variation in adipose cell size. At 300 pm, ^{125}I-labeled CYP binding was 1.7- to 2-times
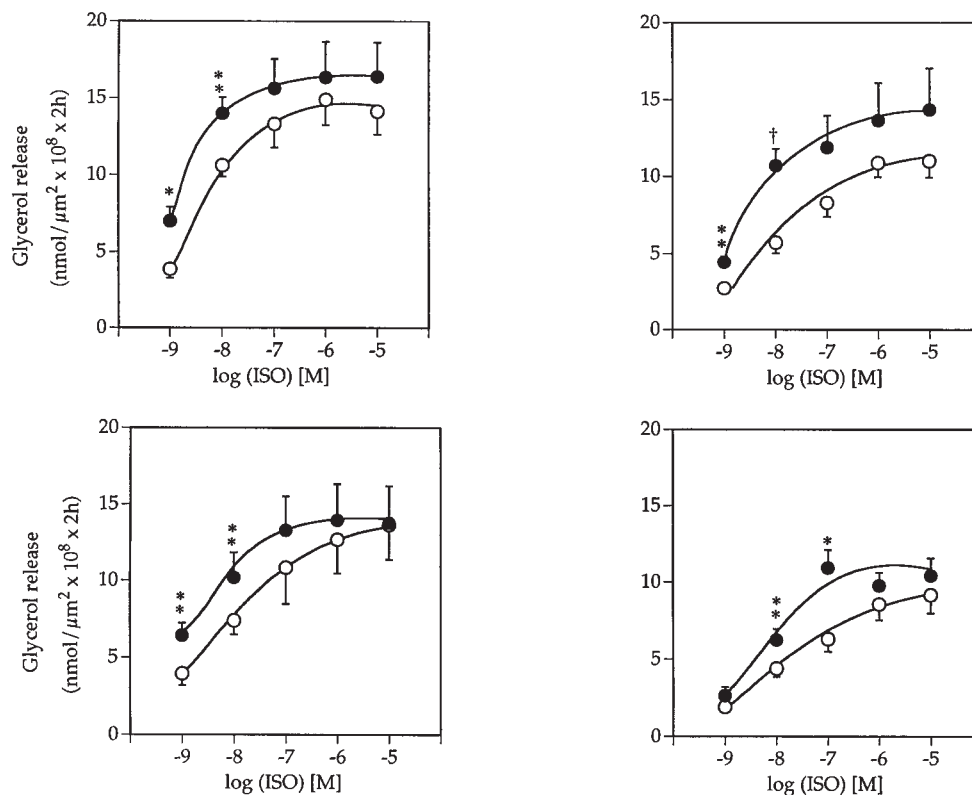
Fig. 3. Isoproterenol (ISO)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (i.e., in standard conditions) and values are means ± SE of (n) experiments performed in duplicate. Significant effect of treatment at $^{†}P < 0.005$, $^{*}P < 0.01$, and $^{**}P < 0.05$. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration–response curves.

higher in adipose tissue membranes of both genders (P values ranging from 0.01 to 0.05), whereas at 5 nm, $[^3H]$-RX binding did not change significantly in response to weight reduction. However, the β-adrenoceptor density was higher in subcutaneous abdominal than in femoral adipose tissue membrane preparations of both genders (P values ranging from 0.001 to 0.05). A greater number of α2-adrenoceptors was also observed in femoral than in subcutaneous abdominal adipose tissue membranes of women, before and after weight loss ($P < 0.01$). The study of potential gender differences revealed a greater estimated number of α2-adrenergic receptors in femoral adipose tissue membranes of women, compared to men, irrespective of the treatment period ($P < 0.05$). Similar results were obtained when binding data were expressed per cell number (not shown). As the evaluation of the functional balance between α2- and β-adrenoceptors appears physiologically relevant (17, 39), the ratio of $[^3H]$-RX to ^{125}I-labeled CYP binding sites was also calculated for each adipose tissue membrane and averaged for the different sites and by gender. The mean ratio of α2- to β-adrenoceptors was always higher in femoral than in subcutaneous abdominal adipose tissue membranes, before and after weight reduction (P values ranging from 0.005 to 0.05) (Table 4).

Lipolytic responses to post-receptor agents and hormone-sensitive lipase assays. As any step in the lipolytic cascade could be

responsible for the site differences observed in catecholamine responsiveness, the effects of agents acting at well-defined post-adrenoceptor sites were also investigated (**Fig. 6**). There was no regional variation nor any gender difference when lipolysis was stimulated at maximum concentrations of dibutyryl-cyclic AMP (10^{-3} m), forskolin (10^{-5} m), theophylline (10^{-3} m), or cilostamide (10^{-3} m) at baseline. In addition, the rates of glycerol release reached in the presence of these compounds were similar before and after weight reduction, when expressed either on an absolute (not shown) or on a relative basis (Fig. 6). Finally, hormone-sensitive lipase activity was not affected by weight reduction in men, although it was significantly increased in response to weight loss in femoral adipocytes of women ($P < 0.005$). The lipase activity was also higher in femoral than in subcutaneous abdominal adipose cells of women, after weight loss ($P < 0.01$) (Table 4). Similar results were obtained when enzyme activity was expressed either per cell number or on the basis of adipocyte triglyceride content (not shown).

DISCUSSION

Until recently, few studies had attempted to elucidate the mechanisms responsible for the regional and gender
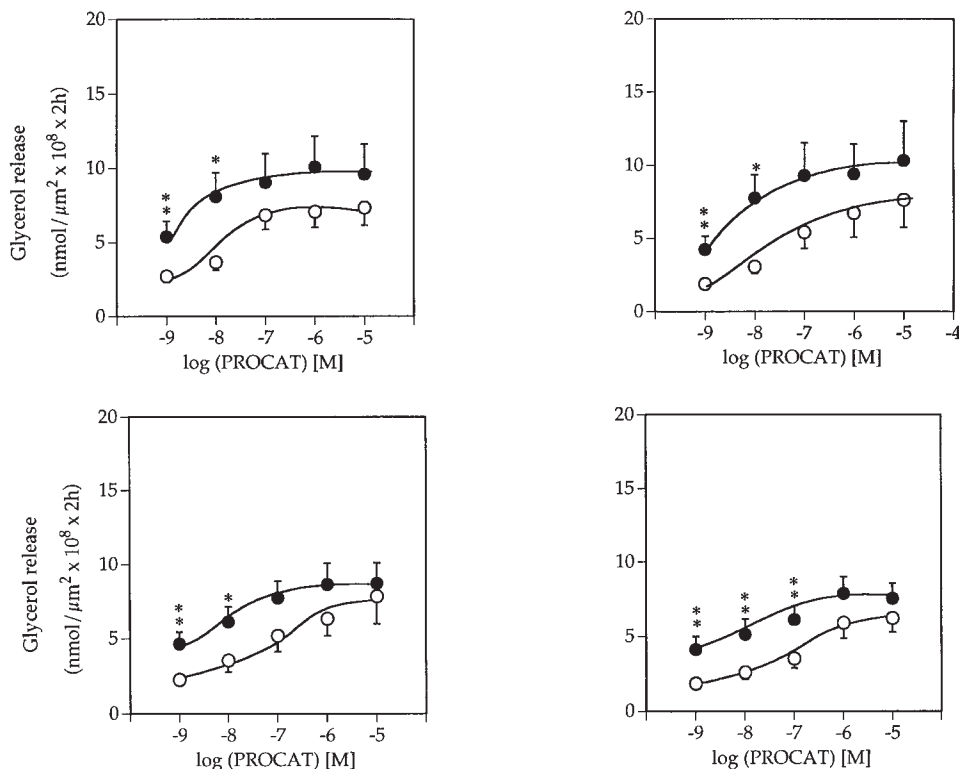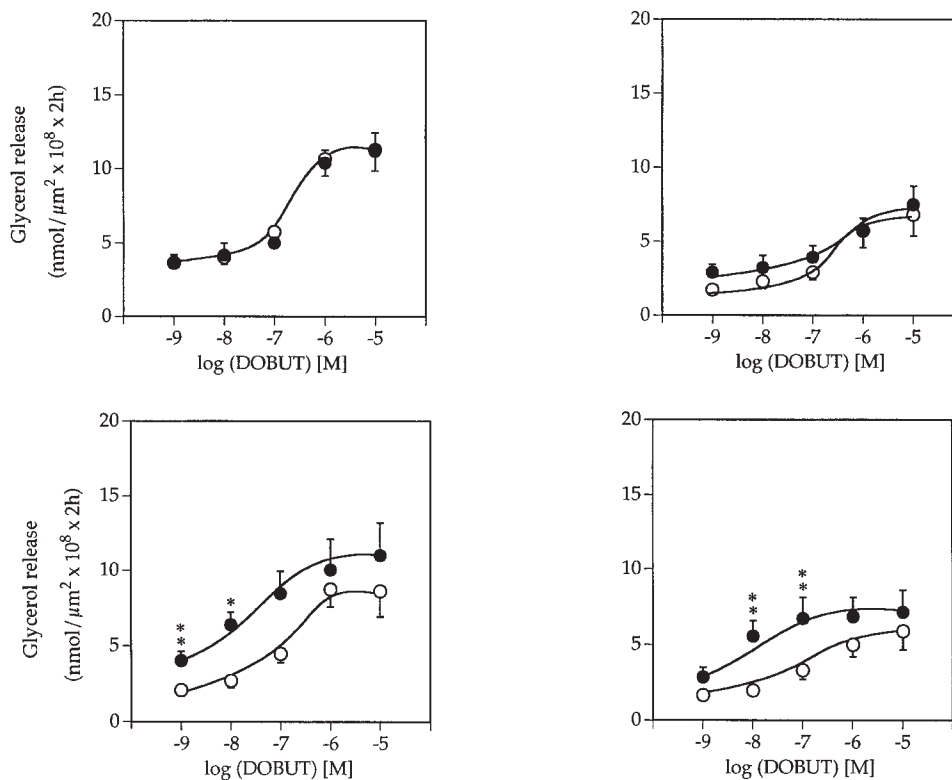
Fig. 4. Procaterol (PROCAT)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 12; upper panels) and women (n = 14; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (i.e., in standard conditions) and values are means ± SE of (n) experiments performed in duplicate. Significant effect of treatment at * $P < 0.01$ and ** $P < 0.05$. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration–response curves.

variation observed in adipose tissue lipolysis of obese individuals subjected to diet-induced weight loss (23, 24). Indeed, most experiments published so far have dealt exclusively with obese women and did not consider site differences as subcutaneous abdominal adipose tissue was the only depot investigated (25–31). On the other hand, controversial findings resulting from in vitro experiments have pointed out either an unchanged (24, 29) or even reduced (28) catecholamine-induced lipolysis as well as an enhanced (25–27, 29, 30) or a decreased (31) basal lipolytic rate in subcutaneous abdominal fat cells of reduced obese individuals. Therefore, to the best of our knowledge, the present study is the first to examine the cellular mechanisms underlying regional variation in adipose cell lipolysis of both overweight men and premenopausal women who underwent dietary treatment for obesity.

The fact that adipose cell weight did not differ according to the anatomic location of fat was probably due to our subjects' high levels of total body fat which is commonly associated with hypertrophy of adipose cells. This finding is concordant with previous observations from our group and other investigators (15, 16, 18, 19). Although femoral adipose cells were larger in women than in men (14–16), this gender variation was no longer significant after weight loss, as adipose cell size reduction was of the same order of magnitude in both genders (Table 1).

The similar basal lipolytic rate, regardless of the adipose depot or the gender, is in agreement with the unchanged ADA-induced lipolysis observed in response to weight loss. This is in clear contrast to findings resulting from short- or long-term very low calorie diet studies (25, 27, 29–31). Assuming that an increased basal lipolysis may represent a physiological adaptation to negative energy balance, the discrepancy observed between our results and earlier data was probably due to the fact that our subjects were examined 4–6 weeks after the end of the treatment when they were weight-stable. Moreover, differences in basal lipolysis among various fat depots or after weight reduction have already been suggested to be partly due to variations in HSL activity (12, 13). In this regard, conflicting results have reported either a decreased (31), an increased (30), or a similar enzyme activity (29; the present study) in subcutaneous abdominal adipocytes of obese women subjected to weight reduction, despite a marked reduction in adipose cell size (29–31; the present study). Once again, the fact that subjects were examined several weeks after the completion of the hypocaloric diet program could explain, at least to a certain extent, the unchanged basal lipolysis and lipase activity that we observed in response to weight loss. In addition, the unchanged adipose cell lipolytic capacity clearly indicated an unaltered hormone responsiveness. Indeed, the similar maximal lipolytic rates

Fig. 5. Dobutamine (DOBUT)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 10; upper panels) and women (n = 14; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (i.e., in standard conditions) and values are means $\pm$ SE of (n) experiments performed in duplicate. Significant effect of treatment at * $P < 0.01$ and ** $P < 0.05$, in women, exclusively. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration–response curves.

promoted by β-agonists or by agents acting selectively at the adenylate cyclase, the lipase–protein kinase A complex, or the phosphodiesterase level, is concordant with unaltered post-adrenoceptor pathways after weight reduction, as previously reported (28, 29, 31). The lack of post-receptor changes is further strengthened by the similar maximal lipase activity, with the exception of the increased enzyme activity found in femoral adipocytes of women after weight loss. As the present assay used for the measurement of lipase activity does not differentiate between the phosphorylated active and the dephosphorylated inactive forms of the enzyme (12, 44), the discrepancy observed between lipolysis and enzyme data in femoral adipocytes of women could be explained either by an increased phosphorylation capacity of HSL (via the cAMP-dependent protein kinase A) or by the regulation of cellular components involved in the translocation of the lipase to the lipid droplet (47, 48). The similar lipolytic rates promoted by a phosphodiesterase (PDE)-resistant (dibutyryl-cAMP) and a PDE-sensitive (theophylline) cAMP-analogues, indicated an unchanged phosphodiesterase activity after weight reduction, in contrast to its marked increase during short-term fasting (49).

Marked effects of body weight reduction on the regula-

TABLE 4. $\alpha2$- versus β-adrenoceptor density and hormone-sensitive lipase activity in subcutaneous abdominal and femoral adipose depots of men and women before and after weight loss

|	Subcutaneous adipose tissue			
	Abdominal		Femoral	
	Before	After	Before	After
Men				
HSL activity (14)	29 ± 6	38 ± 6	43 ± 8	47 ± 5
$\alpha2$-AR sites (12)	16 ± 4	23 ± 5[†]	18 ± 4	22 ± 3
β-AR sites (12)	1.0 ± 0.1[a]	2.1 ± 0.2[b]	0.6 ± 0.1	1.2 ± 0.2*
$\alpha2/\beta$-AR ratio (12)	16 ± 3[a]	13 ± 3[b]	30 ± 6	26 ± 3
Women				
HSL activity (18)	27 ± 6	31 ± 4	34 ± 7	55 ± 8[†,b]
$\alpha2$-AR sites (16)	18 ± 2[b]	21 ± 2[b]	26 ± 2[&]	30 ± 2[&]
β-AR sites (16)	1.0 ± 0.1[a]	1.9 ± 0.2[†,c]	0.7 ± 0.1	1.3 ± 0.2**
$\alpha2/\beta$-AR ratio (16)	24 ± 7[a]	14 ± 3[c]	41 ± 9	36 ± 6

AR, adrenoceptor; HSL, hormone-sensitive lipase.

Values are means $\pm$ SE of (n) separate experiments performed in duplicate. Both radioligand binding data and enzymatic activities were expressed per cell surface area (i.e., in amol/μm^2 and pU/μm^2 cells, respectively).

Significant difference before and after weight loss at [†] $P < 0.005$, * $P < 0.01$, ** $P < 0.05$.

Significant regional variation at [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$.

Gender difference for $\alpha2$-AR sites at [&] $P < 0.05$.
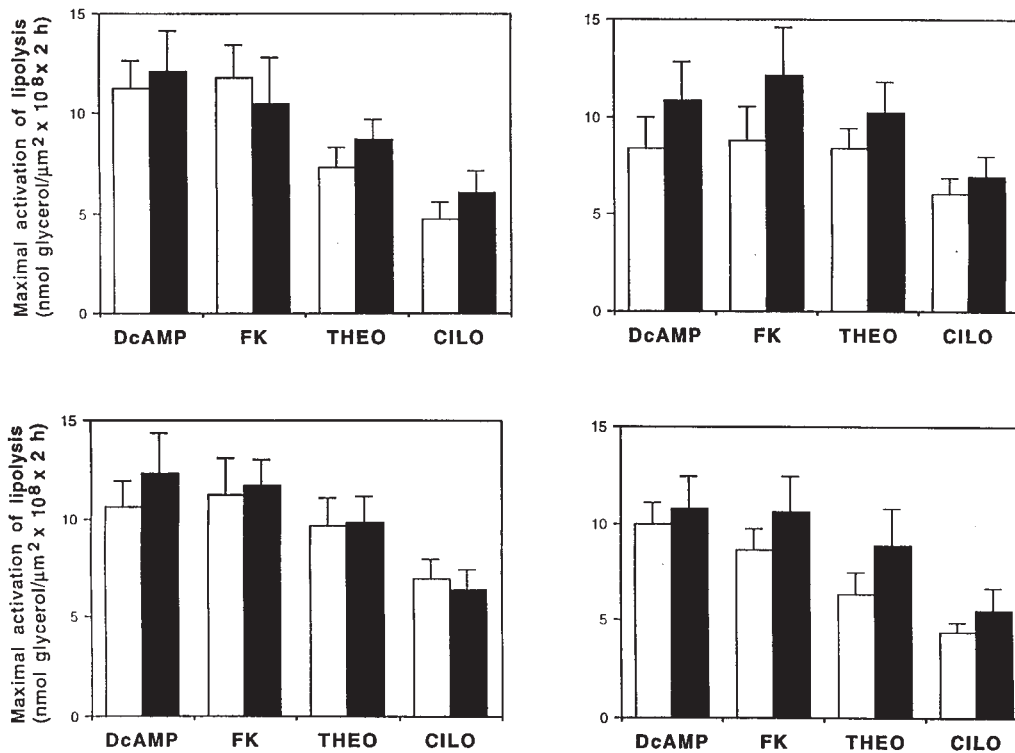
Fig. 6. Lipolytic responsiveness to post-adrenoceptor agents of isolated adipocytes from the abdominal (left) and femoral (right) regions in men (upper panels) (n values ranging from 8 to 14, depending on the agent used) and women (lower panels) (n values ranging from 9 to 18, depending on the agent used) before (open columns) and after (filled columns) weight reduction. Fat cells were incubated without ADA, in the presence of either dibutyryl-cyclicAMP (DcAMP) (10^{-3} m), forskolin (FK) (10^{-5} m), theophylline (THEO) (10^{-3} m) or cilostamide (CILO) (10^{-5} m). Previous experiments revealed that the concentrations of the different drugs used were maximally effective doses. Values are means $\pm$ SE of (n) experiments performed in duplicate and basal glycerol release has already been subtracted.

tion of catecholamine-induced lipolysis were observed in the present study. However, the greater lipid mobilization in subcutaneous abdominal than in femoral fat depots of both genders re-emphasizes the notion that the typical "female" fat storage depot is highly resistant to slimming (11, 14, 15, 17, 23–25). The greater sensitivity of subcutaneous abdominal than femoral adipose cells to catecholamine stimulation, which could be attributed to the higher number of β-adrenergic receptors in the former adipocytes, is consistent with previous observations in obese (15, 18, 19) and healthy normal-weight subjects (14, 16, 20). However, our results demonstrate a similar biphasic epinephrine response profile in all cell types that probably reflects the interaction of the hormone with both types of adrenoceptors. This finding supports the notion of the differential recruitment of α2- than of β-binding sites (17–19). The functional α2-/β-adrenoceptor balance seems to be of importance in explaining the different adipose cell lipolytic responses to catecholamines. As subcutaneous adipocytes possess a higher α2- than β-adrenoceptor density, the greater ratio of α2- to β-sites could explain the predominant α2-adrenergic component of epinephrine responsiveness observed irrespective of the gender or of the fat depot. The strong α2-antilipolytic effect noted at the lowest concentrations of epinephrine could not en-

tirely be compensated by the β-adrenergic activity of the hormone, although both enhanced β-adrenoceptor sensitivity and density were observed in men and women after weight reduction.

Our results clearly indicate that the cellular mechanisms responsible for the increased ability of obese individuals to mobilize lipids during dietary energy restriction are mainly localized at the β- (and more particularly β2-) and α2-adrenoceptor levels in both adipose regions and genders. As only a few available α2- and β-adrenoceptors need to be occupied to obtain a maximal effect in human fat cells (50), changes in sensitivity may reflect alterations in hormone action that are located at or near the receptor level whereas alterations in responsiveness are usually linked to changes in hormone action at further intracellular steps in the pathway of the signal. However, whether the higher β-adrenergic lipolytic sensitivity noted in response to weight loss may result from an increased β1/β2-adrenoceptor density and/or an improved coupling efficiency of β-adrenoceptors to the stimulatory GTP-sensitive Gs proteins remains to be established. The increased β-adrenergic sensitivity of adipose tissue that we have noted after weight loss is concordant with the enhanced β-adrenoceptor density determined by radioligand binding assays. This up-regulation of β-adrenergic receptors has al-

ready been attributed to a reduced basal activity of the sympathetic nervous system which is frequently observed after energy restriction (9). Indeed, reduced-weight obese subjects have been shown to be characterized by a lower basal sympathetic activity and a higher lipolytic response to catecholamine infusion than obese or lean individuals (51). Although our findings are concordant with the increased β2-adrenergic lipolytic sensitivity of subcutaneous abdominal adipocytes of obese women after weight loss (31), they contrast with other observations resulting from in vitro and in vivo studies which have shown an unchanged sensitivity to isoproterenol (29, 30). The specific improvement of the β1-adrenergic lipolytic pathway of female adipose cells after weight loss may also appear at variance with previous studies which did not detect any change in this component after weight reduction (29–31). However, Barbe et al. (52) have recently shown an increased β1-adrenergic lipolytic response of the subcutaneous abdominal fat depot in obese women subjected to hypocaloric diet, using the in situ microdialysis technique. Further studies are clearly warranted to address this issue. On the other hand, the decreased adipose cell α2-adrenergic antilipolytic sensitivity observed in subcutaneous abdominal adipocytes of men and in adipose cells of women, after weight loss, could not be explained by a reduction in the α2-adrenoceptor density as the estimated number of α2-adrenoceptors was similar, irrespective of the treatment period (Table 4). It is thus possible that the lower α2-adrenergic sensitivity could be related to a decreased coupling efficiency of these receptors to inhibitory GTP-sensitive Gi proteins, as a minor role of G proteins as modulators of clonidine sensitivity has already been suggested (16). Although it should have been interesting to measure the relative amounts of Gi and Gs proteins, this was beyond the scope of the present study as these methods required large amounts of adipose tissue which cannot be removed for obvious ethical reasons.

Conclusion

A moderate weight loss resulting from 15 weeks of dietary energy restriction leads to an increased lipolytic catecholamine sensitivity, despite a preserved lipolytic hormonal capacity which is mainly reflected by both similar basal lipolysis and hormone-sensitive lipase activity. The resulting higher efficiency in the regulation of subcutaneous adipocyte lipolysis after weight loss is partly mediated by increased β- and β2-adrenergic (combined with a higher β-adrenoceptor density) and decreased α2-adrenergic sensitivities (without any change in the α2-adrenoceptor number) in both men and women. These changes are more pronounced in the subcutaneous abdominal than in the femoral fat depot and suggest that a preferential fat mobilization from the major energy depot storage (i.e., the subcutaneous abdominal adipose region) occurs during dietary caloric restriction, in both genders.

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