Reduced α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes as a modulator of fasting and postprandial triglyceride levels in men

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Abstract **This study examined the postprandial lipemia of two groups of men displaying similar age, body weight, and regional fat distribution, but characterized by either low (n = 11) or high (n = 15) α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes. In addition to fat cell lipolysis, adipose tissue lipoprotein lipase (AT-LPL) as well as postheparin plasma LPL activities were measured in the fasting state. Fasting AT-LPL and PH-LPL activities were similar in both groups. Maximal adipose cell lipolysis induced by isoproterenol (β-adrenergic agonist) as well as the β-adrenergic sensitivity did not differ between both groups of men. The selective α_2-adrenergic agonist UK-14304 promoted a similar antilipolytic response in subcutaneous abdominal adipocytes from both groups. However, the α_2-adrenergic sensitivity, defined as the dose of UK-14304 that produced half-maximal inhibition of lipolysis (IC_{50}), was significantly different between groups ($P < 0.0001$). Men with low versus high subcutaneous abdominal fat cell α_2-adrenergic sensitivity showed higher fasting TG levels. In the whole group, a positive relationship was observed between log-transformed IC_{50} UK-14304 values of subcutaneous adipocytes and fasting TG levels ($r = 0.39$, $P < 0.05$), suggesting that a low abdominal adipose cell α_2-adrenergic sensitivity is associated with high TG levels. After the consumption of a high-fat meal, subjects with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity showed higher TG levels in total, medium, and small triglyceride-rich lipoprotein (TRL) fractions at 0- to 6-h time points than men with high adipocyte α_2-adrenergic sensitivity (P values ranging from 0.01 to 0.05). Stepwise regression analysis showed that the fasting TG concentration was the only variable retained as a significant predictor of the area under the curve of TG levels in total TRL fractions (73% of variance) among independent variables such as body weight, percent body fat, visceral and subcutaneous abdominal adipose tissue accumulation measured by CT, as well as subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity. Taken together, these results indicate that a reduced antilipolytic sensitivity of subcutaneous abdominal adipocytes to catecholamines may increase fasting TG levels, which in turn play a role in the etiology of an impaired postprandial TRL clearance in men.**—Imbeault, P., C. Couillard, A. Tremblay, J-P. Després, and P. Mauriège. **Reduced α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes as a modulator of fasting and postprandial triglyceride levels in men.** ***J. Lipid Res.*** **2000.** 41: **1367–1375.**

Supplementary key words adipose cell lipolysis • lipase activities • regional fat distribution • lipoproteins

Alterations in plasma lipid and lipoprotein levels are prominent features of obesity, especially abdominal obesity (1–3). Indeed, individuals displaying a substantial accumulation of abdominal adipose tissue show greater plasma triglycerides (TG), very low density lipoprotein (VLDL), and apolipoprotein B (apoB) concentrations than nonobese persons. These metabolic alterations probably result from an increased free fatty acid (FFA) flux to the liver and a major culprit seems to be visceral adipose tissue, because it is characterized by a high lipolytic activity and a low antilipolytic response to insulin (4, 5).

As α_2-adrenoceptors inhibit and β-adrenoceptors stimulate adipose tissue lipolysis (6, 7), the fact that subcutaneous adipocytes possess more α_2- and fewer β-adrenoceptors than visceral fat cells explains in part their lower lipolytic capacity (8, 9). Moreover, subcutaneous adipocytes do not display the same potential as visceral adipose cells to deliver FFA into the portal circulation because of their anatomic location. However, some in vitro studies have already emphasized that subcutaneous fat cell lipolysis may also contribute to the development of metabolic perturbations in abdominally obese patients. In this re-

Abbreviations: ADA, adenosine deaminase; apo-B, apolipoprotein-B; ASP, acylation-stimulating protein; AT-LPL, adipose tissue lipoprotein lipase; AUC, area under curve; AUIC, area under incremental curve; CT, computed tomography; FFA, free fatty acid; HSL, hormone-sensitive lipase; LDL, low density lipoprotein; PH-LPL, postheparin plasma lipoprotein lipase; RP, retinyl palmitate; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

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gard, Arner et al. (10) have previously reported that low β_2-adrenoceptor sensitivity in subcutaneous abdominal fat cells was related to high plasma VLDL-TG and apoB levels. Furthermore, we have demonstrated that men with high femoral fat cell lipolysis (i.e., a low α_2-adrenergic component) were characterized by increased fasting plasma insulin, low density lipoprotein (LDL)-cholesterol (C), and LDL-apoB levels (11), suggesting that high femoral adipose tissue lipolysis may be associated with an enhanced cardiovascular disease (CVD) risk profile in men.

Evidence from in vivo studies has demonstrated that in the postprandial state, the enzyme responsible for adipocyte intracellular TG hydrolysis, hormone-sensitive lipase (HSL), is normally suppressed by insulin, whereas lipoprotein lipase (LPL) is activated by this hormone, thus leading to the release of FFA, which either enter the tissue for reesterification and storage or are delivered into the systemic circulation (12). These coordinated changes appear important in the regulation of FFA movement and postprandial lipoprotein metabolism. However, to the best of our knowledge, no study has yet verified whether the antilipolytic efficiency (i.e., the α_2-adrenoceptor sensitivity) of subcutaneous abdominal fat cells measured in vitro could be associated with the fate of triglyceride-rich lipoproteins (TRL) in the postprandial state. Therefore, the aim of this study was to verify whether postprandial levels of various TRL fractions differ between two groups of men characterized by similar body fatness and regional fat distribution but displaying low versus high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity.

MATERIALS AND METHODS

Subjects

Twenty-six white men were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. As two distinct clusters of men were observed, with regard to the antilipolytic sensitivity of their isolated subcutaneous abdominal adipocytes to UK-14304 (α_2-adrenoceptor agonist), low (n = 11) and high (n = 15) α_2-adrenoceptor-sensitive groups were compared in order to isolate the independent contribution of subcutaneous abdominal adipocyte antilipolytic sensitivity to postprandial lipemia. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, or endocrine disorders, or those receiving medication that could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc.), were excluded from the study. All participants were sedentary, nonsmokers, 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 during the last 6 months prior to the study.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique and percent body fat was derived from body density (13). Pulmonary residual volume was measured by the helium dilution method (14). Fat mass was calculated as total body weight minus fat free mass. Waist girth was measured according to procedures recommended at the Airlie Conference (15). Computed tomography (CT) was performed on a Siemens (Erlangen, Germany) Somatom DRH scanner, according to the methodology previously described by Sjöström et al. (16). Briefly, 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) level, using an abdominal scout radiograph to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing AT surfaces with an attenuation range of −190 to −30 Hounsfield units (HU) (17). 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.

Oral lipid tolerance test

After a 12-h overnight fast, an intraveneous catheter was inserted into a forearm vein for blood sampling. Each participant was given a test meal containing 60 g of fat/m^2 body surface area and 60,000 IU of vitamin A (Aquasol A; Astra, Westborough, MA) (18). The meal consisted of eggs, cheese, toast, peanut butter, peaches, whipped cream, and milk. Composition of the meal was 64% fat, 18% carbohydrate, and 18% protein. The test meal was well tolerated by all subjects. After the meal, subjects were not allowed to eat for the next 8 h but were given free access to water. Blood samples were drawn before the meal and every 2 h after the meal over an 8-h period; samples were handled in a dimmed light to avoid deterioration of vitamin A.

Adipocyte isolation and lipolysis

After an overnight fast, participants underwent a biopsy of subcutaneous fat in the periumbilical region. A small cutaneous incision (1 cm) was performed in the abdominal site after local anesthesia (1% lidocaine, without epinephrine) and about 350 mg of subcutaneous adipose tissue was surgically removed from the fat depot.

Samples of 250 mg of adipose tissue were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (19) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus collagenase (1 mg/mL), as previously described (20). 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 three times with 5 mL of KRBA. Isolated adipocytes were finally resuspended 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. Aliquots (50 μL) 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 of KRB were immediately placed on ice and provided 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 of HCl (1 N) was added to all tubes to stop the reaction, and then 50 μL of NaOH (1 N) was added to neutralize the medium. All tubes were stored at −20°C until glycerol determination and NADH concentration was measured by bioluminescence with a luciferase solution, using an automated 2250 Dynatech (Vienna, VA) luminometer (20, 21). 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 sensitive and especially well adapted when only small amounts of adipose tissue are available (20, 21). Adipose cell diameters were determined with a Leitz (Rockleigh, NJ) microscope equipped with a graduated ocular. Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described (22).

The lipolytic activity of the isolated fat cells was tested with epinephrine, which is a mixed agonist (α_2/β) with a higher affinity for α_2- than for β-adrenoceptor (AR) sites (8), UK-14304 (selective α_2-AR agonist), and isoproterenol (nonselective β-AR agonist) (21). Ascorbic acid (0.1 mmol/L) was included in the medium in order to prevent catecholamine degradation. When antilipolytic effects were investigated, the incubation buffer was supplemented with adenosine deaminase (ADA, 5 μg/mL) to remove adenosine released into the incubation medium by the isolated fat cells; this procedure allowed better investigations of α_2-AR-mediated antilipolytic effects (20, 21). Lipolysis was expressed either per cell number (i.e., in micromoles of glycerol/10^6 cells × 2 h) or per unit of cell surface area (i.e., in nanomoles of glycerol/$\mu m^2 \times 10^8 \times 2$ h), the latter mode of expression being used to correct for variation in fat cell size, which is well known to influence the rate of lipolysis (21). When complete dose-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 isoproterenol or forskolin, 10^{-3} M dibutyryl cAMP, or theophylline). The β-adrenergic sensitivity was considered as the concentration of isoproterenol giving half-maximal stimulation of lipolysis (EC_{50}), whereas the α_2-adrenergic sensitivity was calculated as the dose of UK-14304 that produced half-maximal inhibition of lipolysis (IC_{50}). Both were evaluated by logarithmic conversion of each dose-response curve. The higher was the EC_{50} (isoproterenol) or the IC_{50} (UK-14304) value, and the lower was the β- or α_2-adrenergic sensitivity, respectively.

Adipose tissue lipoprotein lipase activity

Samples of approximately 100 mg of adipose tissue from the abdominal site were immediately frozen in liquid nitrogen and stored at −80°C. Heparin-releasable LPL activity was measured within 1 month of freezing storage, according to Savard et al. (23). AT-LPL activity was expressed as micromoles of free fatty acids (FFA) released per h per 10^6 cells. Because AT-LPL activity is associated with fat cell size (23, 24), AT-LPL activity was also expressed per cell surface area (i.e., nanomoles of FFA per h per $\mu m^2 \times 10^8$).

Postheparin plasma LPL activity

Plasma LPL activity was measured on one occasion after a 12-h overnight fast, 10 min after an intravenous injection of heparin (60 IU/kg body weight). The lipase activity was measured by a modification of the method of Nilsson-Ehle and Ekman (25), as previously described (26), and expressed as nanomoles of oleic acid released per mL of plasma per min.

Fasting and postprandial plasma lipoprotein concentrations

Plasma was separated immediately after blood collection by centrifugation at 3,000 rpm for 10 min at 4°C and placed in aluminum foil-wrapped tubes. Triglyceride and cholesterol concentrations in total plasma were determined enzymatically on an RA-1000 Auto-Analyzer (Technicon Instruments, Tarrytown, NY), as previously described (27). Each plasma sample (4 mL) was then subjected to a 12-h ultracentrifugation (50,000 rpm) in a Beckman (Palo Alto, CA) 50.3 Ti rotor at 4°C, in 6-mL Beckman Quickseal tubes, which yielded two fractions: the top fraction containing TRL ($d < 1.006$ g/mL; total) and the bottom fraction consisting of triglyceride-poor lipoproteins ($d > 1.006$ g/mL). Using the distilled water layering technique and modified method of Ruotolo et al. (28), the total TRL fraction was further separated, by a 5-min spin (40,000 rpm) at 4°C with the same tubes and rotor, into three subclasses of TRL, namely, large, medium, and small. A small volume (100 μL) of a $d = 1.019$ g/mL saline solution was added to the total TRL fraction to facilitate water layering. The large TRL fraction was collected by tube slicing and made up to a final volume of 1 mL with 0.15 M NaCl. The next 3 mL of the middle layer was collected by aspiration as medium TRL and the final 2 mL was considered as the small TRL fraction. HDL particles were isolated from the bottom fraction ($d > 1.006$ g/mL) after precipitation of apoB-containing lipoproteins with heparin and $MnCl_2$ (29). The triglyceride and cholesterol contents of each fraction, e.g., large, medium, and small TRL as well as HDL, were quantified on the Auto-Analyzer. All lipoprotein isolation procedures were completed within 2–3 days of the fat load, and samples were protected from light at all times for later assays. Plasma FFA levels were measured at 0, 2, 4, 6, and 8 h, using a colorimetric method (30).

Glucose and insulin concentrations

Fasting and postprandial plasma glucose levels were determined by the glucose oxidase assay (31) (Sigma, St. Louis, MO). Plasma insulin concentrations were measured by a commercial double-antibody radioimmunoassay (Linco Research, St. Louis, MO) that shows little cross-reactivity (<0.02%) with proinsulin (32).

Retinyl palmitate measurements

As retinyl esters, predominantly retinyl palmitate (RP), serve as useful estimates of the intestinally derived component of postprandial lipemia, the RP content of total as well as of large, medium, and small TRL fractions was analyzed by high performance liquid chromatography (HPLC) as previously described (28). Briefly, aliquots of 100 μL of total and large TRL as well as 500 μL of medium and small TRL were used for the analysis. The volume of total and large TRL fractions was adjusted to 500 μL with 0.15 M NaCl. A volume of 200 μL of retinyl acetate (RA, 200 ng/mL; Sigma) was added to each sample as internal standard. The extraction of RP from the samples was obtained by addition of 500 mL of methanol followed by 500 μL of mobile phase buffer prepared from 90 mL of hexane, 15 mL of *n*-butyl chloride, 5 mL of acetonitrile, and 0.01 mL of acetic acid (82:13:5 by volume, with 0.01 mL of acetic acid). Tubes were mixed thoroughly after each addition step. All solvents used were HPLC grade (Caledon Laboratories, Georgetown, Ontario, Canada). Samples were then centrifuged for 15 min at 1,500 rpm (room temperature). This procedure yielded two distinct phases. The upper phase, containing the RP and RA, was carefully removed and placed in separate autosampler vials. Vials were then placed in an autosampler from Shimadzu (Kyoto, Japan) and samples were analyzed with an HPLC system from Waters (Waters Associates, Milford, MA). The RP and RA peaks were detected at 325 nm. The RP concentration (in RA equivalents) of every fraction was calculated according to the equation of Ruotolo et al. (28):

$$\text{RP (ng RA/mL)} = (\text{RP peak area/RA peak area}) \times (1/\text{volume of sample used}) \times 40 \text{ ng RA}$$

Drugs and chemicals

Collagenase, bovine serum albumin, ADA, and enzymes for glycerol assays were obtained from Boehringer Mannheim (Indi-

anapolis, IN). Ascorbic acid, theophylline, forskolin, dibutyryl-cAMP (DcAMP), (−)-isoproterenol bitartrate and (−)-epinephrine bitartrate, and cold triolein were purchased from Sigma. [^{14}C]Triolein was obtained from Dupont NEN (Boston, MA). 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK-14304) was generously provided by D. A. Faulkner (Pfizer, Sandwich, UK). 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 analyses

The Student's *t*-test was utilized for comparisons between men with low and high subcutaneous abdominal adipocyte α_2-adrenergic sensitivity. Associations between two variables were quantified by Pearson's product-moment correlation coefficients. Stepwise multiple regression was also performed to enhance predictability of fasting TG levels and areas under the curve (AUC) of TG. Analysis of variance for repeated measures was used to verify the overall differences in total, large, medium, and small TRL fractions over time. The same procedure was performed with plasma insulin, glucose, and FFA concentrations. The AUC of TG, glucose, insulin, FFA, and RP were determined by the trapezoid method. All analyses were performed with the Jump version 3.2.2 program (SAS Institute, Cary, NC) adapted for Macintosh computers.

RESULTS

Physical characteristics and fasting metabolic variables of both groups are presented in **Table 1**. Men with high or low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity displayed similar age, body weight, fat mass, and regional adipose tissue distribution measured by CT. Moreover, no difference in subcutaneous abdominal fat cell size was observed between groups. Men with high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity showed lower fasting plasma TG levels than men with low adipose cell α_2-adrenoceptor sensitivity ($P < 0.01$). However, fasting plasma insulin, FFA and glucose levels were similar in both groups.

TABLE 1. Physical characteristics and fasting metabolic profile of men with high or low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivy

	Subcutaneous Abdominal Adipocyte α_2-Adrenoceptor Sensitivity	
	High (n = 15)	Low (n = 11)
Age (years)	45 ± 10	48 ± 11
Body weight (kg)	90 ± 16	86 ± 11
BMI (kg/m^2)	30 ± 5	29 ± 3
Fat mass (kg)	24 ± 10	24 ± 7
% Body fat	26 ± 7	27 ± 5
Abdominal adipose tissue areas (cm^2)		
Subcutaneous	291 ± 109	260 ± 57
Visceral	169 ± 50	159 ± 39
Abdominal fat cell weight (μg lipid/cell)	0.56 ± 0.12	0.56 ± 0.11
Triglycerides (mmol/L)	2.0 ± 0.7	3.4 ± 1.8[a]
FFA (mmol/L)	0.7 ± 0.1	0.8 ± 0.1
Insulin (pmol/L)	106 ± 45	104 ± 43
Glucose (mmol/L)	5.2 ± 0.5	5.2 ± 0.7

Data are means ± SD; BMI, body mass index; FFA, free fatty acids.
[a] Significant difference between groups at $P < 0.01$.

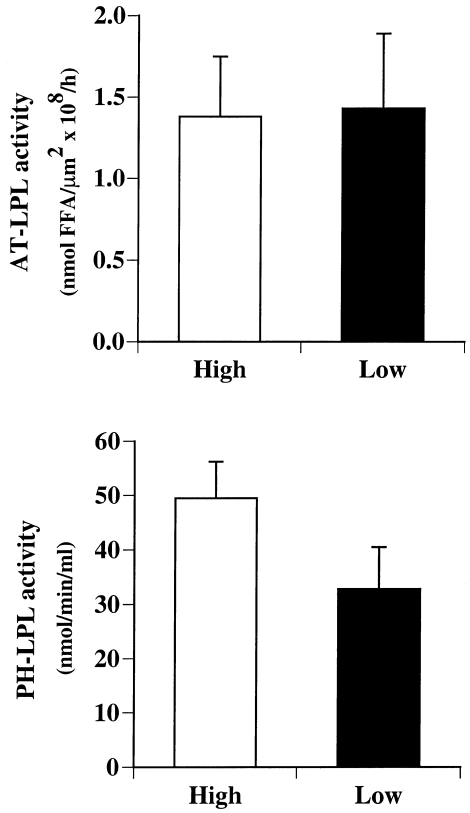

Fig. 1. Adipose tissue lipoprotein lipase (AT-LPL) activity of subcutaneous abdominal region as well as postheparin plasma LPL activity in men with high versus low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE.

AT- and postheparin (PH)-LPL activities did not differ between men with low versus high α_2-adrenoceptor sensitivity (**Fig. 1**). Moreover, neither the basal lipolytic rate nor ADA-stimulated lipolysis was significantly different between groups (**Fig. 2**). As shown in **Fig. 3A**, the effect of epinephrine, which is known for its mixed agonist (α_2/β) properties on lipolysis, was examined in the presence of ADA. The catecholamine initiated a similar biphasic response profile in subcutaneous abdominal adipocytes from both groups: antilipolysis was observed at low concentrations (10^{-9} to 10^{-7} M), this effect being completely reversed at higher doses at which the hormone exerted a lipolytic response (10^{-6} to 10^{-5} M), thus suggesting a differential recruitment of α_2-, then of β-adrenoceptors.

To characterize the α_2-adrenoceptor component, the selective α_2-agonist UK-14304 was also tested on ADA-stimulated lipolysis (Fig. 3B). A similar antilipolytic effect was observed in subcutaneous abdominal adipocytes from both groups. However, the α_2-adrenergic sensitivity (IC_{50}) estimated as the half-maximal antilipolysis induced by UK-14304 was significantly different between groups ($P < 0.001$) (**Fig. 4**). Indeed, men with high subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity showed a

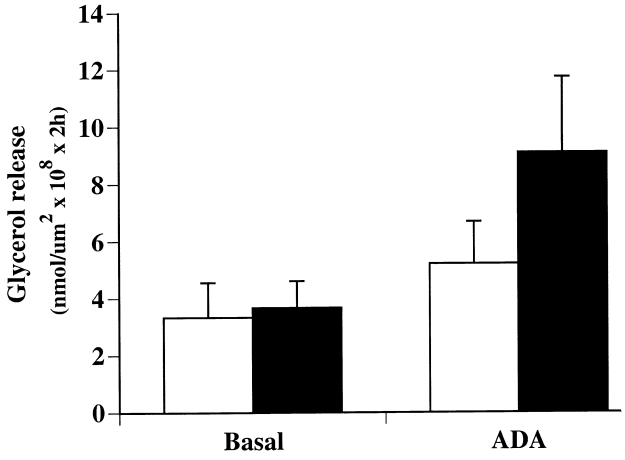

Fig. 2. Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the abdominal region of men with high (open columns) versus low (solid columns) subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE.

16-fold lower IC_{50} value than men with low α_2-adrenoceptor sensitivity. In the whole group, a positive relationship was observed between log-transformed IC_{50} (UK-14304) values and fasting TG levels, suggesting that a low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity was associated with high TG levels ($r = 0.39$, $P < 0.05$) (not shown).

To study the influence of the β-adrenoceptor component, the lipolytic effect of the β-agonist isoproterenol on basal lipolysis was also examined (Fig. 3C). No difference in the maximal lipolytic response to isoproterenol (defined at 10^{-5} M) was observed between groups. Moreover, the β-adrenergic adipose cell lipolytic sensitivity was similar in both groups (22 ± 9 vs. 51 ± 21 nM in men with high vs. low α_2-adrenoceptor adipocyte sensitivity).

Figure 5 shows TG concentrations in total as well as in large, medium, and small TRL fractions before and after the consumption of the high-fat meal. Men with high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity displayed lower values of total, medium, and small TRL fractions at 0- to 6-h time points than men with low abdominal adipocyte antilipolytic sensitivity (P values ranging from 0.01 to 0.05). However, no difference between groups was observed in the respective response above baseline values (area under incremental curve, AUIC) of the TRL fractions.

Changes in RP over the meal in total as well as in large, medium, and small TRL fractions are presented in **Fig. 6**. Although some group differences were observed in RP of large and medium TRL fractions, no difference in RP contained in total and small TRL fractions was observed between men with high versus low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Both groups of men also showed similar RP AUIC for all TRL fractions. Moreover, postprandial insulin, glucose, and FFA levels were similar in both groups (not shown).

To estimate the respective contribution of regional adiposity and subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity in accounting for variance in the AUC of TG levels in total TRL fractions, stepwise multiple regression analysis was performed. Our model included body weight, percent body fat, visceral and sub-

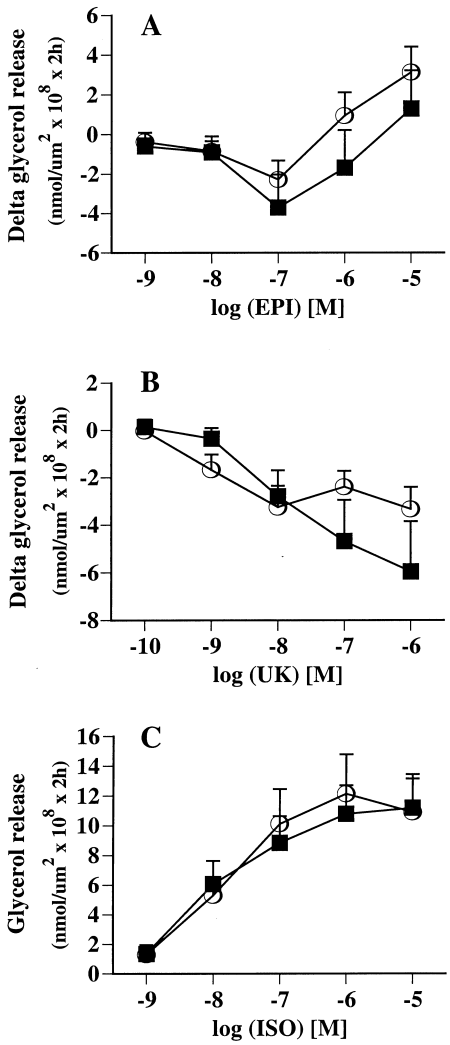

Fig. 3. (A) Effect of epinephrine (EPI) on adenosine deaminase (ADA)-stimulated lipolysis in subcutaneous abdominal adipocytes of men with high (open circles) versus low (solid squares) subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE. Glycerol release was expressed as the difference between stimulated (with EPI) and ADA values. Negative values reflect inhibition of lipolysis. (B) UK14304-induced inhibition of ADA-stimulated lipolysis in subcutaneous abdominal adipocytes of men with high (open circles) versus low (solid squares) subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE. 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 ADA values. Agonist concentrations required for half-maximal inhibition of lipolysis (IC_{50}) were determined from these dose–response curves. (C) Isoproterenol (ISO)-induced lipolysis in subcutaneous abdominal adipocytes of men with high (open circles) versus low (solid squares) subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Fat cells were incubated without ADA and values are means ± SE. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these dose-response curves.

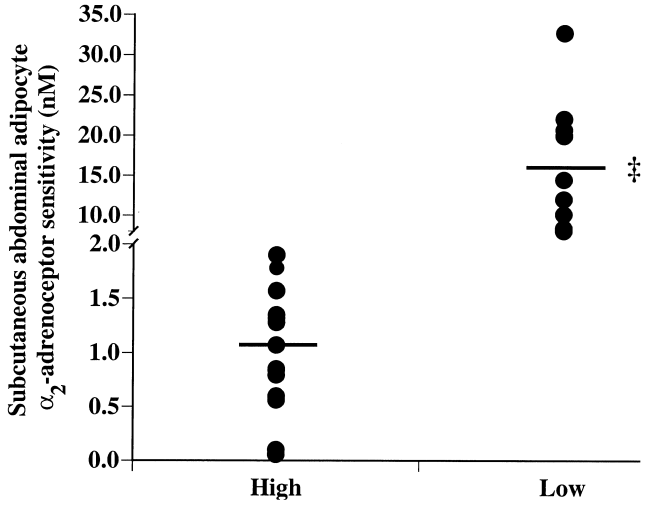

Fig. 4. Individual values of subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity of high or low sensitive men. Significant difference between groups at $^{\ddagger}P < 0.0001$. Horizontal lines represent mean values.

cutaneous abdominal adipose tissue accumulation measured by CT, fasting TG concentration, as well as subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity. Fasting TG concentration was the only variable retained in the model and accounted for 73% of the variance in the AUC of TG levels in total TRL fractions ($P < 0.0001$) (not shown).

DISCUSSION

Results of the present study suggest a potential role of subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity in the etiology of altered postprandial lipemia. Indeed, men with low antilipolytic efficiency showed higher fasting TG levels, which therefore impaired postprandial metabolism after the ingestion of a rich fat meal compared with men with a high adipose cell sensitivity to an α_2-adrenoceptor agonist. This finding is reinforced by the perfect control of factors that could have contributed to adipose tissue lipolysis variation or postprandial lipemia between our groups, such as age, body fat, visceral adipose tissue accumulation, and subcutaneous fat cell size.

We have demonstrated that an excess of visceral adipose tissue accumulation was associated with an impaired postprandial TRL clearance in men (33). The important flux of FFA to the liver from visceral adipocytes is a well-known correlate of this postprandial metabolic alteration. Although subcutaneous abdominal adipocytes do not present the same capacity as visceral fat cells to deliver FFA into the portal circulation (5, 8, 9), large subcutaneous abdominal adipocytes have also been reported to alter

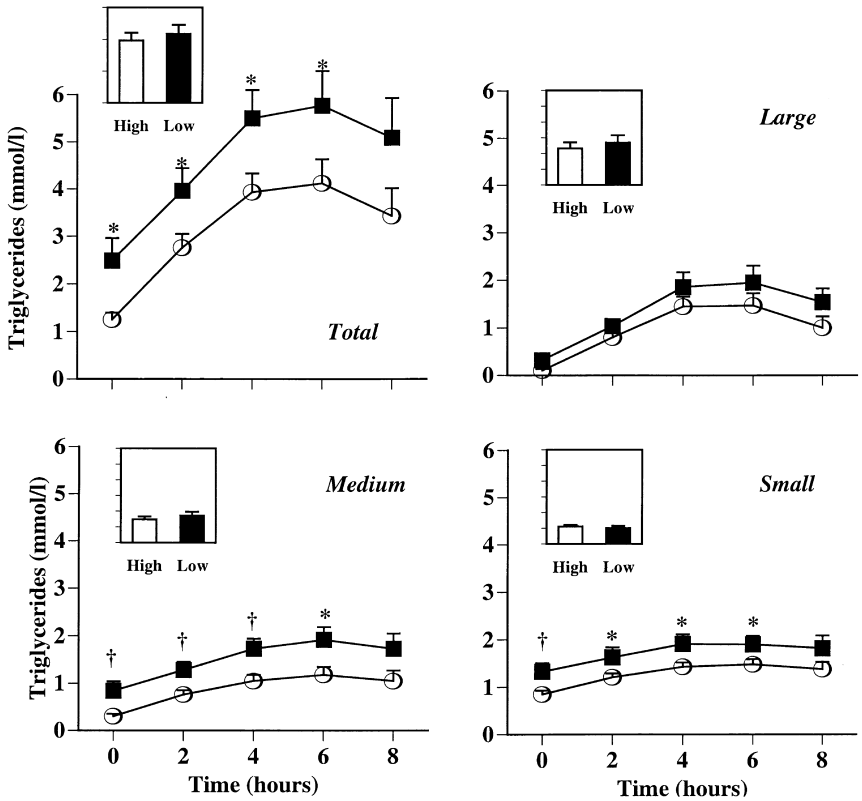

Fig. 5. Postprandial triglyceride concentrations of total, large, medium, and small TRL in men with high (open symbols; n = 15) versus low (filled symbols; n = 11) subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Columns represent the areas under incremental curves (responses) of each subgroup. Data are means ± SE. Significant difference between groups at $^{*}P < 0.05$, $^{\dagger}P < 0.01$.

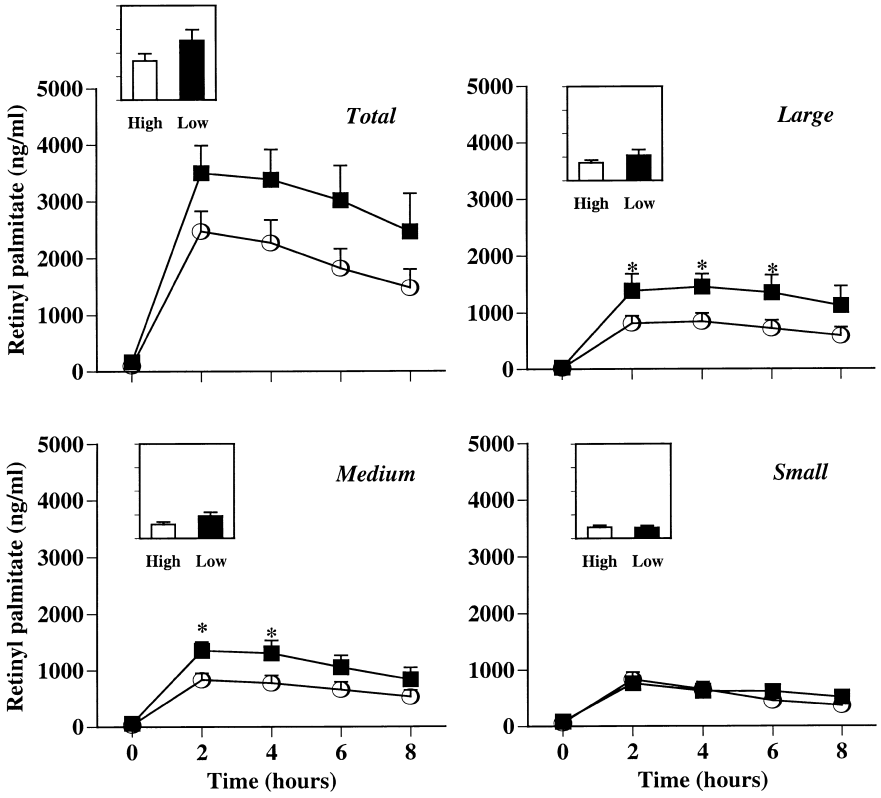

Fig. 6. Postprandial RP concentrations of total, large, medium, and small TRL in men with high (open symbols; n = 15) versus low (filled symbols; n = 11) subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Columns represent the areas under incremental curves (responses) of each subgroup. Data are means ± SE. Significant difference between groups at $^*P < 0.05$.

the metabolic risk profile in men (34). The rationale behind this finding was that large adipocytes are generally characterized by a high lipolytic rate (20, 35, 36), which could lead to an increased adipocyte-hepatocyte FFA flux and therefore to metabolic disturbances. The most striking feature of the present study was the significant contribution of subcutaneous abdominal adipocytes to fasting and therefore to postprandial metabolism of TRL fractions, even after controlling for both fat cell size and visceral adipose tissue accumulation. From a clinical standpoint, all these data suggest that an individual with a high visceral fat deposition and a low α_2-adrenoceptor subcutaneous abdominal adipocyte sensitivity may be at greater risk for alteration of postprandial lipemia.

We found that subcutaneous abdominal adipose cell lipolytic response was similar after stimulation of either α_2- or β-adrenoceptors in both groups of men. The β-adrenergic sensitivity was also comparable between men with low versus high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Taken together, these results suggest that the difference between groups observed in the subcutaneous abdominal adipocyte α_2-adrenergic sensitivity reflects principally alterations in hormone action that are located at or near the α_2-adrenoceptor level (35). Because a selective α_2-adrenergic stimulation promotes antilipolysis, it is possible that at given low catecholamine concentrations, subjects with low subcutaneous abdominal adipocyte α_2-adrenergic sensitivity display a greater fasting FFA release than men with high adipose cell α_2-antilipolytic sensitivity. Consequently, an inappropriate elevated FFA availability might result in an increased esterification of FFA and a reduced hepatic degradation of apolipoprotein B, leading to an increased synthesis and secretion of VLDL particles. This model is supported by the high fasting TG levels observed in men with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. These results are in good accordance with a previous study showing that an acute elevation of plasma FFA stimulates VLDL production in humans (37).

In the postprandial phase, men with low abdominal adipose cell α_2-adrenergic sensitivity could also be characterized by an impaired suppression of HSL activity, resulting in a continued FFA release at a time when FFA mobilization might be substantially reduced in the high α_2-adrenergic sensitivity group. This increased FFA availability could, once again, contribute to the reduced clearance of TRL fractions observed in subjects with low α_2-adrenergic sensitivity. To some extent, these results are concordant with a previous in vivo study reporting that adipose tissue HSL activity of obese subjects fails to respond to insulin in the postprandial state, a potential maladaptation in terms of lipoprotein metabolism and risk for coronary heart disease (38). On the basis of our results, we cannot exclude that the in vitro antilipolytic response of subcutaneous abdomi-

nal adipocytes to insulin was different between our groups. Further studies are needed to investigate this issue.

Another important factor explaining the release of FFA in the postprandial state is the chylomicron hydrolysis by the action of LPL in adipose tissue capillaries. As previously proposed, a good proportion of FFA release in the postprandial period originates from this pathway rather than from the intracellular action of HSL (12). On the basis of studies conducted by his team, Frayn (39) has suggested that FFA generated by LPL will flow into adipocytes only if the concentration gradient is in the appropriate direction. In other words, after an overnight fasted state, when HSL is most active, LPL-derived FFA are principally released into plasma. On the other hand, in the fed state, HSL activity is suppressed by insulin and this may favor entrapment by adipose tissue of LPL-derived FFA, leading then to fat store replenishment. On the basis of this evidence, the regulation of HSL activity appears to be an important determinant of the fate of LPL-derived FFA. Our results showed that men with low adipose cell α_2-adrenergic sensitivity display similar fasting AT-LPL as well as PH-LPL activities compared with men with high adipocyte α_2-adrenergic sensitivity. Moreover, neither group of men showed any difference in basal lipolysis, a well-known determinant of HSL activity (40, 41). Postprandially, our results are concordant with the preceding hypothesis of Frayn's group (12). Indeed, men with low abdominal fat cell α_2-adrenergic sensitivity might be characterized by an inappropriate release of FFA into the circulation due to their expected lower HSL activity suppression induced by the meal. That no difference between groups was observed in postprandial plasma FFA levels may be explained by the fact that FFA are rapidly taken up by hepatocytes and induce hepatic VLDL production, which in turn alters postprandial lipoprotein clearance, as previously reported (42). Another adipose tissue regulator of postprandial FFA metabolism that might have an impact on our observations is the acylation-stimulating protein (ASP) (43). Indeed, ASP production in adipose tissue has been related to postprandial TG clearance and uptake of FFA into adipocyte (44).

Regarding TRL RP concentrations, it is tempting to speculate that high fasting TG levels found in the low fat cell α_2-adrenergic sensitivity group may be the result of the stimulation of hepatic VLDL-TG secretion several hours after meal ingestion. Indeed, the increased TG levels in small TRL observed in men with low adipose cell α_2-adrenergic sensitivity were not accompanied by higher RP concentrations in this TRL fraction, as compared with men with high adipocyte α_2-adrenergic sensitivity. This observation supports the fact that TG from endogenous TRL, presumably VLDL particles, account for most of the increase in small TRL TG levels observed late through the postprandial period (33, 42).

In conclusion, the present study shows that men with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity show elevated postprandial TG concentrations as compared with men with high adipose cell α_2-adrenergic sensitivity. This difference is mainly due to the fact that reduced subcutaneous abdominal adipose cell α_2-adrenergic sensitivity may increase the fasting TG level, which in turn is an important predictor of postprandial TG concentration. These results confer to subcutaneous abdominal adipose cell α_2-adrenergic sensitivity an indirect role in the regulation of postprandial lipemia via its impact on fasting TG levels.

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