Acute exposure to long-chain fatty acids impairs α_2-adrenergic receptor-mediated antilipolysis in human adipose tissue

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Abstract The acute in vitro and in vivo effects of long-chain fatty acids (LCFAs) on the regulation of adrenergic lipolysis were investigated in human adipose tissue. The effect of a 2 h incubation, without or with LCFA (200 μmol/l), on basal and hormonally induced lipolysis was tested in vitro on isolated fat cells. The lipolytic response to epinephrine was enhanced by suppression of the antilipolytic α_2-adrenergic effect. Then, healthy lean and obese male subjects performed a 45 min exercise bout at 50% of their heart rate reserve either after an overnight fast or 3 h after a high-fat meal (HFM: 95% fat, 5% carbohydrates). Subcutaneous adipose tissue lipolysis was measured by microdialysis in the presence or absence of an α-antagonist (phentolamine). In vivo, a HFM increased plasma levels of nonesterified fatty acids in lean and obese subjects. In both groups, the HFM did not alter hormonal responses to exercise. Under fasting conditions, the α_2-adrenergic antilipolytic effect was more pronounced in obese than in lean subjects. The HFM totally suppressed the α_2-adrenergic antilipolytic effect in lean and obese subjects during exercise. LCFAs per se, in vitro as well as in vivo, suppress α_2-adrenergic-mediated antilipolysis in adipose tissue. LCFA-mediated suppression of antilipolytic pathways represents another mechanism whereby a high fat content in the diet might increase adipose tissue lipolysis.—Polak, J., C. Moro, D. Bessière, J. Hejnova, M. A. Marquès, M. Bajzova, M. Lafontan, F. Crampes, M. Berlan, and V. Stich. **Acute exposure to long-chain fatty acids impairs α_2-adrenergic receptor-mediated antilipolysis in human adipose tissue. *J. Lipid Res.* 2007.** 48: **2236–2246.**

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Obesity results from an imbalance between energy intake and energy expenditure. The availability of high-calorie food, mainly rich in lipids, predisposes to weight gain. The consequence of nutrition on lipolysis, particularly acute high fat intake that modifies extracellular FA levels, has not been evaluated in humans. In rodents, it has been shown that the reduction of the level of plasma NEFAs using masoprocol, a lipoxygenase inhibitor, significantly reduced isoproterenol-mediated lipolysis (1). More recently, it was shown that exposure (4–24 h) of rat fat cells to FA does not alter either insulin-stimulated glucose uptake or lipolysis (2). In this animal model, the lipotoxicity linked to FA did not appear in adipose tissue. Other investigations have demonstrated that short-chain FAs rapidly cross the fat cell plasma membrane by passive diffusion and induce an intracellular decrease in pH. Conversely, the permeation of long-chain fatty acids (LCFAs) was mediated by protein transporters at physiological concentrations. The decrease in pH modulates hormonal signaling and leads to an enhancement of triglyceride hydrolysis (3). Apart from these in vitro studies in animals, no such investigations have been conducted in humans. Human fat cells express both lipolytic β- and antilipolytic α_2-adrenergic receptors (α_2-ARs) (4, 5), whereas in rat fat cells, no functional α_2-ARs are found (6). Additionally, a new lipolytic pathway, involving the natriuretic peptides (NPs), atrial natriuretic peptides (ANPs), and brain natriuretic peptides, has been characterized in humans. It was shown that NP-induced lipolysis is a primary function specific to primate and human fat cells (7, 8). In conclu-

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sion, there is no suitable animal model in which to study the integrated regulation of lipolysis subsequent to acute dietary fat intake. In a previous study, we showed that 4 days of a high-fat diet (65% fat, 15% protein, 20% carbohydrate) impaired the α_2-adrenergic antilipolytic effect of catecholamines in human subcutaneous adipose tissue (SCAT) during exercise (9). Additionally, a 48 h incubation of human adipose tissue explants in vitro with bromopalmitate suppressed the antilipolytic α_2-adrenergic effect on lipolysis (10). Whatever the relevance of these previous observations, the effect of acute exposure to LCFAs on human fat cell lipolysis and exercise-induced lipid mobilization has not been studied to date.

The first specific aim of this study was to investigate in vitro, in human fat cells, the short-term effect (2 h) of an acute exposure to LCFAs on the well-characterized lipolytic pathways. The second specific aim was to investigate in vivo whether a high-fat meal (HFM) before exercise might change adipose tissue lipolysis in lean and obese subjects. Exercise promotes the sympathetic nervous system-mediated activation of lipid mobilization in SCAT (11). During exercise, both antilipolytic α_2-ARs and lipolytic β-adrenergic receptors are activated by catecholamines, the antilipolytic effect being higher in overweight and obese subjects (11, 12). This study was designed to evaluate lipid mobilization in lean and obese subjects and to delineate possible differences in the adrenergic regulation of lipolysis during exercise in fasting conditions or after a high fat intake that rapidly increases the concentration of NEFAs in the plasma.

MATERIALS AND METHODS

In vitro studies

Subjects. Human abdominal SCAT was obtained from eight moderately overweight females undergoing plastic surgery. Their mean age was 42.7 ± 2.1 years, and their mean body mass index was 27.0 ± 1.8 kg/m^2 (range, 24.1–31.6). The investigation protocol was approved by the Ethical Committee of Toulouse University Hospital and of the Third Medical Faculty Hospital, Charles University. Written informed consent was also obtained from all subjects before the investigation began.

Adipocyte isolation. Isolated adipocytes were obtained as described previously by Rodbell (13) using collagenase (0.5 mg/ml) digestion of adipose tissue fragments (2–3 g) in Krebs Ringer Bicarbonate buffer containing 10 mmol/l HEPES and 2% fatty acid-free BSA (KRBHA) and 6 mmol/l glucose, pH 7.4, under shaking at 100 cycles/min at 37°C for 30 min. Adipocytes were filtered through a silk mesh (250 μm) and washed three times with KRBHA buffer to eliminate collagenase.

Acute exposure of fat cells to LCFAs. The freshly isolated fat cells were divided into three batches and diluted in 3 ml of KRBHA buffer. The first batch (control) was immediately assayed in FA-free medium (2,000–3,000 cells/assay) for lipolysis. The second batch was incubated in FA-free KRBHA buffer without the addition of FAs for 2 h. The third batch was incubated with the addition of 200 μmol of LCFAs for 2 h in the FA-free KRBHA buffer. The LCFA mixture was composed mainly of saturated and ω-6 polyunsaturated fatty acids, namely lauric acid (18.3%), myristic acid (21.2%), stearic acid (21.3%), linoleic acid (20.6%), and arachidonic acid (18.7%). The type and proportion of each FA was measured by gas chromatography. After the 2 h preincubation period, the control and LCFA cell batches were washed three times with fresh buffer before lipolysis assays.

Lipolysis assays. Fat cells were suspended in a 100 μl of FA-free KRBHA medium (2,000–3,000 cells/assay). The cells were incubated with the addition of 5 μl of ANP (1 μmol/l) or isoproterenol (1 μmol/l) and also incubated with 5 μl of increasing concentrations of epinephrine (0.1, 1, and 10 μmol/l) alone or added with 5 μl of 10 μmol/l RX 821002 (a selective α_2-AR antagonist) for 90 min at 37°C under gentle shaking at 120 cycles/min. For each protocol, 30 and 10 μl aliquots of the medium were taken at the end of the incubation period for the determination of glycerol and FA, respectively, both being used as lipolytic indices. The total lipid content was determined gravimetrically after extraction according to Dole and Meinertz (14).

In vivo studies

Subjects. Eight healthy untrained lean and seven obese male volunteers (25.5 ± 0.4 and 27.3 ± 2.8 years, respectively) participated in the study. The mean body mass index was 22.8 ± 0.6 and 31.5 ± 1.1 kg/m^2 for the lean and obese subjects, respectively. All subjects were healthy and drug-free and had given their written informed consent before the experiments began. The studies were performed according to the Declaration of Helsinki and approved by the ethical committee of the Third Faculty of Medicine, Charles University.

The subjects were examined in the laboratory at 7 AM on two occasions. The two investigation days were separated by 1 week according to a crossover procedure. Subjects performed a 45 min bout of exercise on an electrically braked ergometer (Ergometrics 800s; Ergoline) at 50% of their heart rate reserve (calculated using the formula 220 − age) at 11 AM. The actual heart rate during the exercise sessions was monitored continually with a cardiometer (Polar Accurex Plus Cardiometer). Subjects were investigated on one day under fasting conditions and on the other day after an oral intake of a HFM at 8 AM containing (in percentage of total energy) 95% fat and 5% carbohydrate energy content (50% of estimated resting energy expenditure). The composition was 30% fatty cream and a calculated amount of butter added to the meal so that the caloric value was achieved. The composition of fatty acids in fatty cream and butter was quite similar (66% saturated fatty acids, 30% monounsaturated fatty acids, and 4% polyunsaturated fatty acids). The total calorie content of each meal was adapted to the body weight of each individual subject.

Microdialysis assays. The microdialysis procedure was essentially the same as that described previously (11). The subjects were placed in a semirecumbent position. Two microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20/0.5 mm and 20,000 molecular weight cutoff were inserted percutaneously after epidermal anesthesia (200 μl of 1% lidocaine; Roger-Bellon, Neuilly-sur-Seine, France) into the abdominal SCAT at a distance 10 cm lateral to the right or the left of the umbilicus. The probes were connected to a microinjection pump (Harvard Apparatus, Les Ulis, France). One probe was perfused with Ringer solution (in mmol/l: 139 sodium, 2.7 potassium, 0.9 calcium, and 140.5 chloride) and the second was perfused with Ringer plus 0.1 mmol/l phentolamine (α-AR antagonist). This nonselective α_1/α_2-antagonist, with an efficient α_2-AR antagonist action on human fat cells in vitro, was the only agent allowed by the ethical committee for use in microdialysis in humans (12). The perfusate

solutions were supplemented with ethanol (1.7 g/l). Ethanol was added to the perfusate to estimate changes in the local blood flow of SCAT, as described previously (11, 12, 15).

Exercise protocol. Two 15 min fractions of the outgoing dialysate were collected at rest and at 15, 30, and 45 min after the beginning of the physical exercise of 45 min at a power level corresponding to 50% of the maximal oxygen consumption on a cycle ergometer. Blood samples for plasma analysis were collected at rest and at 30 and 45 min after the beginning of the physical exercise from an indwelling polyethylene catheter inserted into an antecubital vein. Blood was collected into 50 μl of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseille, France) to prevent catecholamine oxidation and processed immediately in a refrigerated centrifuge. The plasma was stored at −80°C until analysis.

Drugs and analytical methods

Isoproterenol hydrochloride, BSA, and crude collagenase were obtained from Sigma Chemical (Paris, France). Phentolamine methanesulfonate (Regitine) was obtained from Cia-Geigy (Rueil-Malmaison, France). RX 821002 was a gift from Reckitt and Coleman (Kingstown-upon-Hull, UK). Human ANP (1–27) was from Neosystem (Stasbourg, France). For the in vitro study, fatty acids were obtained from Sigma, EU (animal component-free). Glycerol in the dialysate and in plasma was analyzed by an enzymatic method (Sigma, St. Louis, MO). Ethanol in the dialysate and perfusate (5 μl) was also determined with an enzymatic method (16). Plasma glucose was determined with a glucose-oxidase technique (Biotrol kit; Merck-Clevenot, Nogent-sur-Marne, France) and nonesterified fatty acids by an enzymatic procedure (Wako kit; Unipath, Dardilly, France). Plasma insulin concentrations were measured using enzymoimmunoassay kits from Mercodia (Sweden). Plasma epinephrine and norepinephrine were measured in 1 ml aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection. The detection limit was 20 pg per sample.

Data analysis

Values are given as means ± SEM. The significance of differences was assessed using Student's paired *t*-test and ANOVA with Bonferroni's and Student-Newman-Keuls tests for post hoc analysis. Significance values are given in the text and figures. $P < 0.05$ was considered statistically significant. All calculations were performed using software statistical packages (Superanova and Statview, Abacus Concepts, Inc., Berkeley, CA).

RESULTS

In vitro studies

This study was performed to investigate whether pretreatment of isolated fat cells with LCFA induces modifications of α_2-adrenergic, β-adrenergic, and ANP-mediated lipolytic responses. After 2 h of incubation, the medium contained 101 ± 67 μmol/l NEFA in the medium initially deprived of FA and 372 ± 127 μmol/l in the medium initially supplemented with 200 μmol/l LCFA, respectively. It was observed that 2 h of preincubation in medium without FA or enriched with 200 μmol/l LCFA activated the spontaneous lipolysis (evaluated by the measurement of glycerol and NEFA released in the incubation medium, the spontaneous lipolysis being greater in the medium previously supplemented with 200 μM FA) (**Figs. 1–3**). The degree of stimulation of lipolysis by 1 μmol/l isoproterenol or ANP was not significantly affected. For example, the isoproterenol-stimulated increase in NEFA was 1.17 ± 0.27 and 0.95 ± 0.35 μmol/100 mg lipid in controls (without LCFA) or after 2 h of preincubation with 200 μM LCFA, respectively. Under similar experimental conditions, the increase in ANP-induced NEFA was not different (0.99 ± 0.47 and 0.83 ± 0.27 μmol/100 mg lipid).

When fat cells were immediately incubated in a FA-free medium, we observed an expected enhancement of the lipolytic effect of epinephrine by the selective α_2-AR antagonist, RX 821002 (6). On the contrary, this potentiating effect of RX 821002 was not observed when fat cells were preincubated for 2 h in a medium without LCFA (but enriched by NEFA released during preincubation) or FA-enriched with 200 μmol/l LCFA (Figs. 2, 3) (17).

Because adenosine is an important regulatory agent of adipose tissue metabolism through the inhibition of lipolysis, we also evaluated the inhibitory effect of the reference A1-adenosine receptor agonist, phenylisopropyladenosine (PIA). To increase basal lipolysis to test the inhibiting effect of PIA, inhibition of lipolysis initiated by adenosine deaminase (4 μg/ml), an enzyme hydrolyzing the free adenosine spontaneously released by isolated adipocytes, was tested. Concentration-dependent changes in the NEFA and glycerol concentrations in the incubation medium were determined. Whatever the experimental condition, spontaneous lipolysis under adenosine deaminase was increased after the preincubation with or without LCFA added to the medium. Increasing concentrations of PIA similarly inhibited the adenosine deaminase-induced lipol-

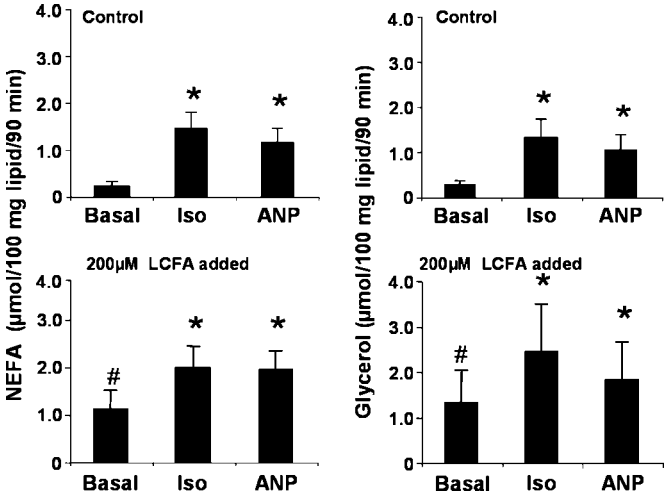

Fig. 1. Effects of incubation of human fat cells for 2 h with 200 μM long-chain fatty acids (LCFAs) on lipolysis induced by 1 μM isoproterenol (Iso) and 1 μM atrial natriuretic peptides (ANPs), compared with unincubated controls. After the 2 h incubation period, cells were washed. In both case, lipolysis was performed for 90 min in a NEFA-free medium. Data are expressed as means ± SEM of eight separate experiments. # $P < 0.05$ compared with basal control values; * $P < 0.05$ compared with basal values.

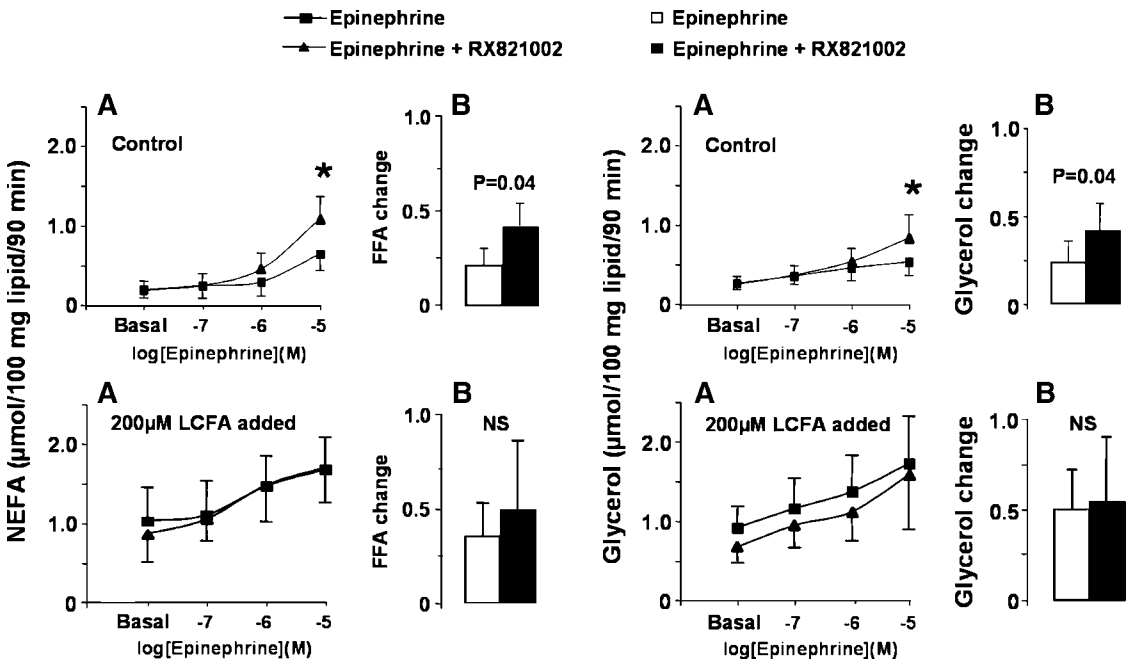

Fig. 2. Effects of 2 h of incubation with 200 µM LCFA on lipolysis in human fat cells, compared with unincubated control cells. After the 2 h incubation period with LCFA, cells were washed. In both cases, lipolysis was performed for 90 min in a NEFA-free medium. Lipolysis was induced by increasing concentrations of epinephrine alone or with 10 µM RX 821002, a selective α_2-adrenergic receptor (α_2-AR) antagonist. A: Effects of increasing concentrations of epinephrine alone or with RX 821002 on lipolysis. B: Change with epinephrine alone or associated with RX 821002 on lipolysis, calculated as the mean increase induced by increasing concentrations of epinephrine minus the basal lipolysis. Data are expressed as means ± SEM of eight separate experiments. * $P < 0.05$ compared with values obtained with epinephrine alone.

ysis in the control study or after 2 h of incubation with 200 µmol/l LCFA (**Fig. 4**).

In vivo studies

Effects of a HFM on plasma parameters. **Table 1** lists the changes in the plasma concentrations of insulin, glucose, glycerol, and NEFA during the 3 h after the high fat intake in lean and obese subjects. During fasting, plasma NEFA and glycerol concentrations were higher in obese than in lean subjects. Three hours after ingestion of the HFM, plasma NEFA levels increased up to 3-fold in both groups ($P = 0.003$). Plasma glycerol values increased after the HFM, with the increase being more pronounced in obese than in lean subjects. Plasma insulin levels were not changed significantly, and glucose concentrations increased slightly, but not significantly, after 120 and 180 min.

Effects of exercise on plasma parameters during fasting and after a HFM. **Figure 5** depicts the time course of plasma glycerol and NEFA concentrations during exercise. In lean subjects, during fasting and after the HFM, plasma glycerol levels increased similarly during exercise, whereas plasma NEFA concentrations did not change in the fasted situation but were reduced slightly at 30 min after the beginning of the exercise after the HFM. In obese subjects, plasma glycerol concentrations increased moderately during exercise in both nutritional conditions. Plasma NEFA decreased at only 45 min after the beginning of the exercise under fasting or after the HFM.

In lean subjects, the HFM did not significantly modify the time course of norepinephrine and epinephrine changes during exercise (**Table 2**). Exercise promoted a similar decrease in plasma insulin in both nutritional conditions in lean subjects. A significant decrease in plasma insulin was observed only after the HFM in obese subjects. Finally, no change in plasma glucose levels was observed in either nutritional condition.

Effects of exercise on SCAT lipolysis in lean and obese subjects. Baseline lipolysis, reflected by the baseline dialysate glycerol concentration (DGC), was similar in lean subjects during fasting and after the HFM (55.6 ± 6.1 and 61.2 ± 8.2 µmol/l, respectively). Baseline lipolysis was identical as well after the HFM in the probe perfused with the α_2-AR antagonist phentolamine (50.6 ± 5.1 and 55.3 ± 10.9 µmol/l, respectively) (**Fig. 6**). Lipolysis increased during exercise regardless of the nutritional condition. However, the exercise-induced lipolysis was enhanced under α-adrenergic blockade ($P = 0.04$) during fasting, but that potentiating effect disappeared after the HFM (Fig. 6).

In obese subjects, baseline lipolysis measured in the control probe was identical (67.3 ± 14.9 and 75.5 ± 9.4 µmol/l, respectively) during fasting and after the HFM. During fasting, the exercise-induced increment of lipolysis was strongly potentiated under α_2-AR blockade by phentolamine ($P = 0.005$) (**Fig. 7**). On the contrary, after the HFM intake, exercise-induced lipolysis was greater than that observed under fasting conditions. Moreover, the en-

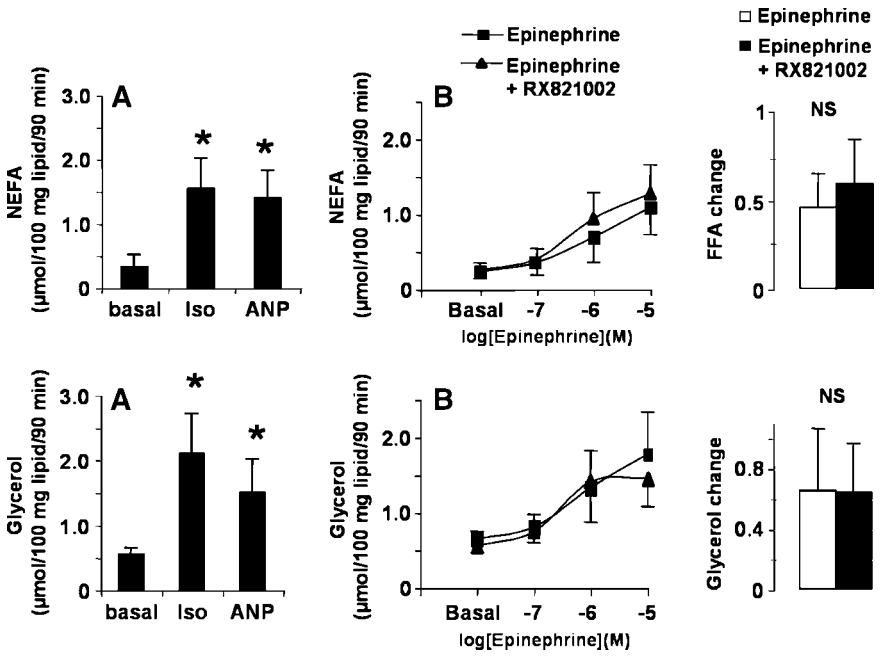

Fig. 3. Effects of 2 h of incubation of human fat cells without LCFA on in vitro lipolysis. Lipolysis was induced by 1 μM isoproterenol (Iso) and 1 μM ANP and with increasing concentrations of epinephrine alone or with 10 μM RX 821002. A: Effects of isoproterenol and ANP on lipolysis. B: Change with epinephrine alone or associated with RX 821002 on lipolysis, calculated as the mean increase induced by increasing concentrations of epinephrine minus the basal lipolysis. Data are expressed as means ± SEM of eight separate experiments. * $P < 0.05$ compared with basal values.

hancing effect of phentolamine on exercise-induced lipolysis was totally blunted after the HFM intake (Fig. 7).

Blood flow in SCAT during exercise. Changes in adipose tissue blood flow occurring in the SCAT microcirculation were evaluated using the method based on the measurement of ethanol escape from the microdialysis probes. The ethanol outflow-to-inflow ratio was calculated by the formula ethanol concentration in the dialysate ÷ ethanol concentration in the perfusate × 100.

At rest, in fasting conditions or after the HFM, the mean average ethanol ratio did not differ in the control probe in lean or obese subjects (72.8 ± 4.7 and 71.5 ± 4.2, and 79.8 ± 4.6 and 80.1 ± 5.6, in lean and obese, respectively). The addition of phentolamine did not modify the ethanol ratio found in fasting conditions or after the HFM in lean or obese subjects (73.7 ± 4.7 and 73.4 ± 6.8, and 84.5 ± 3.9 and 78.9 ± 2.8, in lean and obese, respectively). The adipose tissue blood flow did not change significantly during exercise in either lean or obese subjects.

DISCUSSION

In this study, we investigated the effect of FA on the antilipolytic α_2-AR-dependent effect in human adipose tissue in vitro on adipocytes obtained from moderately overweight women and in vivo using a microdialysis technique. The regulatory balance between β- and α_2-adrenergic effects was investigated in lean and obese subjects during exercise.

In vitro experiments on human fat cells showed that the spontaneous (i.e., FA accumulation during the control incubation period) or experimental enrichment of the incubating medium with FA during 2 h is associated with an alteration in the lipolytic response to catecholamines. An increase in spontaneous lipolysis is observed, as well as a suppression of the antilipolytic α_2-adrenergic effect. Other lipolytic pathways (i.e., lipolysis induced by isoproterenol or ANP) were unchanged. The in vivo study showed that an intake of a HFM at 3 h before exercise promoted an increase in resting plasma NEFA and glycerol levels. Throughout the subsequent exercise period, the HFM affects the exercise-induced lipolysis in SCAT by suppressing the antilipolytic α_2-adrenergic response in lean subjects. The loss of the α_2-adrenergic response is more noticeable in obese subjects, because a potent α_2-adrenergic effect is observed under control conditions.

In a previous study using competition binding studies on adipocyte membranes, we showed that bromopalmitate significantly altered the binding characteristics and affinity of epinephrine for α_2-ARs in human adipocytes after a 24 h incubation of adipose tissue explants with FA. Such data suggested that there was a reduction of the coupling efficiency of α_2-ARs to downstream pathways, leading to a concomitant reduction of the α_2-AR-dependent response of the fat cells. Fatty acids exert a negative effect on the expression and/or activity of Gi proteins, involved in a coupling of α_2-AR to adenylyl cyclase and an inhibition of lipolysis (10). The present study revealed that such an effect appears even after a short-term exposure (2 h) to FA,

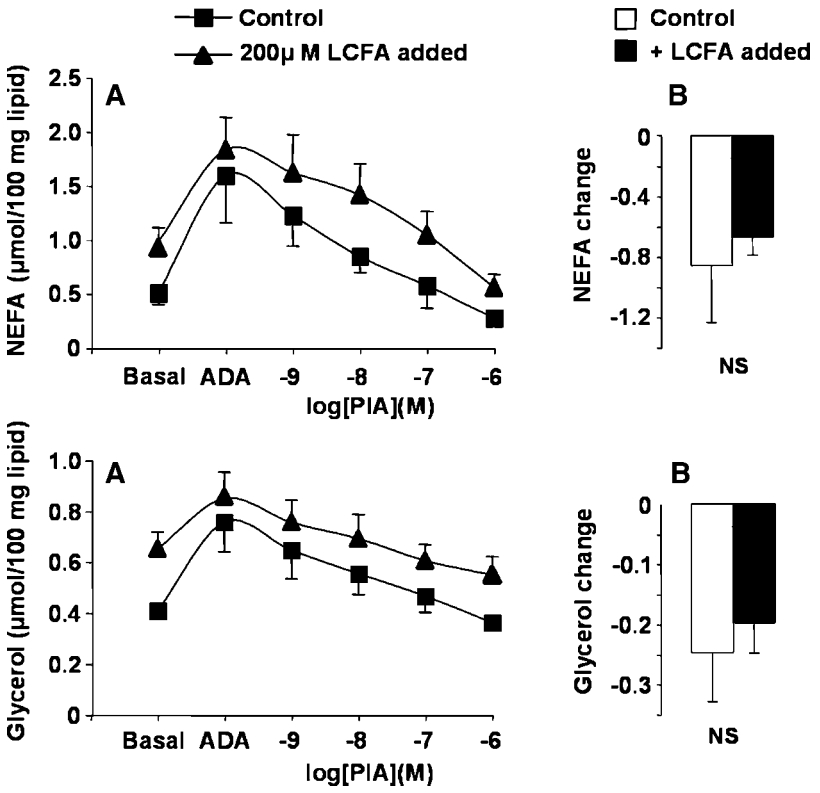

Fig. 4. Inhibitory effects of phenylisopropyladenosine (PIA) on lipolysis induced by 4 μg/100 μl adenosine deaminase (ADA) in human fat cells after 2 h of incubation with 200 μM LCFA, compared with unincubated controls. A: Effects of PIA. B: Change induced by PIA, calculated as the mean decrease induced by increasing concentrations of PIA minus the basal lipolysis. Data are expressed as means ± SEM of six separate experiments.

because the α_2-AR-mediated antilipolytic effect was totally suppressed. As a consequence, the lipolytic effect of epinephrine was enhanced (Fig. 2). A similar result was observed when fat cells were preincubated for 2 h without added FA in the medium (Fig. 3). In this situation, fat cells spontaneously released FA in the medium. The amount of released FA (101 ± 67 μmol/l) was sufficient to suppress the α_2-AR-dependent antilipolytic effect. A previous study showed the composition of the FA released by human fat cells under isoproterenol stimulation (18). It was found that the released FA was a mixture containing 33.8% saturated FA. The epinephrine infusion mainly increased palmitate and oleate (19).

Alterations in fat metabolism are important in the development of obesity. This has been shown in rats: in obesity-prone animals, high-fat diets induced a model of obesity; on the contrary, obesity-resistant animals submitted to a similar high-fat diet did not develop obesity (20).

TABLE 1. Plasma values of insulin, glucose, glycerol, and NEFA after a HFM in lean and obese subjects at rest

	Time				
Parameter	0 min	60 min	120 min	180 min	240 min
Lean					
Insulin (μU/ml)	4.9 ± 0.5	4.8 ± 0.4	5.7 ± 0.6	5.8 ± 0.4	6.4 ± 0.5
Glucose (mmol/l)	3.8 ± 0.4	3.7 ± 0.3	4.0 ± 0.3	4.3 ± 0.7	4.5 ± 0.4
Glycerol (μmol/ml)	83 ± 40	83 ± 25	91 ± 28	93 ± 29[a]	114 ± 39[a]
NEFA (μmol/ml)	202 ± 73	195 ± 52	348 ± 74[a]	513 ± 133[a]	587 ± 142[a]
Obese					
Insulin (μU/ml)	7.1 ± 1.3	7.2 ± 0.9	6.8 ± 1.4	9.1 ± 3.3	9.2 ± 3.4
Glucose (mmol/l)	4.6 ± 0.2	4.4 ± 0.1	5.0 ± 0.5	4.9 ± 0.3	5.1 ± 0.4
Glycerol (μmol/ml)	140 ± 18[b]	145.5 ± 19[b]	230 ± 32[a,b]	314 ± 59[a,b]	295 ± 43[a,b]
NEFA (μmol/ml)	322 ± 44[b]	393 ± 75[b]	449 ± 51[a,b]	743 ± 70[a,b]	768 ± 85[a,b]

HFM, high-fat meal. The HFM was taken at 60 min. Values are means ± SEM of eight separate determinations.
[a] $P < 0.05$ compared with 0 min.
[b] Significant compared with lean subjects.

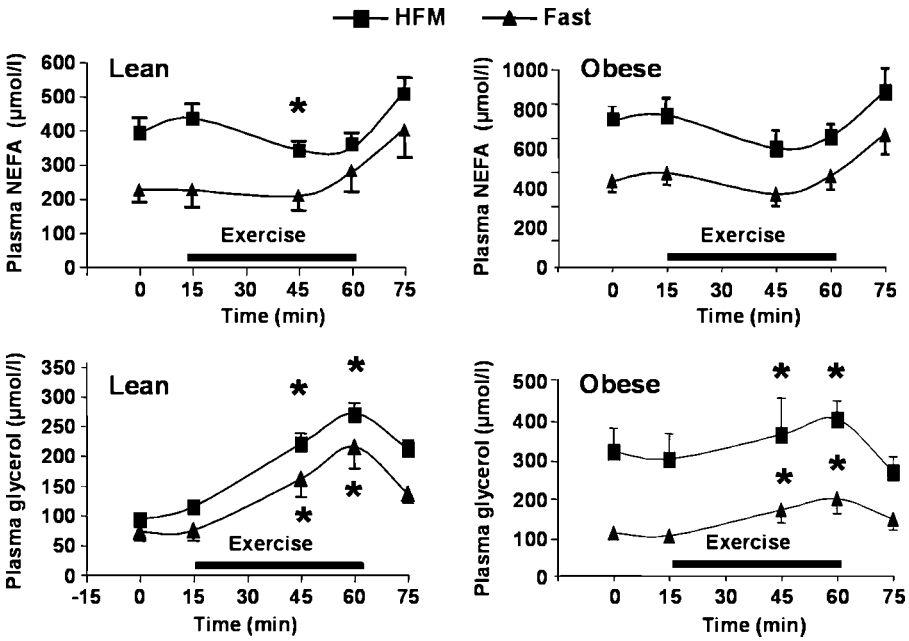

Fig. 5. Plasma glycerol and NEFA concentrations measured at rest, during exercise, and in the recovery period in fasting conditions or 3 h after a high-fat meal (HFM). Data are expressed as means ± SEM of eight (lean) and seven (obese) separate experiments. * $P < 0.02$ compared with values measured at rest.

Fatty acids can pass rapidly through the fat cell plasma membrane by passive diffusion, and the mobilization of triglycerides during stimulatory conditions is linked to the presence of adipocyte lipid binding protein (ALBP; also known as aP2). Coe, Simpson, and Bernlohr (21) have shown that disruption of the aP2 gene impairs fat cell lipolysis and increases FA levels. Thus, it can be postulated that in humans, the level of aP2 can modify the trafficking

TABLE 2. Effects of 45 min of exercise and recovery on plasma insulin, glucose, and catecholamine concentrations under fasting conditions and 3 h after ingestion of a HFM in lean and obese subjects

Parameter	Rest	Exercise	Recovery		
Time (min)	0	15	45	60	75
Lean					
Insulin (μU/ml)					
Fast	6.1 ± 0.6	5.7 ± 0.5	3.9 ± 0.2[a]	4.9 ± 0.4[a]	5.6 ± 0.7
HFM	6.4 ± 0.6	6.4 ± 0.6	5.3 ± 0.4[a]	4.5 ± 0.1[a]	6.9 ± 0.6
Glucose (mmol/l)					
Fast	5.0 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	4.6 ± 0.2	4.6 ± 0.3
HFM	4.8 ± 0.1	4.8 ± 0.2	4.8 ± 0.2	4.9 ± 0.1	4.7 ± 0.1
Norepinephrine (pg/ml)					
Fast	ND	294 ± 29	828 ± 66[a]	901 ± 75[a]	332 ± 34
HFM	ND	281 ± 25	944 ± 123[a]	941 ± 124[a]	320 ± 62
Epinephrine (pg/ml)					
Fast	ND	62 ± 3	91 ± 7[a]	109 ± 9[a]	65 ± 2
HFM	ND	67 ± 4	107 ± 11[a]	118 ± 25[a]	74 ± 5
Obese					
Insulin (μU/ml)					
Fast	7.2 ± 1.1	7.3 ± 1.1	6.3 ± 1.0	7.3 ± 1.6	12.1 ± 4.3
HFM	9.1 ± 3.1	9.2 ± 3.4	6.2 ± 1.73[a]	6.4 ± 1.9[a]	9.2 ± 1.3
Glucose (mmol/l)					
Fast	4.2 ± 0.2	4.3 ± 0.2	4.2 ± 0.1	4.1 ± 0.3	4.5 ± 0.1
HFM	4.9 ± 0.3	5.1 ± 0.3	4.8 ± 0.4	4.9 ± 0.4	5.0 ± 0.2
Norepinephrine (pg/ml)					
Fast	ND	301 ± 32	888 ± 79[a]	987 ± 66[a]	286 ± 37
HFM	ND	324 ± 45	864 ± 121[a]	902 ± 79[a]	349 ± 82
Epinephrine (pg/ml)					
Fast	ND	65 ± 5	130 ± 18[a]	155 ± 21[a]	61 ± 19
HFM	ND	63 ± 12	114 ± 16[a]	147 ± 16[a]	76 ± 20

Values are means ± SEM of eight separate determinations.
[a] $P < 0.05$; significant compared with rest values (15 min).

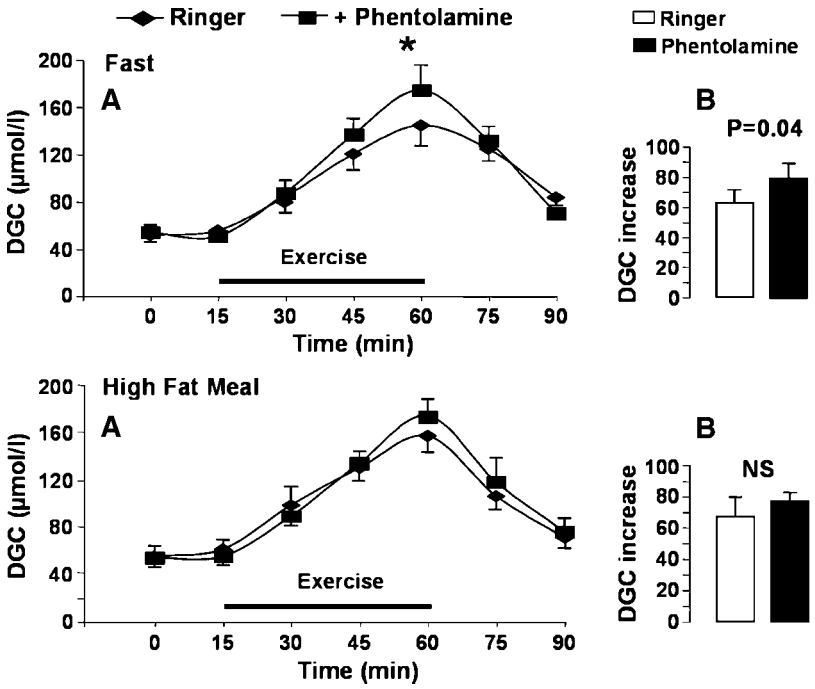

Fig. 6. Dialysate glycerol concentration (DGC) in subcutaneous adipose tissue (SCAT) at rest, during exercise, and during recovery in the control probe (Ringer) or in the probe infused with 0.1 mmol/l phentolamine under fasting conditions or 3 h after a HFM in lean subjects. Data are expressed as means ± SEM of eight separate experiments. A: DGC values. * $P < 0.05$ compared with values measured in the control probe. B: Increase in DGC during exercise. *P* is given compared with values measured in the control probe.

of fatty acids and could finally induce obesity. The decrease in intracellular pH as a consequence of the rapid increase in FA in fat cells could enhance the spontaneous hydrolysis of intracellular triglycerides (3). This point is confirmed in the present study performed on human adipocytes, because spontaneous lipolysis was considerably enhanced after incubation with FA (Figs. 1–3). Nevertheless, it cannot be excluded that an excess of LCFA in the cells via a FA-transporter activity (e.g., CD36) during preincubation could further alter transport activity and augment the accumulation of NEFAs in the incubation medium.

We also studied in vitro the effect of a medium enriched with LCFA on the modifications of the lipolysis pathways in human adipocytes. The results presented in Fig. 2 show that when LCFAs were added to the medium, basal lipolysis was increased but the lipolytic effect of isoproterenol and ANP remained unaffected. However, it was found that the lipolytic effect of epinephrine was enhanced compared with control conditions. The α_2-AR-dependent inhibitory effect (i.e., leading to a reduced lipolytic action of epinephrine) was suppressed, and as a consequence, the epinephrine effect was enhanced. These results show that short-term increases in LCFA only affected the α_2-AR-mediated antilipolytic action of catecholamines. As observed previously with 24 h incubations of fat cells with bromopalmitate (10), LCFA could exert acute actions that only affect the antilipolytic α_2-ARs known to be associated with a Gi protein (the Gi subunit was not determined). Altered coupling of α_2-ARs to Gi could be proposed, as seen previously (10). All of the fat cell receptors coupled to Gi and inhibition of lipolysis do not behave similarly. This is supported by the fact that the antilipolytic effect of the adenosine A1-receptor agonist PIA was not affected by the 2 h incubation of adipocytes with 200 µmol/l LCFA. It is known that the coupling efficiency between inhibitory receptors and Gi subunits can differ according to the nature of the receptor and the αi subunit of Gi involved in the transduction of the effect (6). Apparently, LCFA treatment did not alter the Gi-dependent pathways in human fat cells in a similar way.

The microdialysis method is used to monitor local lipid mobilization in SCAT. It is a suitable method available to perform mechanistic explorations of adipose tissue function in vivo. Exercise promotes both sympathetic nervous system activation and NP release (8), two factors involved in the increase in lipolysis in human fat cells.

In situ investigations of SCAT lipolysis were carried out using microdialysis, and its regulation at rest and during exercise was studied with the use of a pharmacological antagonist compound targeting the α_2-AR (i.e., phentolamine). Our study in vivo showed that plasma NEFA concentrations increased considerably at 3 h after a HFM in lean as well as obese subjects (Table 1). Exercise promoted an increase in DGC in the control probe. Local infusion of phentolamine in the dialysis probe potentiated the increase in DGC during exercise only in the fasting state in lean subjects (Fig. 6) and, with greater response, in obese subjects (Fig. 7). This result confirms previous reports showing the involvement of the antilipolytic α_2-ARs in the regulation of exercise-induced lipolysis in men (11, 12).

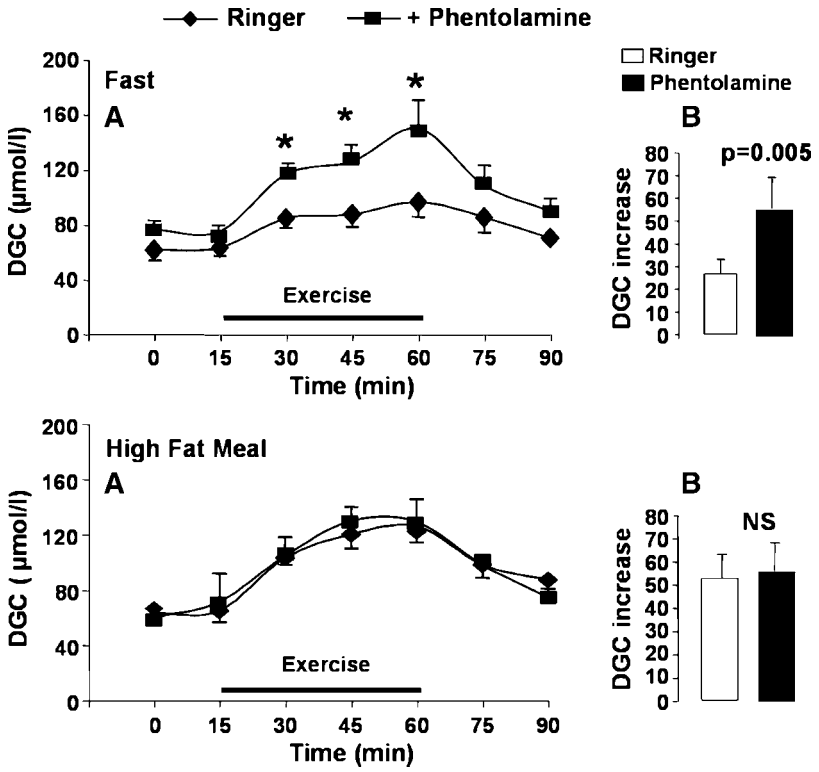

Fig. 7. DGC in SCAT at rest, during exercise, and during recovery in the control probe (Ringer) or in the probe infused with 0.1 mmol/l phentolamine under fasting conditions or 3 h after a HFM in obese subjects. Data are expressed as means ± SEM of seven separate experiments. A: DGC values. * $P < 0.05$ compared with values measured in the control probe. B: Increase in DGC during exercise. *P* is given compared with values measured in the control probe.

Epinephrine exhibits a high affinity for α_2-ARs and activates the antilipolytic α_2-AR in SCAT during exercise. This mechanism is favored in the SCAT of obese people in whom exercise-induced lipolysis in SCAT is severely impaired by the activation of α_2-ARs (12). Our results show that the increase in plasma NEFA resulting from the HFM influences exercise-induced lipolysis in the SCAT of normal-weight and obese subjects; a striking effect was observed in the obese subjects (Fig. 7). Our interpretation is that the acute high fat intake, by promoting an increase in NEFA, modifies the interplay between the antilipolytic α_2- and lipolytic β-adrenergic receptors at the level of the fat cell plasma membrane in SCAT. When considering the results obtained in obese subjects, exercise-induced glycerol production from SCAT was very weak in the fasting state, and the blockade of α_2-ARs by phentolamine considerably increased glycerol production. The main result is that when plasma NEFA increased after a HFM, lipolytic responsiveness is increased in SCAT and phentolamine does not potentiate the exercise-induced glycerol release. The efficacy of the α_2-AR antagonist is lost as a result of weakening of the α_2-adrenergic responsiveness (Fig. 7). Therefore, in agreement with the in vitro studies, it could be postulated that the NEFA increase interferes with exercise-induced responses, leading to suppression of the antilipolytic α_2-adrenergic effect in the SCAT of lean and obese patients.

A previous study has shown that in nonobese subjects subjected to a 4 day high-fat diet, lipolysis was enhanced during exercise, whereas no changes were observed in the in situ responsiveness of SCAT to stimulation by local infusion of isoproterenol (9). These results show that a high-fat diet did not alter the β-AR lipolytic pathway. However, in these conditions, a part of the higher lipolytic response to exercise was also attributable to a greater enhancement of the catecholamine response and to lower plasma insulin levels (10). It was concluded that by selectively influencing α_2-AR sensitivity, 4 days of a high-fat diet modified the balance between β-AR-dependent activation and α_2-AR-dependent inhibition of lipolysis, leading to an alteration in the mobilization of stored fat.

We have shown here that the overall lipolytic processes were unaffected by an acute high fat intake, because the increase in plasma glycerol during the exercise bout was similar at 3 h after the HFM compared with that observed under fasting conditions. Concerning plasma parameters, plasma NEFA levels increased at 3 h after the HFM in both groups (Table 1). Increases in plasma levels of NEFA and glycerol after a HFM reflect the increased hydrolysis of triglycerides in chylomicrons, whose plasma levels increase after a HFM. In the presence of an increased inflow of dietary triglycerides, not all NEFAs mobilized from triglycerides are trapped in the adipose tissue and leak in the circulation (22). Plasma glycerol increased moderately in

lean subjects, but the increase was more pronounced in obese subjects. The fact that obese subjects have higher plasma glycerol could be explained by a low reuptake of plasma glycerol in these subjects. Finally, from these results on plasma levels, it is suggested that an increase in plasma NEFA induced by an acute high fat intake did not alter the whole exercise-induced lipolysis but only suppressed the adrenergic α_2-antilipolytic effect.

Fatty acids can pass rapidly through the fat cell plasma membrane, and the mobilization of lipids during stimulatory conditions is linked to the presence of ALBP; therefore, it is suspected that fatty acids exert a negative effect on α_2-AR coupling. Fisher et al. (23, 24) found that ALBP was differentially expressed in lean and obese subjects, suggesting an important role of this transporter in the development of obesity. During exercise, α_2-AR stimulation strongly blunts lipolysis in obese SCAT (12), as was found in this study. It can be suggested that this α_2-AR-dependent antilipolytic effect exercises a protective action against excessive lipolysis: a limitation of lipolysis prevents an increase in plasma NEFA levels (25). Insulin-resistant obese subjects have an enlarged fat mass, often leading to increased plasma fatty acids levels. Altered storage capacities of the adipocyte and altered buffering capacities of adipose tissue could lead to NEFA overflow into muscle and pancreas β-cells and increase insulin resistance (26). Increased plasma NEFA levels are a risk factor for the development of type 2 diabetes mellitus (25). The accurate control of lipolysis is essential to keep a normal level of plasma NEFA (22, 27).

The major impact of our study is to show that an acute high fat intake alters the protective function of α_2-ARs against increased lipolysis in obese subjects. These patients normally exhibit a major spontaneous antilipolytic α_2-adrenergic effect in fat cells [i.e., as revealed during a calibrated exercise bout (present study and Ref. 12)]. Saturated and unsaturated fatty acids are capable of exerting clear-cut different metabolic actions in in vitro and in vivo studies. It is unknown whether such an effect could be extended to other tissues expressing α_2-ARs, such as vessel muscle cells and/or myocardium, which are the putative target tissues for adrenoceptor-blocking antihypertensive drugs. Following on from our results, it will be interesting to evaluate the effect of the intake of selected meals with the modulation of saturated and unsaturated fatty acid content. The regulation of exercise-induced lipolysis in lean and obese subjects could be used as a functional test to delineate whether the fatty acid composition of a HFM is able to modulate the effects described here. Preservation of α_2-adrenergic responsiveness in human fat cells will represent a major benefit to avoid the deleterious effects of plasma NEFAs.

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