In vitro and in vivo impairment of alpha2-adrenergic receptor-dependent antilipolysis by fatty acids in human adipose tissue.

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ABSTRACT

The aim of the present study was to study the influence of fatty acids on adrenergic control of lipolysis both *in vitro* and *in vivo*. Human subcutaneous adipose tissue explants were cultured for 48h in the presence of 100 µM bromopalmitate (BrPal), and lipolysis was measured in isolated adipocytes. In control conditions, β-AR-dependent activation of lipolysis by epinephrine was almost undetectable, and could fully be restored by pharmacological blockade of α2–AR-dependent antilipolysis. After BrPal treatment, epinephrine became fully lipolytic and was no more influenced by α2–AR-blockade. Radioligand binding analysis revealed that BrPal treatment led to a significant reduction in the coupling of α2–AR to G proteins. In parallel, a chronic and significant increase in plasma fatty acids resulting from a 4 days high fat diet (HFD), was accompanied by an impairment of the amplifying effect of the α2–AR antagonist phentolamine on exercise-induced lipolysis (measured in the subcutaneous adipose tissue with the use of a microdialysis probe) normally observed after a low fat diet. In conclusion, *in vitro* and *in vivo* studies showed that fatty acids impairs α2–AR-dependent antilipolysis.

INTRODUCTION

Catecholamines (epinephrine and norepinephrine) are important regulators of adipocyte lipolysis via activation of adrenergic receptors (AR) that couple positively (β−AR) and negatively (α2−AR) to adenylyl cyclase and hormone-sensitive lipase [16]. In humans, adipocytes isolated from subcutaneous adipose tissue predominantly express α2−ARs over β−ARs. This is characterised by a weak lipolytic response of adipocytes to catecholamines which can be overcome by pharmacological blockade of α2−ARs with specific antagonists such as phentolamine or RX821002 [15]. This tonic inhibitory effect of α2−ARs on lipolysis, which was initially demonstrated *in vitro*, has recently been confirmed *in vivo* by using the microdialysis method [19, 20]. This method allows direct measurement of glycerol in the extracellular compartment of adipose tissue with the use of a microdialysis probe perfused with a physiological fluid containing or not pharmacological agents [1]. With such a method, it was recently demonstrated that perfusion of the α2−AR antagonist phentolamine in microdialysis probe strongly enhanced the lipolytic response generated by an exercise-induced activation of the sympathetic nervous system [19, 20]. In addition, the enhancing effect of phentolamine on exercise-induced lipolysis was more pronounced in obese versus lean subjects [19]. These investigations clearly demonstrated the tonic inhibitory action of α2−AR stimulation *in vivo*, on lipid mobilisation of fat stored in adipose tissue particularly in obese people.

Whereas the physiopathological importance of the balance between β− and α2−AR in adrenergic control of lipolysis has been clearly established, hormonal or nutritional factors controlling this balance remains poorly defined in human. It has been shown that exercise-induced release of glycerol in plasma was enhanced after 5 days of low carbohydrate-high fat diet as compared to a low fat-high carbohydrate diet [13]. However, in this previous study, the lipolytic response of the adipocytes to catecholamines, and the active components of the diets leading to an alteration of exercise-induced lipolysis, were not explored. Fatty

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acids being suspected to play a predominant role in metabolic and trophic adaptations of the organism to a high fat diet [14], we attempted to test their possible influence on adrenergic control of adipocyte lipolysis. This was performed by a double approach based on utilisation of human adipose tissue culture and *in situ* microdialysis. *In vitro* and *in vivo* studies showed that fatty acids enhanced adrenergic activation of lipolysis by impairing α2–AR-dependent antilipolysis.

METHODS

In vitro studies

Human adipose tissue culture

Human adipose tissue was obtained from healthy, drug free patients (9 females / age: 43.8 ± 4.5 years / body mass index: 24.4 ± 0.8), undergoing abdominal dermolipectomy for plastic surgery. Adipose tissue was carefully dissected out and cut in small pieces (average weight 50 mg) under sterile conditions. Explants were placed at 37°C in a humidified atmosphere containing 7% CO_2 in 100 mm diameter dishes (7g in 10 ml) in DMEM supplemented with 10% foetal calf serum plus 100 µM bromopalmitate or vehicle (ethanol: 0.1% final concentration). The medium was changed every day. After 48h culture, adipocytes were quickly isolated by collagenase digestion using the method previously described [21] in DMEM containing 2% BSA, 6 mmol/l glucose, and 0.5 mg/ml collagenase.

In vitro lipolysis measurement

Isolated adipocytes were washed three times, and the cells were used for lipolysis measurements in Kreb's Ringer Bicarbonate Hepes Albumine (KRBHA) buffer. Concentration-response curves were obtained using epinephrine alone or in the presence of 10 µM RX821002 (a selective α2−AR antagonist also called 2-methoxy idazoxan). All pharmacological compounds were added to a 5-µl volume at the start of the incubation performed with 2,000-3,000 isolated fat cells in a final volume of 100 µl KRBHA. The incubation was run for 30 min, and 30 µl of infranatant were removed for the determination of glycerol (lipolytic index). Lipolytic activity was expressed as micromoles of glycerol released per 100 mg lipid for 90 min as previously described [7].

Ligand binding assays

Crude membranes from isolated adipocytes were obtained by lysing the cells in a hypotonic buffer and the particulate fraction was prepared as described previously [7]. The α2−adrenergic receptors were identified with the selective antagonist radioligand [3H]RX821002. The incubation was carried out at 25°C for 20 min under a constant shaking

in a medium consisting of 100 µl of radioligand and 100 µl of membrane suspension made up to a final volume of 400 µl with binding buffer (50 mM Tris-HCl, pH 7.5 containing 0.5 mM MgCl2). After incubation, membrane-bound radioligand was separated from free ligand by rapid filtration through GF/C filters (Whatman Inc, Clifton, NJ, USA) placed on a Millipore manifold. Filters were washed with cold binding buffer, air dried, transferred into vials and counted for radioactivity by liquid scintillation spectrometry. Specific binding was defined as the difference between total and non-specific binding determined in the presence of 10 µM phentolamine. For competition studies, indicated concentrations of epinephrine were added to the incubation mixture prior to addition of the membrane suspension. IC50 values were calculated from computer-assisted analysis of the saturation data using the EDBA-LIGAND program [17].

Clinical protocols

Subjects. Seven healthy untrained male volunteers (22.1 ± 1.1 years) participated in the study. Mean body weight of the subjects was 77.0 ± 2.9 kg. Mean body mass index was 22.8 ± 0.6 kg/m^2 . All subjects were 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 Third Faculty of Medicine (Prague, Czech Republic).

Diets

All food was consumed as breakfast, lunch, dinner and 1 snack per day. The high fat diet (HFD) contained (in percentage of total energy) 65% fat, 15% protein, 20% carbohydrate. The low fat diet (LFD) was composed of 70% carbohydrate, 15% protein, 15% fat. The total calorie content of each diet was calculated for each individual so that it corresponded to the calorie content of his habitual diet as assessed before the beginning of the study. The Progena system based on Progena composition tables was used for all calculations.

Microdialysis assays

Microdialysis procedure was essentially the same as described previously [19]. The subjects were investigated at 08:00 after an overnight fast and were placed in a semi-recumbent position. Microdialysis probes (Carnegie Medecin, Stockholm, Sweden) of 20 / 0.5 mm and 20,000-molecular wt cut-off were inserted percutaneously after epidermal anesthesia (200 μl of 1% lidocaine, Roger-Bellon, Neuilly-s-Seine, France) into the abdominal SCAT at a distance of 10 cm immediately to the right of the umbilicus. Two probes, separated by at least 10 cm, 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 with Ringer plus 0.1 mmol/l phentolamine (α-AR antagonist). This non-selective α1−/α2−antagonist, having an efficient α2−AR antagonist action in human fat cells *in vitro*, was the only agent allowed by the ethical committee for use in microdialysis assays in humans. The two perfusate solution were supplemented with ethanol (1.7 g/l). Ethanol was added to the perfusate to estimate changes occurring in the local blood flow of SCAT, as previously described [9, 10]. After a 30-min equilibration period, a 30-min fraction of dialysate was then collected at a flow rate of 0.5 μl/min. Then, the perfusion was set at 2.5 μl/min for the remaining experimental period. A calibration procedure using various perfusion rates for determination of interstitial glycerol concentration in SCAT has already been reported by our group [2, 3]. A simplified, but relevant and less time-consuming method was selected in this study. The estimated extracellular glycerol concentrations were calculated by plotting (after log transformation) the concentration of glycerol in the dialysate measured at 0.5 and 2.5 μl/min against the perfusion rates. After the calibration of the probes, one 15-min fraction of the outgoing dialysate was collected at rest and during the last 15 min before the end of an imposed physical exercise of 60 min at a power level corresponding to 50% of their heart rate reserve on a cycle ergometer. The heart rate was continuously monitored with a Baumann BHL 6000 cardiometer during the exercise. Water intake was allowed ad libitum during the experimental period.

Blood samples for plasma analysis were collected at rest and at the end of the physical exercise, from an indwelling polyethylene catheter inserted into an antecubital vein. The catheter was kept patent by slow infusion of saline. Blood was collected on 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

Phentolamine methanesulfonate (Regitine) was obtained from Ciba-Geigy (Rueil-Malmaison, France). Glycerol in dialysate (10 µl) and in plasma (20 µl) was analysed with an ultra-sensitive radiometric method [6]; the intra-assay and inter-assay variabilities were 5% and 9%, respectively. Ethanol in dialysate and perfusate (5µl) was determined with an enzymatic method [5]; the intra-assay and inter-assay variability were 3% and 4%, respectively. Plasma glucose was determined with a glucose-oxidase technique (Biotrol kit, Merck-Clevenot, Nogent-sur-Marne, France) and non-esterified fatty acids by an enzymatic procedure (Wako kit, Unipath, Dardilly, France). Plasma insulin concentrations were measured using RIA kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France). Plasma epinephrine and norepinephrine were assessed in 1-ml aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection [8]. The detection limit was 20 pg/sample. Day-to-day variability was 4% and within run variability 3%.

Adipose tissue biopsies and RNA extraction

At the end of each diet period, intradermal anaesthesia was performed with 50 µl 1% lidocaine, and a biopsy of abdominal adipose tissue was performed with a 2 mm-diameter needle. Adipose tissue (200-300 mg) was drawn by successive suctions into a syringe containing 2 ml saline solution. Samples were frozen immediately in liquid nitrogen and stored at -80°C until total RNA extraction using the RNeasy kit (Qiagen, Courtaboeuf, France).

RT-PCR analysis

Total RNA (500 ng) was treated with 1U RNAse-free Dnase I (Gibco BRL) for 15 min at room temperature followed by further inactivation with 1µl EDTA (25 mM) 10 min at 65°C. Then, RNA was reverse transcribed for 60 min at 37°C using Superscript II (Life Technologies) RNase H ⁻ Reverse Transcriptase (RT) and subjected to amplification. A minus RT reaction was performed in parallel to ensure the absence of genomic DNA contamination. Polymerase chain reaction (PCR) was carried out in a final volume of 50 µl containing 1.5 µl of cDNA, 1 µl dNTP (10mM), 5 µl 10x PCR buffer (10 mM Tris-HCl, pH 9, 50 mM Kcl and 0,1% Triton X-100), 3 µl MgCl2 (25 mM), 1.5 µl sense and antisense specific oligonucleotide primers (10 µM) and 1.25 unit of Taq DNA polymerase (Promega). Conditions for the PCR reaction were: initial denaturation step at 94°C for 2 min, followed by 34 cycles for α2−AR or 25 cycles for cyclophilin, consisting in 1 min at 94°C, 1 min at 54°C (α2−AR), 60°C (cyclophilin), and 72°C for 90 s. After a final extension at 72°C for 6 min, PCR products were separated on 1.5% agarose gel and amplification products were visualized with ethidium bromide.

Primers used for α2−AR mRNA detection were: sense, 5′-CTACTGGTACTTCGGCAAG-3′ and antisense, 5′-CGTACCACTTCTGGTCGTTGATCT-3′ (size of amplicon 301 bp). Primers used for cyclophilin detection were: sense, 5′-ATGGCACTGGTGGCAAGTCC-3′ and antisense, 5′-TTGCCATTCCTGGACCCAAA-3′ (size of amplicon 243 bp).

Statistical analysis.

All the values are means ± SEM. The responses to exercise were analysed using a paired-t test and ANOVA when appropriate.

RESULTS

1- Influence of bromopalmitate on α2–AR expression and function in adipocytes from cultured human adipose tissue.

Abdominal subcutaneous adipose tissue explants were prepared from patients undergoing plastic surgery, and were maintained in culture for 48 hours in the presence or absence of 100 µM of a stable analogue of saturated long chain fatty acid bromopalmitate (BrPal) [12]. The influence of this treatment on adrenergic control of lipolysis in adipocytes isolated from the explants, was measured.

At day 0 and day 2 in the absence of BrPal, 1 µM isoproterenol (a selective β–AR agonist) induced a significant increase in glycerol release, but epinephrine (a mixed β–α2–AR agonist) was essentially inactive (Figure 1A, B). In the presence of 10 µM RX821002 (a selective α2–AR antagonist) epinephrine became lipolytic with a maximal effect close to that generated with isoproterenol. These results confirmed previous reports [15] showing the existence, in adipocytes from subcutaneous adipose tissue, of antilipolytic α2–ARs the stimulation of which counteracts β–AR-dependent activation of lipolysis.

After 48 h culture in the presence of 100 µM BrPal, epinephrine alone became able to activate glycerol release, with a maximal response similar to that obtained with isoproterenol (Figure 1C). In addition, RX821002 had no more influence on epinephrine action, showing that BrPal led to a disappearance of α2–AR-dependent antilipolysis with no alteration of β–AR-dependent lipolysis. This conclusion was in agreement with the observation that BrPal treatment did not significantly influenced the release of glycerol generated by the β-adrenergic agonist isoproterenol (Figure 1). Similarly, the lipolytic response generated by the adenylyl cyclase activator, forskolin, as well as the antilipolytic response generated by insulin, were not significantly altered by BrPal treatment (data not shown).

The influence of BrPal was dose-dependent. Treatment with 1, 10 and 100 µM BrPal led to respectively to 43 ± 5, 61 ± 6, and 89 ± 5 % (n=3) reduction of α2–adrenergic-dependent antilipolysis. In parallel, BrPal did not modified the lipolytic response generated

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by the adenylate cyclase activator forskolin nor the antilipolytic activity of insulin (data not shown). These results were not in favour of a cytotoxic effect of BrPal on adipocytes.

In order to determine, whether BrPal altered adipocyte α2–AR expression and/or coupling, radioligand binding assays were performed with the α2–AR antagonist radioligand [3H]RX821002 on isolated adipocyte membranes prepared from treated and non-treated adipose tissue explants. Saturation binding assays revealed neither significant modification in maximal binding (Bmax) nor in affinity (equilibrium dissociation constant) (Kd) of [3H]RX821002 between day 0, and day 2 control, and day 2 with BrPal treatment (Table 1). Epinephrine promoted a concentration-dependent inhibition of [3H]RX821002 specific binding. At day 0 and day 2 without BrPal, computerised analysis of inhibition could be resolved as a two site curve. As expected, the existence of two distinct classes of binding sites was revealed: a high affinity class characterised by a IC50H value in the nM range, and a low affinity class characterised by a IC50L value in the µM range. High and low affinity classes have previously been demonstrated to correspond to G-protein-coupled and G-protein-uncoupled α2–adrenergic receptors respectively [11]. In the presence of BrPal a significant right shift of the competition curve with an increase in IC50H (7 fold), and to a less extent in IC50L values (2.6 fold), was observed (Table 2). Above results, showed that BrPal-treatment had no influence on adipocyte α2–adrenergic receptor density nor on their affinity for antagonists. However, BrPal-treatment induced a reduction in adipocyte α2–adrenergic receptor coupling efficiency to G-proteins.

BrPal has been reported to be a strong activator of transcriptional factors belonging to the Peroxysome Proliferator Activated Receptor (PPAR) [4]. In order to test a possible involvement of a PPAR in the effect of BrPal in our experiments, the influence of a PPARγ agonist, rosiglitazone, on adrenergic control of adipocyte lipolysis, was tested. Forty eight hours treatment of adipose tissue explants with 1 µM roziglitazone, led to a marked inhibition (77 ± 11% n=3) of the α2-adrenergic-dependent antilipolysis, suggesting that the transcriptional factor PPARγ could be involved in the effect of BrPal.

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2- Influence of high fat diet α2-AR-dependent antilipolysis *in vivo*

In vivo approaches were performed to investigate whether α2-AR-antilipolysis could also be altered when plasma fatty-acids were chronically elevated by a nutritional protocol. A group of 8 healthy volunteers was successively (in a random order) submitted to the following sequence of treatments: (1) a low fat diet (LFD) for 4 days; (2) a 7-days wash out period; (3) a high fat diet (HFD) for 4 days. At rest, HFD was associated with a significant increase (1.7 fold) in plasma non-esterified fatty acids (NEFA) as compared to LFD: 521 ± 39 μM vs 313 ± 39 μM, P<0.05).

After each diet period volunteers underwent a calibrated physical exercise (50% of maximal heart rate during 60 min), in order to get a physiological activation of the sympathetic nervous system (SNS) and subsequent lipolysis [19, 20]. After each type of diet, exercise led to a significant increase in plasma norepinephrine and epinephrine concentrations, as compared to rest (Table 3). However, after HFD, the plasma levels at the end of exercise tended to be higher when compared to LFD: the difference being significant for norepinephrine (P<0.04) but not for epinephrine (P<0.07) (Table 3).

Exercise-induced lipolysis was determined by measuring extracellular production of glycerol in the adipose tissue with the use of a microdialysis probe, implanted in the subcutaneous adipose tissue, and perfused with a physiological fluid containing or not the α2–AR-antagonist phentolamine. The purpose of phentolamine was to evaluate the relative contribution of β–AR and α2–ARs in adrenergic control of lipolysis *in vivo.* Blocking antilipolytic α2–ARs by phentolamine restores a full β–AR-dependent activation of lipolysis [20]. After LFD, exercise led to an increase in lipolysis, which was significantly enhanced (1.4 fold), in the presence of phentolamine (Figure 2 left part). After HFD, whereas resting basal lipolysis was not modified, the exercise-induced lipolysis was enhanced (4.2 fold) as compared to LFD (2.5 fold) (Figure 2 right part). Phentolamine had no more incidence on exercise-induced lipolysis after HFD (Figure 2 right part), conversely to what was observed

in LFD. <u>The possible influence of the diets on local blood flow of adipose tissue (evaluated as the ratio between ethanol outflow and ethanol inflow: Material and Methods) was determined at rest and after 60 min exercise. No significant differences were observed: in LFD resting and exercise blood flow were 43.6 ± 7.3 and 44.1 ± 7.2 % respectively; in HFD resting and exercise blood flow were 48.3 ± 5.6 and 48.1 ± 5.9 % respectively. These results showed that HFD specifically impaired α2−AR-dependent antilipolysis <i>in vivo.</i></u>

In order to determine whether the expression of adipose tissue α2−ARs of the volunteers was altered by HFD, needle biopsies of subcutaneous adipose tissue were performed on each subject at the last day of each diet, total RNA were extracted, and α2−AR mRNAs were analysed by comparative RT-PCR analysis, in parallel with an house-keeping mRNA cyclophilin. Ratio of expression between the mRNAs were 0.36 ± 0.11 and 0.44 ± 0.10 in LFD and HFD respectively, and the difference was not significant. This showed that HFD did not modify α2−AR mRNA gene transcription in adipose tissue.

DISCUSSION

The aim of the present study was to investigate whether fatty acids could modify adrenergic control of lipolysis in human adipocytes. *In vitro* and *in vivo* studies showed that fatty acids enhanced adrenergic activation of lipolysis by impairing the α2−AR-dependent antilipolysis.

As previously demonstrated, antilipolytic α2−ARs predominate over lipolytic β−ARs in subcutaneous adipose tissue. This is characterized by a very weak, and often inefficient, activation of lipolysis by catecholamines. A full β−AR-dependent activation of lipolysis by catecholamines can be restored by blocking α2−AR-dependent antilipolysis with an α2−AR antagonist [15]. The results obtained in the present study from control abdominal adipose tissue are in complete agreement with these previous conclusions. The original observation made in the present study was that, *in vitro* treatment of adipose tissue explants with BrPal, led to a full β−AR-dependent activation of lipolysis by epinephrine, which did not require the presence of an α2−AR antagonist. Indeed, in the presence of BrPal, the addition of an α2−AR-antagonist had no influence on epinephrine-induced activation of lipolysis. Therefore, incubation of adipose tissue with a fatty acid led to dramatic alteration in adipocyte sensitivity to epinephrine which very likely results from a specific impairment of the α2−AR-dependent antilipolysis.

The relative contribution of β− and α2−ARs in adrenergic stimulation of lipolysis can also be evaluated *in vivo* in human. As previously demonstrated exercise-induced activation of the sympathetic nervous system leads to an increase in extracellular glycerol concentration measured *in situ* in subcutaneous adipose tissue with the use of a microdialysis technique [19, 20]. Perfusion of an α2−antagonist in the microdialysis probe enhances exercise-induced lipolysis, as the result of the local blockade of adipocyte antilipolytic α2−ARs. The results obtained from LFD-fed volunteers are in complete agreement with previous results of the laboratory. The originality of our work was to demonstrate that, after HFD, exercise-induced lipolysis was no more influenced by the blockade of antilipolytic α2−ARs by phentolamine.

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These results strongly suggested that α2–AR-dependent antilipolysis was impaired after HFD.

Fatty acids are known for their involvement in the metabolic and trophic adaptations of the organism to HFD [14]. HFD was accompanied by a 1.7 fold increase in total plasma fatty acids. The fact that impairment of α2-AR-dependent antilipolysis can be reproduced *in vitro* with BrPal strongly suggests that fatty acids could be involved, in the effect of HFD on exercise-induced lipolysis *in vivo*. However, it is considered that only unbound fatty acids are biologically active. As previously described [18] the proportion of unbound NEFA is tightly dependent on the ratio between total NEFA and albumin concentrations. Assuming that plasma concentration of albumin is 600 μM, the concentration of unbound plasma NEFA in subjects undergoing LFD can be estimated at 10 nM. In subjects undergoing HFD, unbound plasma NEFA can be estimated at 40 nM. Because *in vitro* experiments were performed in 10% serum, we assume that albumin concentration was 60 μM. In this condition, the concentration of unbound BrPal were estimated at 5 and 60 nM for total concentration of BrPal 10 and 100 μM respectively. These estimations show that the concentration of unbound BrPal used in *in vitro* experiments are compatible with the increase in concentration of unbound fatty acids resulting from HFD *in vivo*.

What could be the mechanisms involved in the impairment of α2-adrenergic-dependent antilipolysis by fatty acids? Binding studies with an α2–adrenergic radioligand on adipocyte membranes, revealed that BrPal had no influence on the density of α2–ARs. RT-PCR analysis in adipose tissue biopsies revealed that α2–AR mRNA level was not modified after HFD. These observations showed that fatty acids and HFD did not influence α2–AR expression. Competition binding studies on adipocyte membranes showed that BrPal significantly reduced binding characteristics and affinity of epinephrine for α2–ARs. These data suggest that BrPal induced a reduction in the coupling of α2–ARs to downstream pathways leading to antilipolysis. Whereas the mechanism of action of this regulation remains to be determined, it is possible that fatty acids exert a negative effect on the

expression and/or activity of Gi proteins, involved in coupling α2−AR to adenylyl cyclase and inhibition of lipolysis. The hypothesis are supported by a previous report showing that long chain saturated fatty acids induced a reduction in pertussis-toxin catalysed ADP-ribosylation of G proteins in breast cancer cells [22]. Further studies will be necessary to determine whether such a regulation occurs in adipocytes. In addition, the effect of BrPal being reproduced by roziglitazone, suggests a possible involvement of the transcription factor PPARg in the alteration of the expression and/or activity of Gi proteins in adipocytes..

In conclusion, the complementary *in vitro* and *in vivo* approaches reported in the present study outline the importance of fatty acids in modulating adipose tissue sensitivity to catecholamines. By selectively influencing α2−AR sensitivity, fatty acids modify the balance between β−AR-dependent activation and α2−AR-dependent inhibition of lipolysis, thus leading to an alteration in the mobilisation of fat stored in adipose tissue. What could be the physiological consequence of our findings? The fatty acids released from the fat stores are considered as the main energetic substrates for muscle contraction during physical exercise, particularly when performed below or equal to 50% heart rate that is considered as an aerobic condition. The stronger exercise-induced lipolysis observed after HFD will provide a higher amount of fatty acid to the muscle, therefore allowing a sustained physical exercise with limited production of lactate in the muscle. However, if the physical exercise is not maintained over a long enough period of time, fatty acids originating from lipolysis will not be burned in the muscles and will accumulate in the plasma generating deleterious effects such as insulin resistance or lipid deposits in the vessels. Therefore, short term HFD, as performed in the present study, can be considered useful only if it is associated with prolonged aerobic physical exercise.

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LEGEND OF FIGURES

Figure 1: Effect of bromopalmitate-treatment on adrenergic control of lipolysis in human adipocytes.

Adipocytes were isolated from human adipose tissue explants maintained in culture in 10% fetal calf serum supplemented DMEM for 0 (A, Days 0) or 48 h (B and C, Day 2) in the absence (B) or presence (C) of 100 µM bromopalmitate. Lipolysis was analysed on adipocytes isolated from cultured explants. Glycerol release was quantified after 30 min incubation at 37°C of isolated adipocytes without (Basal) or with 1 µM isoproterenol (Iso), or increasing concentrations of epinephrine with or without 10 µM RX821002 . Basal glycerol concentrations were 0.12±0.02, 0.20±0.02 , and 0.10±0.01 µmoles/100 mg lipid in A, B, and C respectively. Values are means ± SE from n separate experiments. *$P < 0.001$ (ANOVA) compared the effect of epinephrine to the effect of epinephrine plus RX821002.

Figure 2: Influence of diet (LFD and HFD) and phentolamine on exercise-induced changes in extracellular glycerol concentration in microdialysate of human adipose tissue.

After LFD and HFD, two microdialysis probes were implanted in the abdominal subcutaneous adipose tissue. One probe was perfused with Ringer solution (-) and the second with Ringer plus phentolamine (+). Outgoing dialysates (15 min fractions) were collected at rest (Rest) and 15 min before the end of a 60 min calibrated physical exercise (50% maximal heart rate) (Exercise). In situ lipolytic activity was determined by measuring glycerol concentration in the outgoing dialysate. Values are means ± SE. # and * correspond to $P<0.05$ when using a Student's paired t test for statistical analysis. NS: non significant.

TABLES

Table 1: Influence of bromopalmitate-treatment on adipocyte α2–AR density and affinity.

	Bmax (fmole/mg protein)	Kd (nM)
Day 0	559 ± 45	1.5 ± 0.5
Day 2	532 ± 159	1.3 ± 0.5
Day 2 + BrPal	630 ± 128	1.3 ± 0.5

Crude membranes were prepared from adipocytes isolated from human subcutaneous adipose tissue the day of the surgery (Day 0) or after 2 days culture in the absence (Day 2) or in the presence of 100 μM bromopalmitate (Day 2+ BrPal). Adipocyte membrane α2–AR densities (Bmax) and affinities (Kd) were determined by equilibrium binding analysis using [^{3}H]RX821002 as radioligand. Values are means±SE, n=3.

Table 2: Influence of bromopalmitate-treatment on high and low affinity binding sites of epinephrine on adipocyte α2–AR.

	Day 0	Day 2	Day2 + BrPal
High affinity sites			
IC50 (nM)	7.89 ± 0.05	8.46 ± 0.04	59.90 ± 0.10 **
Low affinity sites			

IC50 (nM)	2.04 ± 0.15	1.55 ± 0.11	4.13 ± 0.26 *

Membrane samples were the same as in Table 1. High and low affinity sites were determined by computerised analysis of the competition curves of [³H]RX821002 (1 nM) by increasing concentrations of epinephrine. Values are means ± SE. * P<0.05 when compared with control.

Table 3: Influence of 60 min exercise on plasma catecholamines after LFD and HFD.

	Rest	Exercise (60 min)
Norepinephrine (pg/ml)		
LFD	210 ± 13	892 ± 92 *
HFD	186 ± 11	1054 ± 61 *, #
Epinephrine (pg/ml)		
LFD	25 ± 4	92 ± 11 *
HFD	24 ± 3	127 ± 25 *

At the end of each diet period (LFD and HFD), volunteers underwent a 60 min calibrated physical exercise (50% maximal heart rate). Blood samples were collected at rest (Rest) and at the end of exercise (Exercise 60 min) for plasma catecholamines determination. Values are means ± SE. * $P < 0.05$ when comparing to Rest values ; # $P < 0.05$ when comparing with LFD values.