

Acute exposure to long-chain fatty acids impairs alpha₂-adrenergic receptor-mediated antilipolysis in human adipose tissue

Jan Polak⁴, Cédric Moro^{1,3,4}, David Bessièrè^{1,3,4}, Jindra Hejnova⁴, Marie A. Marquès^{1,4}, Magda Bajzova⁴, Max Lafontan^{1,2,4}, Francois Crampes^{1,4}, Michel Berlan^{1,3,4} and Vladimir Stich⁴.

¹ Inserm U858, Institute of Molecular Medicine Ranguel, IFR31- Louis Bugnard Institute
Toulouse, France

² Paul Sabatier University, Toulouse, France

³ Faculty of Medicine Purpan, Laboratory of Medical and Clinical Pharmacology, Toulouse,
France

⁴ Franco-Czech Laboratory for Clinical Research on Obesity, 3rd Medical Faculty, Charles
University in Prague and INSERM, Prague, Czech Republic

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Correspondence to: Dr. Max LAFONTAN

Inserm U858, I2MR-Institut de Médecine Moléculaire de Ranguel, 1, Av. Jean Poulhès,
BP84225, 31432 Toulouse cedex 4, France

Phone : 33 5 62 17 29 51

Fax : 33 5 61 33 17 21

e-mail: Max.Lafontan@toulouse.inserm.fr

Abstract

The acute *in vitro* and *in vivo* effects of long-chain fatty acids (LCFA) on the regulation of adrenergic lipolysis were investigated in human adipose tissue. The effect of a 2-hour incubation, without or with LCFA (200 $\mu\text{mol/l}$), 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 hours 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 alpha-antagonist (phentolamine). *In vivo*, a HFM increased plasma levels of non-esterified fatty acids in lean and obese subjects. In both groups, the HFM did not alter hormonal responses to exercise. Under fasting condition, 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. LCFA 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.

Key words: Long-chain fatty acid, lipolysis, catecholamines, high fat meal, exercise, microdialysis, adrenergic receptors, lipid mobilization,

Introduction

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 the extracellular fatty acid (FA) levels, has not been evaluated in man. In rodents, it has been shown that the reduction of the level of plasma non-esterified fatty acids (NEFA) using masoprocol, a lipooxygenase inhibitor significantly reduced isoproterenol-mediated lipolysis (1). More recently, it has been shown that exposure (4 to 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 FA 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 (LCFA) 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 beta- and antilipolytic alpha₂-adrenergic receptors (alpha₂-ARs) (4, 5), whereas in rat fat cells no functional alpha₂-AR can be found (6). Additionally, a new lipolytic pathway involving the natriuretic peptides (NP), atrial-NP (ANP) and brain-NP (BNP), has been characterized in humans. It was shown that NP-induced lipolysis is a primary function specific to primate and human fat cells (9, 10). In conclusion, there is no suitable animal model to study the integrated regulation of lipolysis subsequent to acute dietary fat intake. In a previous study, we have shown that four days of a high fat diet (65% fat, 15% protein, 20% carbohydrate) impaired the alpha₂-adrenergic antilipolytic effect of catecholamines in human subcutaneous

adipose tissue (SCAT) during exercise (7). Additionally, a 48 h incubation of human adipose tissue explants *in vitro* with bromo-palmitate suppressed the antilipolytic α_2 -adrenergic effect on lipolysis (8). Whatever the relevance of these previous observations, the effect of acute exposure to long-chain fatty acids (LCFA) on human fat cell lipolysis and exercise-induced lipid mobilization has never been so far studied.

The first specific aim of this study was to investigate *in vitro* in human fat cells, the short-term effect (2 hours) of an acute exposure to LCFA on the well-characterized lipolytic pathways. The second specific aim was to investigate *in vivo* whether a high fat meal (HFM) prior to exercise might change adipose tissue lipolysis in lean and obese subjects. Exercise promotes sympathetic nervous system (SNS)-mediated activation of lipid mobilization in SCAT (11). During exercise, both antilipolytic α_2 -ARs and lipolytic beta-adrenergic receptors are activated by catecholamines; the antilipolytic effect being higher in overweight and obese subjects (11, 12). The present 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 NEFA in the plasma.

MATERIAL AND METHODS

In vitro studies

Subjects. Human abdominal subcutaneous adipose tissue was obtained from 8 moderately overweight females undergoing plastic surgery. Their mean age was 42.7 ± 2.1 years and their mean body mass index 27.0 ± 1.8 kg/m² (range: 24.1-31.6). The investigation protocol was approved by the Ethical Committee of Toulouse University Hospital and of the 3rd Medical Faculty Hospital, Charles University in Prague. Written informed consent was also obtained from all the subjects before the investigation began.

Adipocyte isolation. Isolated adipocytes were obtained as previously described 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, 2 % fatty acid free bovine serum albumin (KRBHA) and 6 mmol/l glucose at 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 3 times with KRBHA buffer to eliminate collagenase.

Acute exposure of fat cells to LCFA. The freshly isolated fat cells were divided into three batches and diluted in 3 ml KRBHA buffer. The first batch (control) was immediately assayed in a FA-free medium (2000-3000 cells/assay) for lipolysis. The second batch was incubated in FA-free KRBHA buffer without addition of FA for 2 hours. The third batch was incubated with addition of 200 µmol LCFA for 2 hours in the FA-free KRBHA buffer. The LCFA mixture was composed mainly of saturated and omega-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 hours preincubation period, the control and LCFA cell batches were washed three times with fresh buffer prior to lipolysis assays.

Lipolysis assays. Fat cells were suspended in a 100 μl FA-free KRBHA medium (2000-3000 cells/assay). The cells were incubated with the addition of 5 μl of ANP (1 $\mu\text{mol/l}$) or isoproterenol (1 $\mu\text{mol/l}$) and also incubated with 5 μl of increasing concentrations of epinephrine (0.1, 1 and 10 $\mu\text{mol/l}$) alone or added with 5 μl of 10 $\mu\text{mol/l}$ RX821002 (a selective α_2 -AR antagonist) for 90 min at 37°C under gentle shaking at 120 cycles/min. For each protocol, 30 μl 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 method (14).

In vivo studies

Subjects. Eight healthy untrained lean and seven obese male volunteers (25.5 \pm 0.4 and 27.3 \pm 2.8 years, respectively) participated in the study. The mean body mass index (BMI) was 22.8 \pm 0.6 and 31.5 \pm 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 Third Faculty of Medicine, Charles University (Prague, Czech Republic).

The subjects were examined in the laboratory at 7 a.m. on two occasions. The two investigation days were separated by one 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 continually monitored with a cardiometer (Polar Accurex Plus Cardiometer, Monitor, France). Subjects were investigated one day under fasting conditions and on the other day after an oral intake of a high fat meal (HFM) at 8 a.m. 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% mono-unsaturated 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 previously described (Stich 1999) (11). The subjects were placed in a semi-recumbent position. Two microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20/0.5 mm and 20,000-molecular weight cut-off were inserted percutaneously after epidermal anesthesia (200 μ l of 1% lidocaine, Roger-Bellon, Neuilly-s-Seine, France) into the abdominal SCAT at a distance of 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 with Ringer plus 0.1mmol/l phentolamine (alpha-AR antagonist). This non-selective alpha₁/alpha₂-antagonist, with an efficient alpha₂-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 previously described (11, 12, 15).

Exercise protocol. Two 15-min fractions of the outgoing dialysate were collected at rest and 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, 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 μ l 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, bovine serum albumin, 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 (1-28) 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, Saint Louis, USA). Ethanol in the dialysate and perfusate (5 μ l) 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 non-esterified fatty acids by an enzymatic procedure (Wako kit, Unipath, Dardilly, France). Plasma insulin concentrations were measured using EIA 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 \pm standard error of the mean (s.e.m.). The significance of differences was assessed using Student's paired t-test and analysis of variance (ANOVA) with Bonferroni's and Student-Newman-Keuls' tests for post hoc analysis. Significance values are quoted 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, USA).

RESULTS

In vitro studies. The study was performed in order to investigate if pretreatment of isolated fat cells with LCFA induces modifications of α_2 -, beta-adrenergic and ANP-mediated lipolytic responses. After two hours incubation, the medium contained 101 ± 67 $\mu\text{mol/l}$ of NEFA in the medium initially deprived of FA and 372 ± 127 $\mu\text{mol/l}$ in the medium initially supplemented with 200 $\mu\text{mol/l}$ LCFA, respectively. It was observed that 2 hours of pre-incubation in medium without FA or enriched with 200 $\mu\text{mol/l}$ 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 (Fig. 1, Fig. 2 and Fig. 3)). The degree of stimulation of lipolysis by 1 $\mu\text{mol/l}$ 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 $\mu\text{mol}/100$ mg lipid, in control (without LCFA) or after 2 hours pre-incubation with 200 μM of LCFA, respectively. Under similar experimental conditions, the increase in ANP-induced NEFA was not different (0.99 ± 0.47 and 0.83 ± 0.27 $\mu\text{mol}/100$ 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, the RX 821002 (6). On the contrary, this potentiating effect of RX 821002 was not observed when fat cells were pre-incubated for two hours in a medium without LCFA (but enriched by NEFAs released during preincubation) or FA-enriched with 200 $\mu\text{mol/l}$ of LCFA (Fig. 2 and Fig. 3).

Since adenosine is an important regulatory agent of adipose tissue metabolism through inhibition of lipolysis, we also evaluated the inhibitory effect of the reference A1-adenosine receptor agonist, phenylisopropyladenosine (PIA). In order to increase basal lipolysis to test the inhibiting effect of PIA; inhibition of lipolysis initiated by adenosine

deaminase (4 $\mu\text{g/ml}$), 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 (17). Whatever the experimental condition, spontaneous lipolysis under adenosine deaminase (ADA) was increased after the pre-incubation with or without LCFA added to the medium. Increasing concentrations of PIA similarly inhibited the ADA-induced lipolysis in the control study or after 2 hours incubation with 200 $\mu\text{mol/l}$ LCFA (Fig. 4).

In vivo studies

Effect of a HFM on plasma parameters. Table 1 depicts the changes in the plasma concentration of insulin, glucose, glycerol and NEFA during the three hours 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 the 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 significantly changed and glucose concentrations increased slightly, but not significantly, after 120 and 180 min.

Effect 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 slightly reduced 30 min after the beginning of the exercise after the HFM. In obese subjects plasma glycerol concentration increased moderately during exercise in both nutritional conditions. Plasma NEFA decreased 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 only observed after the HFM in obese subjects. Finally, no change in plasma glucose levels was observed in either of the nutritional conditions.

Effect 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 $\mu\text{mol/l}$, 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 $\mu\text{mol/l}$, respectively) (Fig. 6). Lipolysis increased during exercise whatever the nutritional condition. However, the exercise-induced lipolysis was enhanced under alpha-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 $\mu\text{mol/l}$, 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 enhancing 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 (ATBF) occurring in the SCAT microcirculation was 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 divided by the ethanol concentration in the perfusate x 100.

At rest, in fasting condition 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 in 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 ATBF did not change significantly during exercise in either lean or obese subjects.

DISCUSSION

In the present 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 beta- 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 hours 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 3 hours prior to exercise promoted an increase in resting plasma NEFA and glycerol levels. Throughout the subsequent exercise, 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 since 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-hour 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 inhibition of lipolysis (8). The present study revealed that such an effect even appears after a

short-term exposure (2 hours) to FA since 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 pre-incubated for 2 hours without added FA in the medium (Fig 3). In this situation fat cells spontaneously released FA in the medium. The amount of released FA ($101 \pm 67 \mu\text{mol/l}$) 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 since in obesity-prone animals; high-fat diets induce a model of obesity in animals. On the contrary, obesity-resistant animals submitted to a similar high-fat diet did not develop obesity (20). 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 et al, (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 of fatty acids and could finally induce obesity. The decrease in intracellular pH as the 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 since spontaneous lipolysis was considerably enhanced after incubation with FA (Fig. 1, Fig. 2 and Fig. 3). Nevertheless, it cannot be excluded that excess of LCFA into the cells via a FA transporter activity (e.g. CD36 for example) during preincubation could alter further transport activity and augment 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 showed that when LCFA 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 when 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 previously observed with 24-hour incubations of fat cells with bromopalmitate (8), LCFA could exert acute actions which 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 previously seen (8). All 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-hour incubation of adipocytes with 200 $\mu\text{mol/l}$ 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 α_i 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 (SNS) activation and natriuretic peptides release (10), two factors involved in the increase in lipolysis in human fat cells.

In situ investigations of subcutaneous adipose tissue lipolysis was 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 3 hours after a HFM in lean as well in 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 (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). Epinephrine exhibits a high affinity for α_2 -ARs and activates the antilipolytic α_2 -AR in SCAT during exercise. This mechanism is favoured in the SCAT of obese people in whom exercise-induced lipolysis in subcutaneous adipose tissue is severely impaired by 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, through promoting an increase in NEFA, modifies the interplay between the antilipolytic α_2 - and lipolytic beta-adrenergic receptors at the level of 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 did not potentiate the exercise-induced glycerol release. The efficacy of the α_2 -AR antagonist is lost due to weakening of the α_2 -adrenergic responsiveness (Fig. 7). So, 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 non-obese subjects submitted 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 (7). These results have shown that a HF diet did not alter the beta-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 (8). It was concluded that by selectively influencing alpha₂-AR sensitivity, 4-days of a high fat diet modified the balance between beta-AR-dependent activation and alpha₂-AR-dependent inhibition of lipolysis, thus leading to an alteration in the mobilization of stored fat.

We have shown in the present investigation that the overall lipolytic processes were unaffected by an acute high fat intake since the increase in plasma glycerol during the exercise bout was similar 3 hours after the HFM when compared with that observed under fasting conditions. Concerning plasma parameters, plasma NEFA levels increased 3 hours after the HFM in both groups (Table 1). Increases in plasma levels of NEFA and glycerol after a high fat meal reflect the increased hydrolysis of triglycerides in chylomicrons whose plasma levels increase after a high fat meal. 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 (26). Plasma glycerol increased moderately in lean subjects but the increase was more pronounced in obese subjects. The fact that obese subjects have a 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 alpha₂-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 and it

can be suspected that fatty acids exert a negative effect on the α_2 -AR coupling. Fisher, (22, 23) 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 (11), and in the present 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 (24). Insulin-resistant obese subjects have an enlarged fat mass often leading to elevated 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 beta-cells and increase insulino-resistance (25). Increased plasma NEFA levels are a risk factor for the development of type 2 diabetes mellitus (24). The accurate control of lipolysis is essential to keep a normal level of plasma NEFA (26, 27). The major impact of our study is to show that an acute high fat intake alters the protective function of α_2 -adrenoceptors against increased lipolysis in obese subjects. These patients normally exhibit a major spontaneous antilipolytic α_2 -adrenergic effect in fat cells (i.e. revealed during a calibrated exercise bout: present study and (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 if 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 anti-hypertensive drugs. Following on from our results, it will be interesting to evaluate the effect of the intake of selected meals with 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 if the fatty acid composition of a high fat meal 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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Figure legends

Figure 1. Effect of incubation of human fat cells for 2 hours with 200 μ M LCFA on lipolysis induced by 1 μ M Isoproterenol (Iso) and 1 μ M ANP (ANP), compared with un-incubated controls. After the 2hour incubation period, cells were washed. In both case, lipolysis was performed for 90 min in a NEFA free medium. Data are expressed as mean \pm s.e.m of 8 separate experiments. # $p < 0.05$ when compared to basal control values. * $P < 0.05$ when compared to basal values.

Figure 2. Effect of 2 hours incubation with 200 μ M LCFA on lipolysis in human fat cells, comparison with un-incubated control cells. After the 2 hour incubation period with LCFA, cells were washed. In both case, lipolysis was performed for 90 min in a NEFA free medium. Lipolysis was induced by increasing concentrations of epinephrine alone or with 10 μ M of RX 821002, a selective α_2 -adrenergic receptor antagonist. **A:** Effect 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 mean \pm s.e.m of 8 separate experiments. * $P < 0.05$ when compared to values obtained with epinephrine alone.

Figure 3. Effect 2 hours incubation of human fat cells without LCFA on *in vitro* lipolysis. Lipolysis was induced by 1 μ M isoproterenol (Iso) and 1 μ M ANP (ANP) and with increasing concentrations of epinephrine alone or with 10 μ M RX 821002, a selective α_2 -adrenergic receptor antagonist. **A:** Effect 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 mean \pm s.e.m of 8 separate experiments. * $P < 0.05$ when compared to basal values.

Figure 4. Inhibitory effect of phenylisopropyladenosine (PIA) on lipolysis induced by 4 $\mu\text{g}/100 \mu\text{l}$ of adenosine deaminase (ADA) in human fat cells after 2 hours incubation with 200 μM LCFA, compared with un-incubated controls. **A:** Effect 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 mean \pm s.e.m of 6 separate experiments.

Figure 5. Plasma glycerol and NEFA concentrations measured at rest, during exercise and in the recovery period in fasting conditions or three hours after a high fat meal.

Data are expressed as mean \pm s.e.m of 8 (lean) and 7 (obese) separate experiments. * $P < 0.02$ when compared to values measured at rest.

Figure 6. Dialysate glycerol concentration (DGC) in subcutaneous adipose tissue (SCAT) at rest, during exercise and recovery in the control probe (Ringer) or in the probe infused with 0.1 mmol/l phentolamine under fasting conditions or three hours after a high fat meal in lean subjects. Data are expressed as mean \pm s.e.m of 8 separate experiments. **A:** DGC values * $P < 0.05$ when compared to values measured in the control probe. **B:** Increase in DGC during exercise. P value when compared to values measured in the control probe. NS: non-significant.

Figure 7. Dialysate glycerol concentration (DGC) in subcutaneous adipose tissue (SCAT) at rest, during exercise and recovery in the control probe (Ringer) or in the probe infused with 0.1 mmol/l phentolamine under fasting conditions or three hours after a high fat meal in obese subjects. Data are expressed as mean \pm s.e.m of 7 separate experiments. **A:** DGC values* $P < 0.05$ when compared to values measured in the control probe. NS: non-significant. **B:** Increase in DGC during exercise. P value when compared to values measured in the control probe. NS: non significant.

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

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

High fat meal was taken at time 60min.

Values are mean \pm s.e.m. of 8 separate determinations.

*p<0.05 when compared to time 0 min.

Significant when compared to lean subjects.

Table 2. Effect of 45 min exercise and recovery on plasma insulin, glucose and catecholamine concentrations under fasting conditions and 3 hours after ingestion of a high fat meal in lean and obese subjects.

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

Values are mean \pm s.e.m. of 8 separate determinations

* Significant when compared to rest values (15 min)

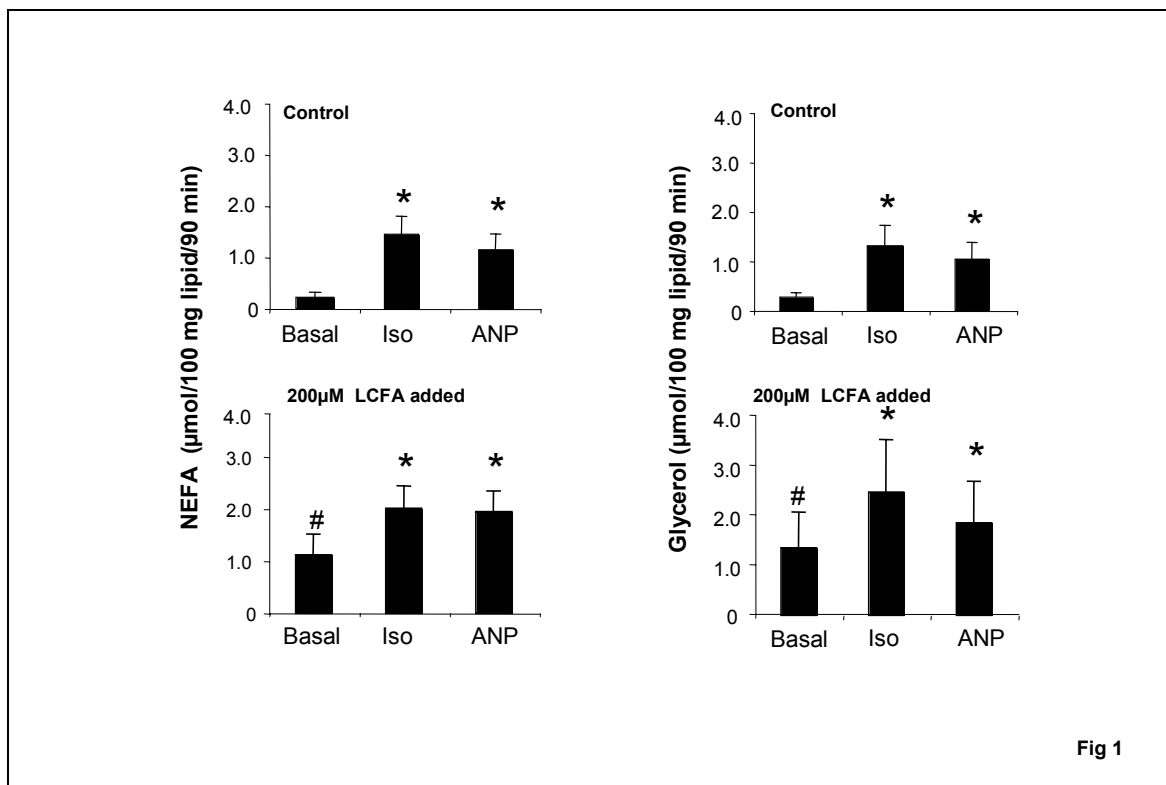


Fig 1

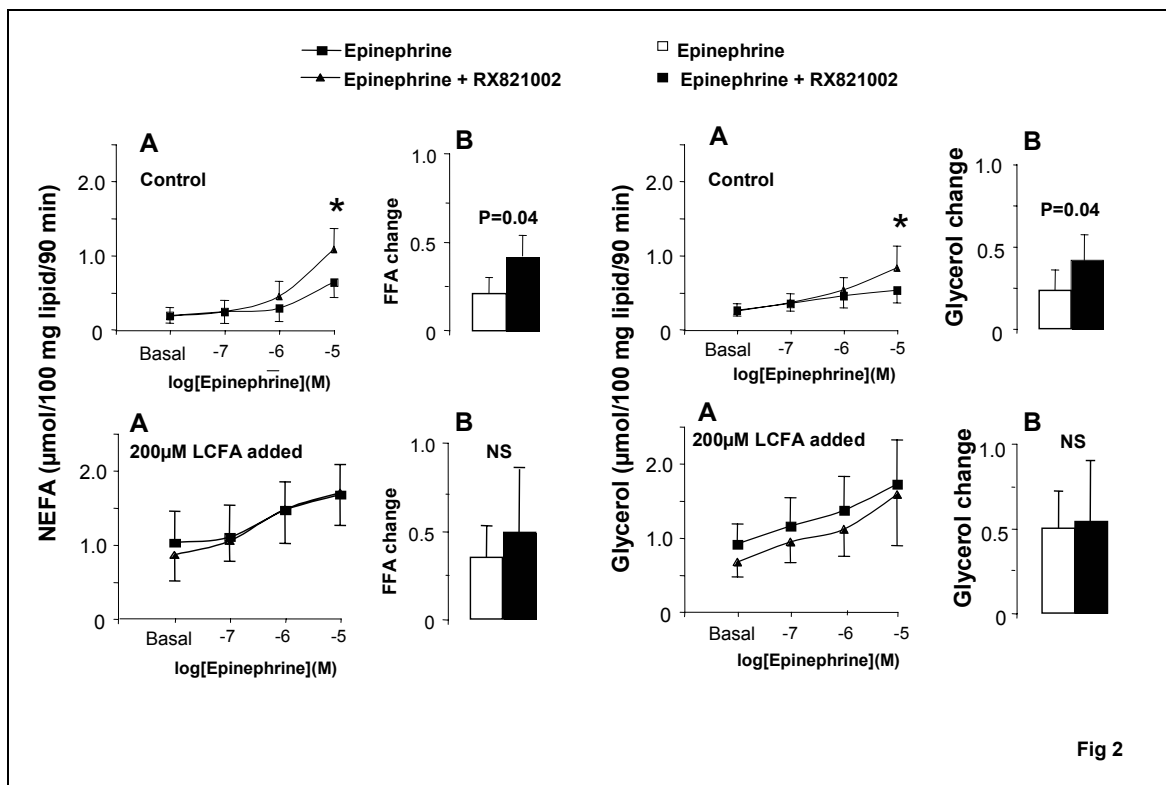


Fig 2

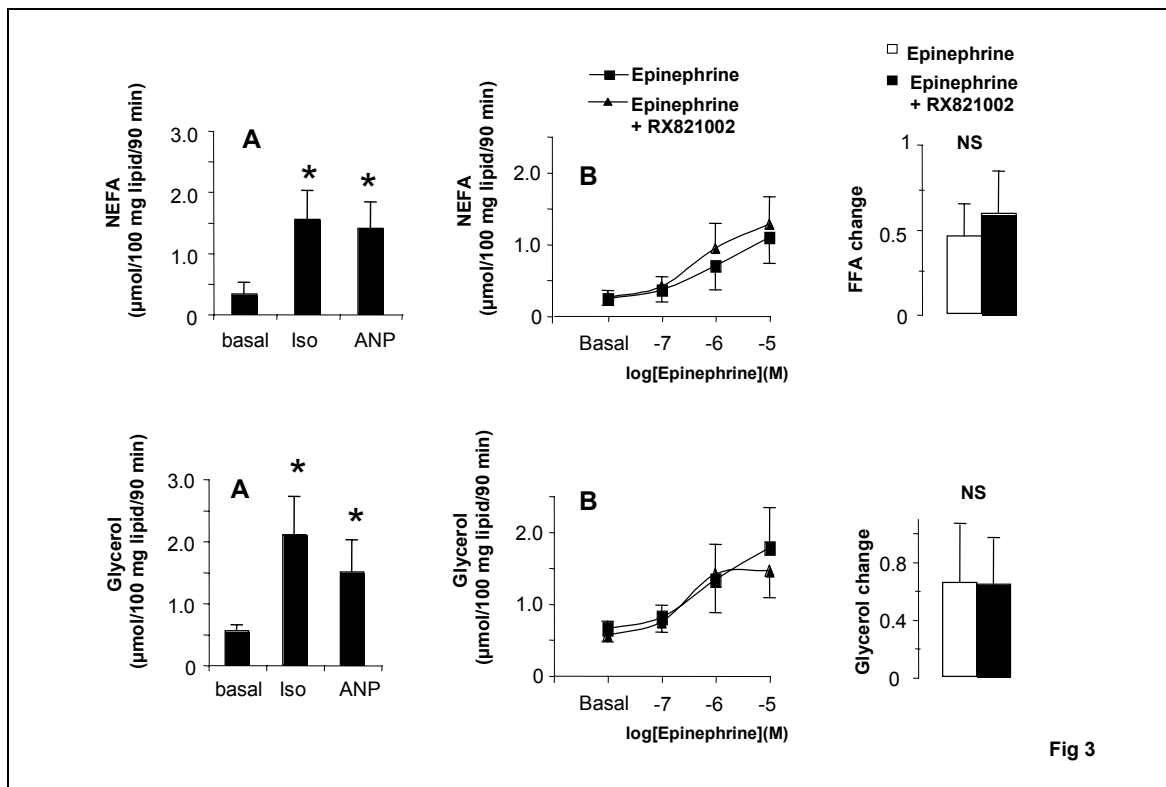


Fig 3

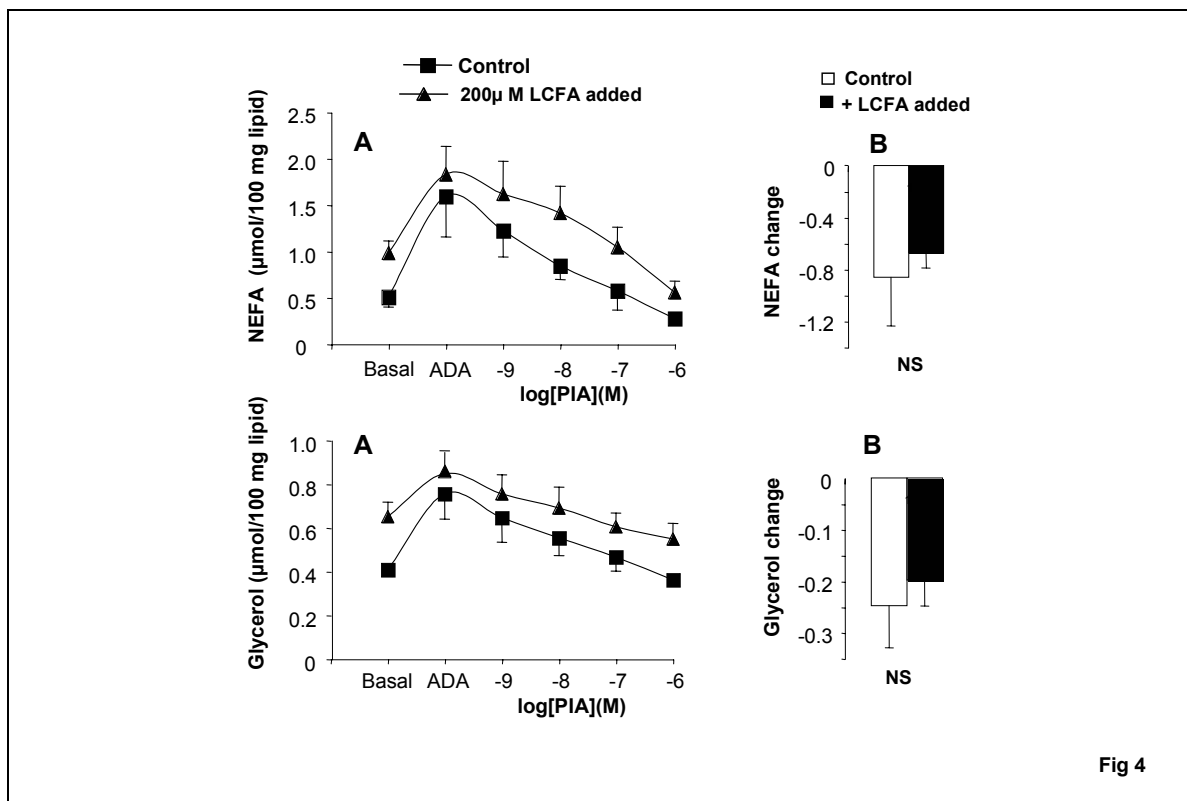
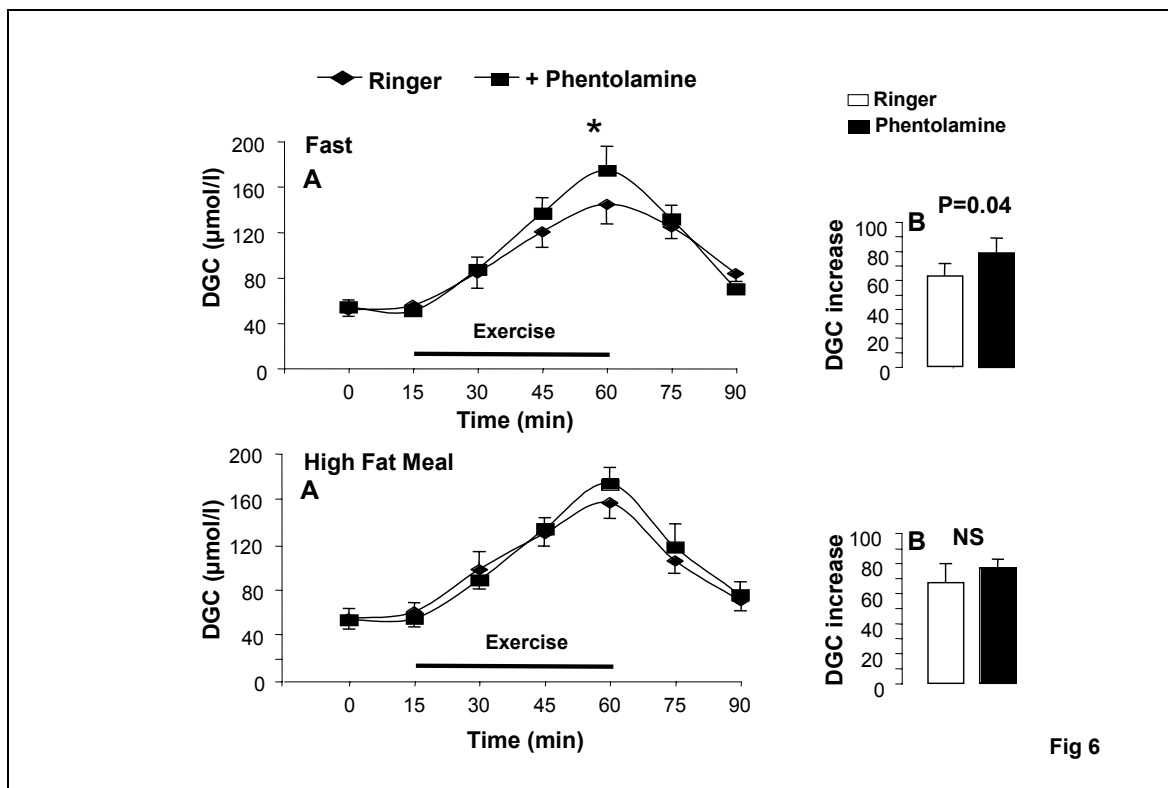
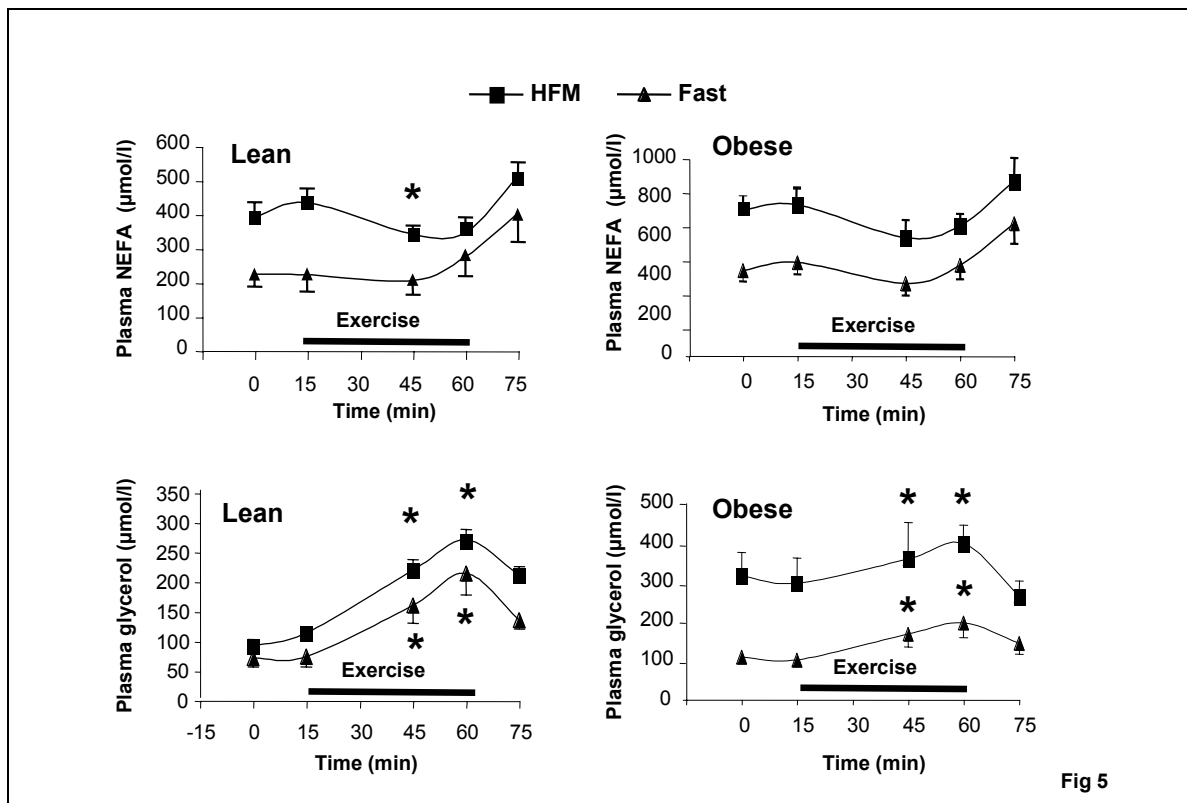


Fig 4



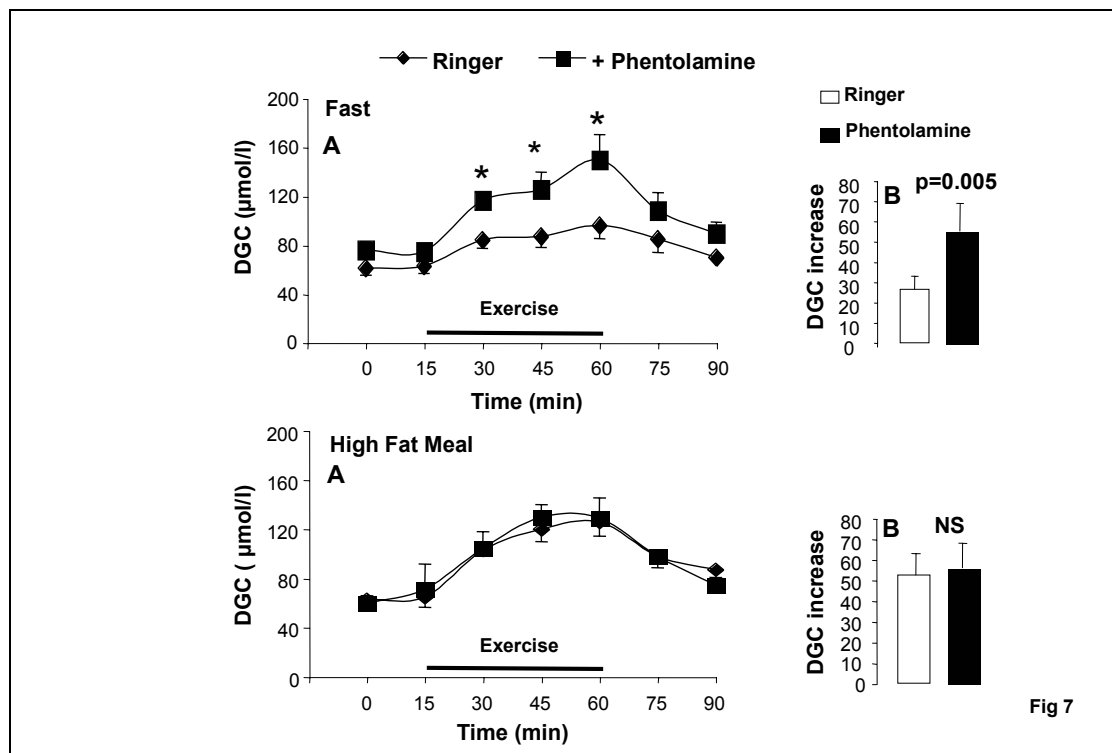


Fig 7