Effect of Macronutrient Composition of the Diet on the Regulation of Lipolysis in Adipose Tissue at Rest and During Exercise: Microdialysis Study

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The aim of the present study was to elucidate, using a microdialysis technique, whether modifications in the proportion of fat in the diet influence lipid mobilization from adipose tissue in situ. Nine healthy volunteers (age, 23.4 ± 0.2 years; body mas index [BMI], 23.5 ± 1.6 kg/m^2) were fed, in random order, with a high-fat diet (HFD) (65% of energy content fat, 15% protein, 20% carbohydrate) or a high-carbohydrate diet (HCD) (70% carbohydrate, 15% protein, 15% fat) for 5 days, with a washout period of 10 days between the diets. Subjects were studied in the fasting state on the morning following days 4 and 5 of each diet. We measured the concentration of extracellular glycerol (EGC) in adipose tissue in response to (1) pharmacologic stimulation with isoprenaline (1 and 10 μmol/L) in situ, (2) stimulation with intravenous infusion of epinephrine (0.0375 μg/min/kg body weight), and (3) submaximal aerobic exercise (50% $\dot{V}O_2$max, 60-minute duration). No effect of the diet composition was found in the increases of EGC in response to isoprenaline (area under the curve [AUC]: HFD, 1,534 ± 370 μmol/90 min; HCD, 1,108 ± 465 μmol/90 min; not significant [NS]) or epinephrine stimulations (AUC: HFD, 190 ± 92 μmol/30 min; HCD, 251 ± 298 μmol/30 min; NS). The exercise-induced increase in EGC was higher during the HFD (AUC: HFD, 1,641 ± 181 μmol/60 min; HCD, 963 ± 156 μmol/60 min; $P < .05$) and was associated with a higher exercise-induced response of norepinephrine ($P < .05$) and epinephrine ($P = .056$) and lower insulinemia during exercise. The results suggest that macronutrient composition of diet does not affect the beta-adrenergic responsiveness of adipose tissue to catecholamine action at rest. During exercise, the HFD promotes higher lipolysis in adipose tissue and this is associated with a higher catecholamine response and lower insulinemia.

HIGH-FAT DIETS (HFD) have been shown to be associated with a higher risk of obesity.[1] Thus, in the treatment of obesity, it is currently recommended to reduce both the energy intake and the proportion of fat in the diet. Diets to treat obesity are expected to promote the reduction of fat mass through mobilization of triglycerides from fat depots. During hypocaloric diets, the mobilization of free fatty acids (FFA) and glycerol from adipose tissue is facilitated by an enhanced responsiveness of this tissue to catecholamine lipolytic action.[2,3] Little is known as to whether modifications of fat content in the diet without changing the total calorie intake would change the sensitivity of adipocyte to catecholamine action, ie, whether lowering the proportion of fat would enhance and increasing the proportion would blunt the lipolytic response to catecholamine stimulation. One in vitro study of catecholamine action on adipocytes[4] did not find changes in beta- and alpha$_2$-adrenergic pathways when comparing HFD and low-fat diets. On the other hand, a few in vivo studies[5,6] have shown a rise in resting plasma glycerol and FFA levels and an increase in exercise-induced response of these metabolites during a HFD, which suggests an increase in the rate of whole body lipolysis at rest and during exercise. A recent study using the stable isotope technique in humans[7] showed a higher whole-body lipolysis during exercise in fasted subjects, as assessed by the rate of whole-body glycerol appearance, during a diet with higher fat proportion (22%) compared with an extremely low-fat diet (2%). Another in vivo study in humans using the same stable isotope technique[8] did not find any difference in the whole body rate of apperance of plasma-derived FFA during rest and exercise between subjects consuming the HFD and low-fat diet. Thus, the results are conflicting and do not seem to answer the question about the effect of the macronutrient composition of the diet on lipolysis regulation in adipose tissue, which remains of interest for the treatment of mild obesity as well as for sportsmen and physically active people.

The aim of this study was, therefore, to investigate the effect of the macronutrient composition of the diet on the responsiveness of subcutaneous adipose tissue (SCAT) to catecholamine lipolytic action in vivo. Microdialysis of SCAT was used for this purpose as it enables in situ evaluation of lipolysis. In order to evaluate the phenomenon on different levels, various adrenergic stimulations were used: pharmacologic stimulation either with isoprenaline perfused in the microdialysis probes in situ or with epinephrine administered intravenously, and physiologic stimulation with a single bout of exercise.

MATERIALS AND METHODS

Subjects

Nine nontrained men were selected for the study. All were drug-free and, when entering the study, had been on their habitual diet which could be considered as a low fat (fat in the range of 30% to 40% of the total calorie intake). The characteristics of the subjects are given in

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Table 1. Characteristics of the Subjects

Age (yr)	23.4 ± 0.2
Weight (kg)	78.1 ± 3.1
BMI (kg/m^2)	23.5 ± 1.6
Fat percentage (%)	14.3 ± 1.1
$\dot{V}o_2$max (ml/kg/min)	45.3 ± 3.7

Table 1. All subjects had given informed consent before the study and the investigation protocol was approved by the Ethical Committee of The Third Faculty of Medicine in Prague.

Experimental Protocols

Each subject successively followed 2 diets that differed in macronutrient composition; each diet lasted 5 days. The 2 diets were conducted in random order and were separated by 10 days during which the subjects consumed their habitual diet. The subjects were given their defined meals at the Clinical Ward of the University Hospital and were instructed to refrain from eating elsewhere and from consuming alcohol and caffeine. During the diet period they maintained their habitual physical activity and refrained from any strenuous exercise. On days 5 and 6 of each protocol, ie, on the morning following days 4 and 5 of the diet, the subjects were investigated in the laboratory at 8 AM after an overnight fast. The day before each examination they had their last snack containing 500 kJ (or 120 kcal) at 10:30 PM.

On day 5, with subjects in the supine position, microdialysis probes were inserted into SCAT and perfusion of the in situ microdialysis probes was begun. Then an indwelling polyethylene catheter was introduced into an antecubital vein and perfused with saline solution to keep patency. The catheter was used for blood sampling throughout the study protocol. Next, the subjects were placed in a semirecumbent position and stayed at rest for 180 minutes. During this period the microdialysis probes were calibrated and baseline microdialysis and blood samples collected (see respective sections in Methods). Then, the response to isoprenaline perfused in one of the microdialysis probes was followed. Afterwards, the subjects started a 60-minute bout of exercise and the response to exercise was followed in the probe that had not been perfused with isoprenaline. After the end of exercise, subjects were monitored during rest in a semirecumbent position for another 60-minute period.

On day 6, the subjects were examined again using one microdialysis probe. The beginning of the protocol was identical to day 5, but 2 intravenous catheters were inserted: one for blood sampling and the other for intravenous infusion. After the initial rest period, an intravenous infusion of epinephrine was performed and the responses in the microdialysis probe and in plasma were followed. The subjects stayed in the resting position for 1 hour after the end of the epinephrine infusion.

Exercise

The bout of exercise was performed on an electromagnetically braked bicycle ergometer (Ergometrics 800s Ergoline, Jaeger, Höchberg, Germany) at a power output corresponding to 50% of the individual maximal oxygen uptake ($\dot{V}o_2$max). The exercise duration was 6O minutes. During exercise, heart rate was monitored continuously (Polar Accurex Plus Cardiometer Monitor, Bayonne, France) and oxygen uptake was measured ($\dot{V}$max; Sensor Medics, Yorba Linda, CA) at 10, 25, and 50 minutes of exercise for 6 minutes.

Epinephrine Infusion

Epinephrine (Adrenalin Spofa, Spofa, Prag, Czech Republic) was diluted in saline solution (Sol. isotonica, Spofa) and administered intravenously using the perfusion pump at a rate of 0.0375 μg/kg/min for 30 minutes.

Diets

All food was consumed as breakfast, lunch, dinner, and 1 snack per day. The HFD contained (in percentage of total energy) 65% fat (25% saturated fatty acids, 30% monounsaturated fatty acids, 7% polyunsaturated fatty acids), 15% protein, 20% carbohydrate (12% complex carbohydrates). The high-carbohydrate diet (HCD) contained 70% carbohydrate (36% complex carbohydrates), 15% protein, 15% fat (5% saturated fatty acids, 4% monounsaturated fatty acids, 4% polyunsaturated fatty acids). 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 softwear system (Ostrasoft, Ostrava, Czech Republic) was used for calculations.

$\dot{V}o_2$max

One week before the investigation period, $\dot{V}o_2$max) was assessed using a graded test on an electromagnetically braked bicycle ergometer. The initial power output was 50 W and was increased by 25 W every 3 minutes until exhaustion. $\dot{V}o_2$ was measured using a $\dot{V}$max apparatus (Sensor Medics) during the test and the highest $\dot{V}o_2$ value was considered as $\dot{V}o_2$max. The performance characteristics of the subjects are given in Table 1.

Microdialysis

Lipolysis in SCAT was assessed with a microdialysis technique that has been described previously.[9] Microdialysis probes (Carnegie Medecin, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cut-off were inserted percutaneously after epidermal anesthesia (200 μL of 1% lidocaine, Roger-Bellon, Neuilly sur Seine, France) into the SCAT at a distance of 10 cm immediately to the right of the umbilicus. The probes were connected to a microinjection pump (Harvard Apparatus, South Natick, MA) and perfused with sterile Ringer's solution (sodium 154 mmol/L, potassium 4 mmol/L, calcium 2.5 mmol/L, chloride 160 mmol/L) at a flow rate 2.5 μL/min. Dialysate collection was started 40 min after probe insertion. Ethanol (1.7 g/L) was added to the perfusate in order to estimate changes in the local blood flow of SCAT, as previously described.[10,11] The glycerol and ethanol concentrations were measured in the outgoing dialysate. The ratio of ethanol concentration in the samples of outgoing dialysate to that in the ingoing perfusate was calculated (outflow/inflow ratio) and taken as an index of the adipose tissue blood flow changes during the course of the experiment as previously described.[10,12]

First, the recovery ratio was determined for each probe using a modification of the "zero flow" calibration procedure at various perfusion rates. This procedure was recently applied for interstitial glycerol concentration in muscle and adipose tissue[13] and has been previously described by our group.[3,14] Briefly, the probes were perfused at a rate 0.5 μL/min, and then 2.5 μL/min, for 30 minutes at each rate and dialysate collected. Glycerol concentrations in the 2 dialysate samples were plotted (after log-transformation) against perfusion at the rates of 0.5 and 2.5 μL/min. Using a straight line fit, the glycerol concentration at zero flow, corresponding to the interstitial glycerol concentration, was calculated. Values given in the results were in agreement with those determined using a higher number of perfusion rates in lean subjects.[3,14,15]

Thereafter, during the entire duration of the study, the probes were perfused at a rate of 2.5 μL/min and 15-minute fractions of outgoing dialysate were collected. After having sampled 2 basal fractions, the isoprenaline was added to perfusate in one of the probes in 2 rising concentrations (1 μmol/L and 10 μmol/L). Each dose was applied for

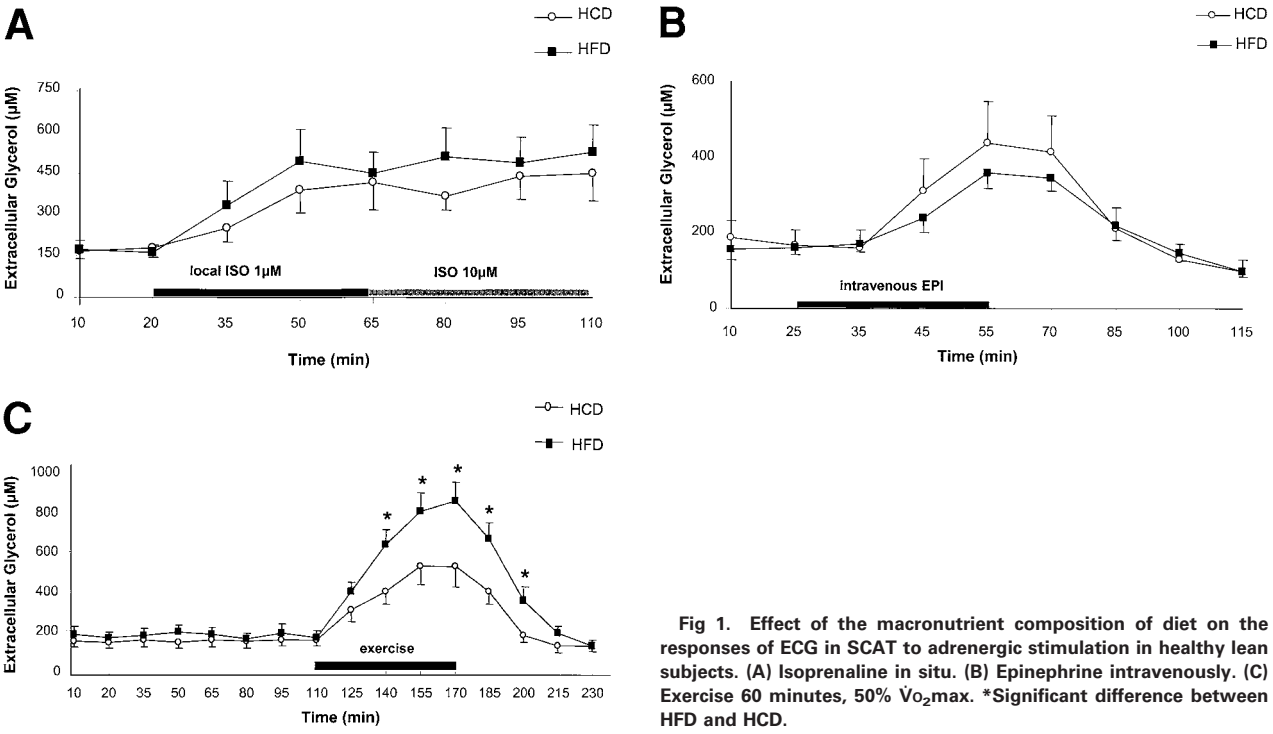

Fig 1. Effect of the macronutrient composition of diet on the responses of ECG in SCAT to adrenergic stimulation in healthy lean subjects. (A) Isoprenaline in situ. (B) Epinephrine intravenously. (C) Exercise 60 minutes, 50% Vo_2max. *Significant difference between HFD and HCD.

45 minutes. All fractions were kept on ice during the experiment. After the end of the study, an aliquot of dialysate was taken from each tube for an immediate assay of ethanol. The remaining dialysate in each tube was kept frozen at -80°C until glycerol analysis.

Blood Sampling

Blood samples were drawn from the intravenous catheter 10 minutes before and again immediately before the first bout of exercise. Thereafter blood sampling was performed in 15-minute intervals. Blood was collected on 50 μL of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseille, France) in order to prevent the oxidation of catecholamines and immediately centrifuged in a cold-refrigerated centrifuge and the plasma stored at -80°C until analysis.

Analytical Methods

Glycerol in dialysate (10 μL) and in plasma (20 μL) was analyzed with an ultrasensitive radiometric method[16]; the intra-assay and interassay variabilities were 5.0% and 9.2%, respectively. Ethanol in dialysate and perfusate (5 μL) was determined with an enzymatic method.[17] Plasma glucose and nonesterified fatty acids (NEFA) were determined with a glucose-oxidase technique (Biotrol, Paris, France) and an enzymatic procedure (Wako, Unipath, Dardilly, France), respectively. Plasma insulin concentrations were measured using radioimmunoassay (RIA) kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France). Plasma epinephrine and norepinephrine were assayed in 1-mL plasma aliquots by high-pressure liquid chromatography using electrochemical (amperometric) detection. The detection limit was 10 pg per sample.

Statistical Analysis

All values are means ± SEM. Paired *t* test and repeated-measures analysis of variance (ANOVA) with diet and time as factors were used for statistical comparisons. When appropriate, plasma and extracellular responses were calculated as the total changes over baseline values (areas under the curves [AUC]) from time corresponding to the beginning of pharmacologic (epinephrine or isoprenaline perfusion) or physiologic (exercise) activation to time corresponding to the end of activation. AUC was calculated using a trapezoidal method. Significance values are quoted in tables and figures. $P < .05$ was considered to be statistically significant. Statistical analysis was performed with a statistical softwear package (Statview II, Abacus Concepts, Berkeley, CA).

RESULTS

General Observations

There were no significant variations in body weight and body fat (assessed using skinfold measurement)[18,19] during the diets when compared to basal conditions before each diet. The mean energy intake was 5,000 ± 1,460 kJ (2,200 ± 350 kcal) and was the same during both diet regimens (see Methods).

Microdialysis of SCAT

The mean concentration of glycerol in dialysate was 61.3 ± 10.3 μmol/L. Using the calibration procedure described above, the recovery ratio and the actual concentration of glycerol in the extracellular compartment were calculated for each probe. The average concentration of extracellular glycerol (ECG) at rest was 214.4 ± 22.6 μmol/L. This value was 3 to 5 times higher than the resting glycerol concentration in plasma, indicating that there was a net release of glycerol from adipose tissue.

Local isoprenaline perfusion at rest. Isoprenaline perfused at 1 μmol/L and, subsequently, 10 μmol/L in one of the probes induced an increase of ECG during both dietary regimens (Fig 1A). The increase over the baseline values became significant

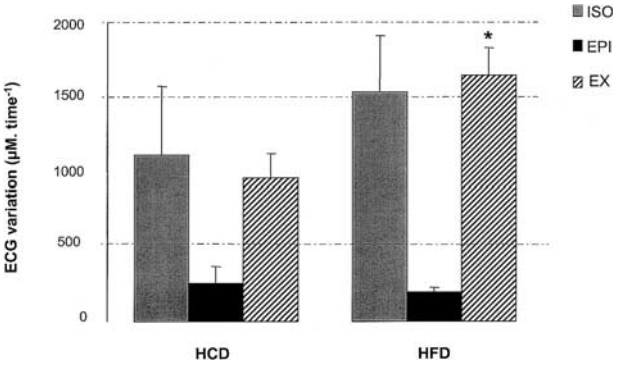

Fig 2. Effect of the macronutrient composition of the diet on the variations of extracellular glycerol concentrations (μmol/L · time^{-1}) during local isoprenaline perfusion (ISO), systemic epinephrine perfusion (EPI), and exercise (EX). *Significant difference between HFD and HCD.

in both diets at concentrations above 1 μmol/L ($P < .05$). ECG levels for the given concentration of isoprenaline tended to be higher on the HFD, although the difference was not significant. The curves representing the isoprenaline-induced increase of ECG concentrations during the HFD and HCD were not different when assessed with AUC method ($P = .2$; Fig 2).

Intravenous epinephrine infusion at rest. Epinephrine infusion elicited significant increase in ECG concentration (Fig 1B). However, when assessed with the AUC method, no difference between the epinephrine-induced increase of glycerol during the HFD and HCD was found (Fig 2).

Exercise. A single bout of exercise induced a significant increase of ECG concentration starting from the 15th minute of exercise ($P < .05$) until the highest value was reached at the 60th minute of exercise (Fig 1C). The value at 60 minutes of exercise was significantly higher during the HFD diet (HFD, 857 ± 91 μmol/L; HCD, 523 ± 93 μmol/L; $P < .05$). During the subsequent 60-minute recovery period, the ECG concentration decreased to values of 127 ± 30 and 126 ± 31 μmol/L for HCD and HFD, respectively, which were not different from those before exercise in any of the diets.

When assessed using the AUC method (Fig 2), the exercise-induced responses of ECG during the HFD were markedly higher than during HCD (1,641 ± 181 *v* 963 ± 156 μmol/L · 60 min^{-1}, $P < .05$).

SCAT blood flow. The nutritive blood flow in adipose tissue was evaluated using the ethanol outflow/inflow ratio. No change due to diet was found in basal values. The outflow/inflow ratio decreased ($P < .05$) in a concentration-dependent manner during isoprenaline perfusion in both diets, indicating the vasodilating effect of isoprenaline. The curves representing responses of the outflow/inflow ratio to local isoprenaline perfusion were not different during the 2 diets (ANOVA with repeated measures with diet and time as factors), indicating that diet did not influence the vasodilating effect of isoprenaline in adipose tissue (Fig 3). Similarly, there was no difference in the vasodilating effect of intravenously administered epinephrine and/or the exercise between the 2 diets (Fig 3).

Plasma Glycerol, FFA, and Glucose Levels

Baseline plasma glycerol levels (92 ± 13 *v* 107 ± 14 mmol/L) did not differ between the 2 diets, while plasma NEFA levels (398 ± 57 *v* 606 ± 92, $P < .05$) were higher during HFD (Table 2).

During intravenous epinephrine infusion, plasma glycerol levels increased significantly throughout the 30-minute infusion. The relative increase of plasma glycerol (expressed in percentage of basal value) in response to epinephrine was not different in the 2 diets (at 30 minutes: HFD, 310% ± 40%; HCD, 290% ± 30%; not significant [NS]).

During exercise, plasma glycerol and NEFA levels increased from the 15th minute of exercise (Table 2). From the 30th minute of exercise, the levels of both metabolites during HFD were higher when compared with HCD. The overall exercise-induced responses when assessed using AUCs were higher for both glycerol and NEFA during HFD. AUCs for glycerol were 792 ± 48 and 320 ± 64 mmol/L for HFD and HCD, respectively ($P < .001$), and for NEFA 844 ± 81 and 339 ± 156 mmol/L for HFD and HCD, respectively ($P < .05$).

Baseline blood glucose was significantly lower during HFD (3.99 ± 0.14 *v* 4.48 ± 0.14 mmol/L, $P < .05$). During both diets, exercise failed to induce a significant change from the baseline levels. However, blood glucose levels throughout the exercise remained lower during HFD in comparison to HCD.

Plasma Hormonal Levels

No differences were found between the 2 diets in the baseline plasma epinephrine and norepinephrine levels. Both epinephrine and norepinephrine increased significantly during the exercise (Table 2) The exercise-induced responses for both catecholamines were higher during the HFD: the difference (assessed by AUC method) was significant for norepinephrine (1,029 ± 115 *v* 860 ± 132 pg/mL, $P < .05$) and was borderline significant for epinephrine (126 ± 26 *v* 91 ± 11, $P = .056$). Insulin levels were lower during the HFD (2.92 ± 0.16 *v* 3.88 ± 0.14 mU/L, $P < .05$). The exercise-induced decreases

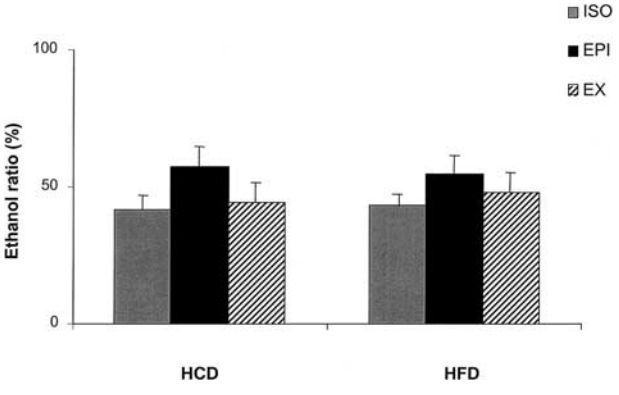

Fig 3. Effect of the macronutrient composition of diet on the ethanol outflow/inflow ratio in subcutaneous adipose tissue during isoprenaline perfusion (last 15 min), epinephrine perfusion (last 10 min), and exercise (last 15 min). #Significantly different from rest value. *Significant difference between high-fat and high-carbohydrate diet.

Table 2. Plasma Metabolic and Hormonal Responses to Exercise (60 minutes at 50% $\dot{V}O_2$max) After 5 Days of High-Fat and High-Carbohydrate Diets

		Exercise		Recovery	
	Rest	30 min	60 min	90 min	120 min
NEFA (μmol/L)					
HCD	398.1 ± 57.2	437.7 ± 67.5	467.1 ± 77.7	409 ± 55.4	400 ± 49.2
HFD	605.9 ± 91.9*	848.4 ± 101.1*	937.7 ± 93.7*	824.2 ± 83.0*	442.7 ± 47.9
Glycerol (μmol/L)					
HCD	91.7 ± 13	180.3 ± 23.7	233.6 ± 36.3	99.3 ± 17.9	102.7 ± 15.3
HFD	106.6 ± 13.9	334.3 ± 20.4*	412.1 ± 27.9*	162.2 ± 20.4*	127 ± 22.7
Glucose (mmol/L)					
HCD	4.48 ± 0.14	5.11 ± 0.17	4.57 ± 0.18	4.42 ± 0.19	4.44 ± 0.15
HFD	3.99 ± 0.14*	4.25 ± 0.15*	4.07 ± 0.2*	3.97 ± 0.15*	3.81 ± 0.16*
Insulin (μU/mL)					
HCD	3.88 ± 0.23	4.06 ± 0.33	3.5 ± 0.22	4.03 ± 0.45	3.42 ± 0.20
HFD	2.92 ± 0.16*	3.21 ± 0.20*	2.78 ± 0.14*	4.00 ± 0.45	2.94 ± 0.18
Epinephrine (pg/mL)					
HCD	25.1 ± 3.7	78.9 ± 8.9	94 ± 10.6	23.4 ± 4.7	24.6 ± 5.3
HFD	24.4 ± 3.4	102.1 ± 19*	118.1 ± 24.7	28.5 ± 4.6*	29.1 ± 2.8
Norepinephrine (pg/mL)					
HCD	209.6 ± 13.9	734.5 ± 92.2	871 ± 88.6	223.6 ± 12.3	220.6 ± 19.2
HFD	185 ± 10.9	822.8 ± 67.7	963.8 ± 106.6	240.1 ± 17.5	227.1 ± 17.5

*$P < .05$, significant difference between HFD and HCD.

in plasma insulin were not different for either diet; nevertheless, plasma insulin was significantly lower throughout exercise during the HFD.

DISCUSSION

The aim of this study was to investigate whether a short-term alteration of the macronutrient composition of diet could change the metabolic characteristics of adipose tissue in vivo, namely, adrenergic regulation of lipolysis. Microdialysis of subcutaneous adipose tissue was used for assessment of lipolysis in situ during pharmacologic and physiologic stimulations of adipose tissue lipolysis.

The main results of the study suggest that a short-term HFD, when compared to a HCD, does not change the in situ responsiveness of adipose tissue to pharmacologic adrenergic stimulation. However, in response to exercise stimulus, the lipolytic response is higher during the HFD. This enhancement of stimulated lipolysis is associated with changes in the hormonal environment of the adipocyte produced by the HFD, namely, an increase in exercise-induced catecholamine responses and lower insulinemia.

The absence of the effect of the HFD on the adipocyte's responsiveness to the beta-adrenergic agonist isoprenaline, demonstrated in this study under in vivo conditions, is in agreement with previous results obtained with in vitro techniques.[4] In the latter study, a lack of the effect of HFD on the responsiveness to beta-receptor–mediated stimulation in isolated adipocytes from subcutaneous tissue was found. In our study, the lack of change of beta-adrenergic responsiveness was demonstrated directly in SCAt by in situ isoprenaline perfusion. However, any comparison of in vivo and in vitro results must be made with caution, as the in vivo adipocyte responsiveness to catecholamines is influenced by its hormonal environment, specifically by insulin and by local agents (adenosine, prostaglandins, etc), which may all modify adipocyte responsiveness to adrenergic lipolytic stimuli.[20-22]

The other pharmacologic approach we used for stimulation of the adipose tissue lipolysis was a 30-minute intravenous epinephrine infusion. Several studies have demonstrated the stimulation of lipolysis in SCAT in situ by intravenous infusion of epinephrine using a microdialysis technique.[23,24] Although epinephrine infusion increased both extracellular and plasma glycerol concentrations, we did not find any significant differencies between the responses during HFD and HCD. Therefore, in resting conditions, it was shown that, whatever the pharmacologic adrenergic stimulation procedure (local or general) used, the composition of the diet did not alter the adipose tissue lipolytic responsiveness.

Contrary to resting conditions, during exercise the increase of ECG concentration was higher during HFD when compared to HCD.

ECG concentrations are influenced by local blood flow in adipose tissue: the higher adipose tissue blood flow contributes to lowering ECG levels.[25] In this study, the ethanol wash-out technique, which has been shown to give a good semiquantitative assessment of the changes of adipose tissue blood flow,[10] was used. The diet composition did not influence the responses of adipose tissue blood flow to catecholamine stimulation, either by isoprenaline in situ or by epinephrine in vivo, or by exercise. Therefore, changes (or lack of changes) in the responses of extracellular glycerol to catecholamine stimuli and to exercise do reflect alterations of lipolysis in adipose tissue.

The enhancement of exercise-induced lipolytic response in adipose tissue during HFD—when compared with HCD—evaluated in our study by direct microdialysis, could correspond to higher exercise-induced response of plasma glycerol (and NEFA), which was found during HFD in previous studies.[5,6,26] However, when assessing the lipolytic response to

exercise by the responses of plasma metabolites we have to take into account that plasma levels are determined not only by lipolysis in adipose tissue but by utilization of products of lipolysis as well.

Measuring the glycerol rate of appearance (Ra) with the stable isotope technique is reported to give a more reliable index of whole body lipolysis than plasma metabolites levels.[27] Using this technique Coyle et al[7] showed that the exercise-induced whole body lipolytic response (Ra) was lower during a very-low-fat (2%) diet when compared with a diet containing 22% fat. In this last study, the Ra of NEFA did not differ in the 2 diets. The same finding was reported in another study[8]: the Ra of plasma palmitate was not modified either at rest or during exercise after a 7-day HFD. However, the Ra of FFA may not be the best index of lipolytic activity because a portion of the fatty acids released during lipolysis are re-esterified and thereby retained within adipose tissue. It seems that glycerol Ra provides a better index of whole-body lipolytic rate.[27] All of the above mentioned studies provided an index of whole-body lipolytic rate while in the present study the responses of ECG provided an index of local lipolytic rate in subcutaneous adipose tissue. Nevertheless, the enhancement of exercise-induced lipolysis during the HFD found in the present study appears to be consistent with the findings of exercise-induced responses of glycerol variables in the above-mentioned studies. Moreover, our study showed that at rest the adipose tissue responsiveness to catecholamine was not altered by the macronutrient composition of the diet. Therefore, it might be suggested that the HFD-induced increase in lipolytic response during exercise is not due to a variation of adipose tissue adrenergic sensitivity but to changes in hormonal response during the HFD.

In agreement with other studies investigating the effects of short-term HFD, we observed that HFD induced a higher norepinephrine and epinephrine responses to exercise and lower insulinemia at rest and during exercise.[5,6,26] Several factors contributing to the higher catecholamine response during the HFD may be suggested. The blood glucose level during exercise was shown to influence the catecholamine responses to exercise.[6,28] Indeed, maintained hyperglycemia during exercise was shown to suppress catecholamine, growth hormone, and glycerol responses to exercise.[29] Furthermore, oral sucrose supplementation during exercise blunted in situ lipolytic and catecholamine exercise-induced responses.[30] In our study, blood glucose levels were lower during the HFD and, thus, could contribute to the higher catecholamine responses during the HFD. Another mechanism involved in the higher epinephrine response might be found in the lower insulinemia during HFD. Galbo et al suggested that the availability of insulin in the period preceding exercise might be an important determinant of the catecholamine response.[6,31]

Partial depletion of glycogen, which was shown to be produced by the HFD of the same duration as that in the present study,[5,26] could play a role in the differences in the hormonal and lipolytic responses between the 2 diets. The glycogen depletion influences the lipid metabolism per se[32,33] or through its effect on blood glucose and hormonal response to exercise.[5,6] The results of the present study are in agreement with our previous finding of enhancement of exercise-induced lipolysis in subcutaneous adipose tissue after a preceding glycogen-depleting exercise bout.[34]

In conclusion, the present study demonstrates that the macronutrient composition of a short-term diet does not alter the in situ responsiveness of SCAT to beta-adrenergic and/or epinephrine lipolytic action. On the other hand, exercise-induced lipolysis in adipose tissue was higher during the HFD compared to the HCD. This enhancement of lipolytic response is associated with changes in hormonal environment of adipocytes: a higher exercise-induced catecholamine response and lower insulinemia.

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