

Sex Differences in Lipolysis-Regulating Mechanisms in Overweight Subjects: Effect of Exercise Intensity

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Abstract

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Objective: To explore sex differences in the regulation of lipolysis during exercise, the lipid-mobilizing mechanisms in the subcutaneous adipose tissue (SCAT) of overweight men and women were studied using microdialysis.

Research Methods and Procedures: Subjects matched for age, BMI, and physical fitness performed two 30-minute exercise bouts in a randomized fashion: the first test at 30% and 50% of their individual maximal oxygen uptake (VO_{2max}) and the second test at 30% and 70% of their VO_{2max} .

Results: In both groups, an exercise-dependent increment in extracellular glycerol concentration (EGC) was observed. Whatever the intensity, phentolamine [α -adrenergic receptor (AR) antagonist] added to a dialysis probe potentiated exercise-induced lipolysis only in men. In a probe containing phentolamine plus propranolol (β -AR antagonist), no changes in EGC occurred when compared with the control probe when exercise was performed at 30% and 50%

VO_{2max} . A significant reduction of EGC (when compared with the control probe) was observed in women at 70% VO_{2max} . At each exercise power, the plasma non-esterified fatty acid and glycerol concentrations were higher in women. Exercise-induced increase in plasma catecholamine levels was lower in women compared with men. Plasma insulin decreased and atrial natriuretic peptide increased similarly in both groups.

Discussion: Overweight women mobilize more lipids (assessed by glycerol) than men during exercise. α_2 -Antilipolytic effect was functional in SCAT of men only. The major finding is that during low-to-moderate exercise periods (30% and 50% VO_{2max}), lipid mobilization in SCAT relies less on catecholamine-dependent stimulation of β -ARs than on an increase in plasma atrial natriuretic peptide concentrations and the decrease in plasma insulin.

Key words: microdialysis, adipose tissue, atrial natriuretic peptide, catecholamines, α_2 -adrenergic receptor

Introduction

Until now, catecholamine release consecutive to sympathetic nervous system (SNS)¹ activation has been considered to be the unique pathway controlling lipid mobilization in humans. Catecholamines activate fat cell lipolysis through $\beta_{1,2}$ -adrenergic receptor (AR) activation and inhibit the process through stimulation of α_2 -ARs (1–3). There is a growing body of evidence that the control of lipid mobilization during exercise does not involve only catecholamine release and inhibition of insulin secretion. Moro et al. (4) and Hellstrom et al. (5) have demonstrated that a

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¹ Nonstandard abbreviations: SNS, sympathetic nervous system; AR, adrenergic receptor; SCAT, subcutaneous adipose tissue; ANP, atrial natriuretic peptide; NP, natriuretic peptide; NPR-A, NP plasma membrane receptor A; cGMP, 3',5'-cyclic guanosine monophosphate; NEFA, non-esterified fatty acid; VO_{2max} , maximal oxygen uptake; EGC, extracellular glycerol concentration; GH, growth hormone; SE, standard error; ATBF, adipose tissue blood flow.

Table 1. Anthropometric and physical fitness parameters of the subjects

	Men	Women	<i>p</i>
Age (yrs)	31.2 ± 2.3	26.7 ± 2.1	NS
Weight (kg)	83.7 ± 3.4	70.9 ± 2.0	0.005
Height (cm)	171.1 ± 2.6	161.3 ± 2.3	0.007
BMI (kg/m ²)	27.9 ± 0.4	27.2 ± 0.5	NS
Fat (%)	26.3 ± 1.4	40.5 ± 1.8	0.0001
LM (%)	58.6 ± 6.2	39.3 ± 3.5	0.0001
VO _{2max} (l/min)	3.2 ± 0.1	2.1 ± 0.1	0.0001
VO _{2max} /LM (ml/min/kg)	54.5 ± 1.4	53.2 ± 1.9	NS

NS, not significant; LM, lean mass; VO_{2max}, maximal oxygen uptake. Values are means ± standard error.

very significant lipolytic rate still remains during exercise performed under β -blockade. We have shown that β -AR blockade [i.e., a local infusion of propranolol directly into subcutaneous adipose tissue (SCAT) through a microdialysis probe or an oral administration of tertatolol] did not prevent exercise-induced lipid mobilization in young, lean, sedentary men. This exercise-induced lipolysis resistant to β -blockade treatment was attributed to an atrial natriuretic peptide (ANP) effect (4). Therefore, ANP released by the heart during exercise might contribute to the physiological control of lipid mobilization during physical exercise concomitantly with plasma catecholamines and insulin. Indeed, we have shown, using isolated fat cells, that natriuretic peptides (NPs) control human fat cell lipolysis (6). The lipolytic activity of ANP and brain NP is mediated by specific NP plasma membrane receptors (NPR-A subtype) bearing a guanylyl cyclase activity (6,7). When the NPR-A is activated, a quick and sustained increment in intracellular 3',5'-cyclic guanosine monophosphate (cGMP) levels occurs, leading to hormone-sensitive lipase activation and lipolysis stimulation. Plasma concentrations of glycerol and non-esterified fatty acids (NEFAs) are increased by intravenous infusion of human ANP in humans (8,9). Human ANP, when delivered systemically, is able to cross endothelium and has direct access to fat cells to activate lipid mobilization.

Exercise leads to both SNS activation (5,10) and NP release, both events leading to lipid mobilization in SCAT. Sex differences in catecholamine-induced lipid mobilization have been reported previously in obese and non-obese patients. We have shown that activation of α 2-ARs impairs exercise-induced lipid mobilization in SCAT of obese men (11) and that this effect is weaker in obese women (12). In addition, other studies have shown that during exercise, no α 2-antilipolytic effect was observed in lean women, whereas it did appear in lean men (5). Bearing in mind these previous observations and the discovery of the new ANP-

dependent lipolytic pathway, sex differences in adrenergic and non-adrenergic regulation of exercise-induced lipid mobilization were reconsidered and assessed. The present study was designed to evaluate the factors controlling lipid mobilization in SCAT of overweight men and women and to delineate possible differences according to exercise intensity in both sexes. Performing exercise bouts at various intensities was expected to reveal differences in the responses initiated by various hormones released during the exercise bouts, such as catecholamines and ANP, or inhibited by exercise (i.e., insulin).

Research Methods and Procedures

Subjects

Nine young, healthy, overweight men (BMI, 27.9 ± 0.4 kg/m²; range, 26.2 to 28.4) and nine healthy, overweight women (BMI, 27.2 ± 0.5 kg/m²; range, 25.4 to 30.4) were included in the study. Anthropometric and physical fitness variables of the subjects are depicted in Table 1. All subjects were drug-free, and their weight had remained stable for at least 3 months before the beginning of the study. Written informed consent was obtained before the experiment began. The studies were performed according to the Declaration of Helsinki and approved by the Ethical Committee of Toulouse Hospital. One week before the investigation, a maximum exercise test was performed by each subject on a bicycle ergometer (Ergometrics 800; Ergoline; Jaeger, Wuerzberg, Germany) to determine the maximal oxygen uptake (VO_{2max}) of each subject using indirect calorimetry (Oxycon Pro; Jaeger). Each individual value was used to define absolute workload corresponding to 30%, 50%, and 70% of VO_{2max}.

Design of the Study

The study was designed to investigate hormonal mechanisms controlling lipolysis at different exercise intensities.

Table 2. Plasma concentrations of catecholamines, glucose, insulin, lactate, GH, ANP, cortisol, leptin, and adiponectin at rest and during exercise bouts in men and women

	Rest	Exercise (%VO _{2max})		
		30%	50%	70%
Men				
Norepinephrine (pg/mL)	412 ± 20	701 ± 52*	1103 ± 101*	2049 ± 196*
Epinephrine (pg/mL)	66 ± 3	100 ± 11*	156 ± 23*	215 ± 22*
Glucose (mM)	5.0 ± 0.1	5.1 ± 0.1	4.9 ± 0.3	4.8 ± 0.1
Insulin (mU/mL)	15.7 ± 2.8	13.4 ± 1.5	11.2 ± 1.8*	11.4 ± 2.8*
Lactate (mM)	0.8 ± 0.1	0.9 ± 0.2	1.9 ± 0.4*	4.1 ± 0.4*
GH (ng/mL)	4.2 ± 0.4	4.9 ± 0.3	5.8 ± 0.9*	8.2 ± 0.9*
ANP (pg/mL)	106.3 ± 8.3	140.4 ± 10.6*	180.8 ± 15.8*	224.4 ± 23.9*
Cortisol (nM)	235 ± 34	263 ± 26	480 ± 75*	565 ± 39*
Leptin (ng/mL)	8.6 ± 1.5	8.6 ± 1.3	8.8 ± 1.7	8.7 ± 1.4
Adiponectin (μg/mL)	3.6 ± 0.7			
Women				
Norepinephrine (pg/mL)	426 ± 36	578 ± 25*	861 ± 41*†	1708 ± 151*†
Epinephrine (pg/mL)	69 ± 2	79 ± 1*†	104 ± 10*†	166 ± 17*†
Glucose (mM)	4.9 ± 0.1	5.1 ± 0.3	5.1 ± 0.2	4.9 ± 0.1
Insulin (mU/mL)	16.2 ± 1.3	15.0 ± 0.8	13.7 ± 1.2*	12.4 ± 1.4*
Lactate (mM)	0.6 ± 0.1†	0.7 ± 0.1	1.3 ± 0.2*†	3.6 ± 0.4*
GH (ng/mL)	4.4 ± 0.3	4.8 ± 0.4	9.3 ± 0.8*†	14.3 ± 1.8*†
ANP (pg/mL)	90.1 ± 1.1	135.2 ± 15.6*	160.9 ± 15.5*	219.7 ± 24.8*
Cortisol (nM)	348 ± 82	340 ± 79	442 ± 101*	463 ± 79*
Leptin (ng/mL)	32.5 ± 3.1†	33.1 ± 3.0†	33.4 ± 3.0†	33.5 ± 3.0†
Adiponectin (μg/mL)	6.0 ± 0.8†			

GH, growth hormone; ANP, atrial natriuretic peptide.

* $p < 0.05$ when compared with resting values.† $p < 0.05$ when compared with mean values.

Before the investigation, fat mass and lean mass were measured using a total body Dual-Energy X-ray Absorptiometer (Lunar-DPX, Madison, WI). Subjects were investigated at 8 AM after an overnight fast. Two separate experiments were performed. On the first study day, the subjects performed exercise at 30% of their VO_{2max} (low exercise intensity) for 30 minutes, followed by exercise at 50% of their VO_{2max} (moderate intensity) for 30 minutes. On the second study day, subjects performed a similar exercise at 30% of their VO_{2max} (low intensity) for 30 minutes, followed by an exercise at 70% of their VO_{2max} (high intensity) for 30 minutes. The days for the experiments were separated by one month according to a double-blind randomized cross-over procedure. Women were investigated at the beginning of their menstrual cycle.

For each exercise session, three microdialysis probes were inserted into the abdominal SCAT at a distance of 10 cm from the umbilicus and on either side of the umbilicus.

The probes were connected to a microinjection pump (Harvard Apparatus, Les Ulis, France). One probe (control probe) was perfused with Ringer's solution (Braun Medical, Boulogne, France). The second was supplemented with 100 μM phentolamine (a non-selective α₁/α₂-AR antagonist). This drug was used to prevent activation of fat cell α₂-AR during exercise (11). It is the only available agent with α₂-AR antagonist properties for use in microdialysis assays. The third probe was perfused with 100 μM phentolamine plus 100 μM propranolol (a non-selective β-AR antagonist). All perfusate solutions were also supplemented with ethanol (1.7 g/L) to assess changes occurring in SCAT blood flow in the vicinity of the probes, using the previously validated ethanol escape method (13,14). After a 60-minute equilibration period, the simplified calibration method selected for this study was applied as previously described (11). The average recovery of the probes was 29 ± 3% (mean ± standard deviation); individual values were used

Table 3. Dialysate concentration of cGMP (picomolar) and glycerol (micromolar) at rest and during exercise bouts in men and women in probes perfused with phentolamine and propranolol

	Rest	Exercise (% VO_{2max})		
		30%	50%	70%
Men				
cGMP	3.3 ± 0.81	3.43 ± 0.35*	6.03 ± 0.76*	10.02 ± 1.45*
Glycerol	220.6 ± 17.0	304.9 ± 25.5*	357.4 ± 25.8*	428.1 ± 28.7*
Women				
cGMP	3.1 ± 0.6	6.64 ± 1.9*	11.4 ± 5.4*	12.4 ± 5.2*
Glycerol	209.4 ± 14.1	305.4 ± 21.7*	360.5 ± 16.9*	375.4 ± 27.4*

cGMP, 3',5'-cyclic guanosine monophosphate.

* $p < 0.05$ when compared with resting values.

for calculation of extracellular glycerol and cGMP concentrations. The extracellular glycerol concentrations (EGCs) found in the present study fit with previous determinations (15).

In each probe, a 30-minute fraction of the outgoing dialysate was collected for determination of resting values. Then, the subjects performed successive exercise bouts on a bicycle ergometer. During each exercise bout and the recovery periods, 30-minute fractions of the dialysate were collected in each probe. Before exercise, and at the 10th and 30th minutes of each exercise bout, 10 mL of blood was collected from an in-dwelling polyethylene catheter inserted into an antecubital vein for plasma determinations. Blood was collected on 50 μ L of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseilles, France) to prevent catecholamine oxidation and processed immediately in a refrigerated centrifuge. The plasma was stored at -80°C until analysis. At rest and during exercise, blood pressure was measured with an exercise-adapted monitor, the Tango Stress Test BP Monitor (Suntech Medical Instruments, Inc., Raleigh, NC).

Drugs and Biochemical Determinations

Phentolamine methanesulfonate (Regitine) and propranolol chlorhydrate (Avlocardyl) were obtained from Novartis (Reuil-Malmaison, France) and Zeneca Pharma (Cergy, France), respectively. Glycerol in dialysate and in plasma was analyzed with an ultrasensitive radiometric method (16). Ethanol in dialysate and perfusate (5 μ L) was determined with an enzymatic method (17). Plasma glucose was determined with a glucose-oxidase technique (Biotrol kit; Merck-Clevenot, Nogent-sur-Marne, France) and NEFA by an enzymatic procedure (Wako kit; Unipath, Dardilly, France). Plasma insulin concentrations were measured using a radioimmunoassay kit from Sanofi Diagnostics Pas-

teur (Marnes la Coquette, France). Plasma epinephrine and norepinephrine were assayed in 1-mL aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection. The detection limit was 5 pg/sample. Plasma ANP and growth hormone (GH), collected on EDTA (1 mg/mL) and aprotinin (500 KIU/mL), respectively, were determined using radioimmunoassay kits from Peninsula Laboratories (San Carlos, CA).

Statistical Analysis

Data are presented as means \pm standard error (SE). Extracellular glycerol, cGMP, and plasma hormones and metabolites were measured before and during exercise. ANOVA with or without repeated measures models were fitted to test for differences between sex (between-factor), exercise intensities (within-factor, repeated), or probes (within-factor, repeated). All values were tested for normality using the Shapiro-Wilk test for normality. A probability of 0.05 was used as the level of significance for ANOVA tests. When a significant main effect was found, significant pair-wise differences were tested using an ANOVA model for each of the factors controlling the effect, with a level of significance set with the Bonferroni's correction. Statistical analyses were performed using software package Stata version 6.0 (StataCorp, College Station, TX).

Results

General Observations

Overweight men and women had a similar BMI, but the percentage of fat mass was higher in women than in men (Table 1). The VO_{2max} was higher in men but was similar when the oxygen consumption value was adjusted for individual free fat mass. As expected, women had a higher

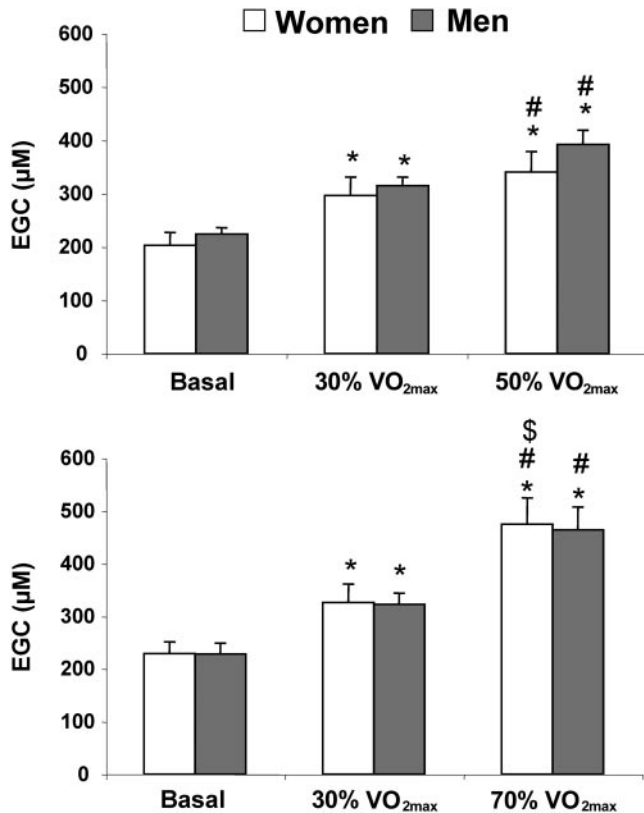


Figure 1: Comparative effect of exercise bouts of increasing intensity on EGC in SCAT in overweight men and women. Values were obtained from a control probe perfused with Ringer's solution. Data are expressed as means \pm SE. * $p < 0.002$ when compared with values measured at rest. # < 0.002 when compared with values measured during exercise at 30% VO_{2max} . \$ $p < 0.02$ when compared with values measured during exercise at 50% VO_{2max} .

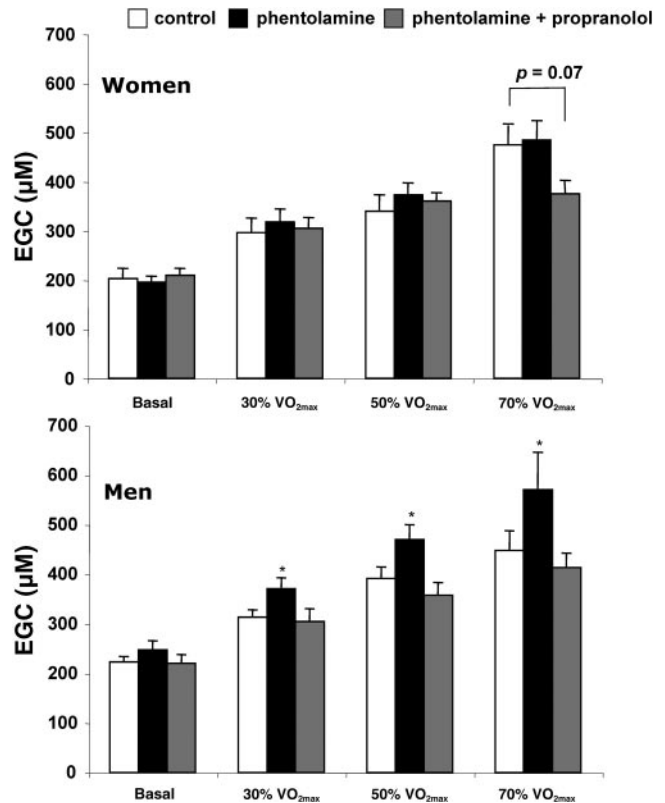


Figure 2: EGC in microdialysis probes implanted in SCAT in overweight men and women. Exercise bouts were performed at increasing intensities. Probes were perfused with Ringer's solution alone (control), Ringer's solution plus phentolamine alone, or Ringer's solution with phentolamine plus propranolol. Values are means \pm SE. For men, * $p < 0.05$ when compared with values obtained with control probe perfused with Ringer's solution. For women, $p = 0.07$ when compared with values obtained with control probe perfused with Ringer's solution.

plasma level of adiponectin (almost double) and leptin (about 4-fold) (Table 2).

Comparative Effect of Exercise on Lipid Mobilization in Adipose Tissue

EGCs at Rest and during Exercise. At rest, the baseline EGC in the control probe or in the probe perfused with phentolamine and propranol was similar in overweight men and women (men, 222 ± 10 and 203 ± 21 , respectively; women, 221 ± 17 and 209 ± 14 μM , respectively). However, a significant difference was observed in the probe perfused with phentolamine, the EGC being higher in men than in women (247 ± 19 and 195 ± 13 μM , respectively; $p = 0.01$).

During exercise, EGC significantly increased according to the exercise intensity in the control probes in the overweight men and women (Figure 1). However, no difference was found in EGC when the exercise was performed at 50% and 70% VO_{2max} in men ($p = 0.14$). The sex-dependent

changes in EGC in SCAT, according to the exercise intensity and drugs infused in the probes, are depicted in Figure 2. In overweight women, EGC increased gradually and significantly in the control probes according to exercise intensity. In overweight men, EGC was significantly increased when comparing exercise bouts at 30% and 50% of VO_{2max} ($p = 0.01$). However, no difference was found when comparing the EGC observed at 50% and 70% VO_{2max} ($p = 0.11$). In overweight men, phentolamine promoted a significant increment in EGC in adipose tissue whatever the exercise intensity when compared with the EGC found in the control probe. Such an enhancement was not observed in SCAT of overweight women. In overweight men, whatever the exercise intensity, the increase in EGC observed in the phentolamine probe was reduced, approaching the level in the control probe, when propranolol was added to phentolamine. Different results were observed in overweight women; i.e., when exercise was performed at 30% and 50%

VO_{2max} , the increase in EGC observed in the probe containing propranolol plus phentolamine was similar to that observed in the control probe or in the probe with phentolamine alone. However, when exercise was performed at 70% VO_{2max} , a tendency of propranolol to promote a reduction of EGC increase when compared with the control probe was observed; however, statistical significance was not reached ($p = 0.07$).

Concentrations of Interstitial cGMP and Glycerol at Rest and during Exercise. cGMP concentration was measured in the probe perfused with propranolol plus phentolamine (i.e., when β - and α_2 -ARs are blocked locally). At rest, extracellular cGMP concentrations in SCAT were equivalent between men and women (3.30 ± 0.81 and 3.10 ± 0.60 pM). Extracellular cGMP and glycerol levels increased in both groups during exercise bouts according to the relative intensity of the workout (Table 3). No significant differences were observed between both sexes whatever the intensity of exercise. A positive correlation was found between mean extracellular cGMP and glycerol values according to exercise intensity ($p < 0.05$).

Adipose Tissue Blood Flow. The ethanol outflow-to-inflow ratio measured at rest was similar in the SCAT of men and women (range, 85% to 90%), irrespective of the microdialysis probe and the experimental session. In the control probe and in both men and women, exercise did not modify the ethanol outflow-to-inflow ratio (Figure 3). In the probe perfused with phentolamine alone, a tendency toward a decrease in ethanol outflow-to-inflow ratio was observed, suggesting an increase in local blood flow when exercise was performed at 30% and 50% VO_{2max} . However, the decrease did not reach significance (p values ranged between 0.06 and 0.07). Such a tendency did not appear when exercise was performed at the high intensity level, at 70% of VO_{2max} , in women. According to some statistics experts, when an intervention is known to change a variable in a known direction, it could be inappropriate to use the Bonferroni correction for multiple comparisons, and the one-sided Student's t tests could be more appropriate. On this assumption, when applying the one-sided Student's t tests, the reduction of the inflow/outflow ratios in the probe with phentolamine was significant ($p < 0.02$) at 30% and 50% VO_{2max} compared with the basal values for both sexes (Figure 3). Finally, in the probes containing phentolamine plus propranolol, whatever the statistical test used, no significant change in ethanol ratio occurred at any exercise level in both sexes.

Comparative Effect of Exercise on Plasma Values

Plasma Glycerol and NEFA Values. Plasma glycerol and NEFA concentrations were quite similar at rest in both groups. An intensity-dependent increment in plasma glycerol concentrations occurred during exercise in both sexes (Figure 4). This response was significantly stronger in

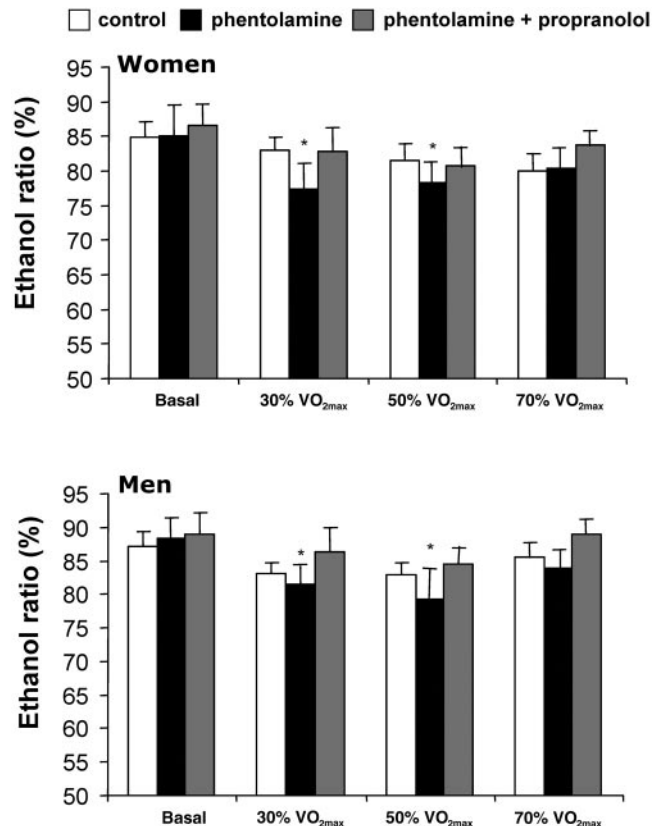


Figure 3: Ethanol ratio in SCAT during exercise bouts performed at increasing intensities in overweight men and women. Probes were perfused with Ringer's solution alone, Ringer's solution plus phentolamine alone, or Ringer's solution with phentolamine plus propranolol. Values are means \pm SE. A one-sided Student's t test was used given that the physiological effects of phentolamine are predictable. * $p < 0.05$.

women than in men. In men, plasma NEFA concentrations did not change during exercise bouts performed at 30% and 50% VO_{2max} . A significant reduction was observed 10 minutes after the beginning of the exercise bout performed at 70% VO_{2max} . In contrast, in women, plasma NEFA levels increased 15 minutes after the beginning of the exercise performed at 30% VO_{2max} and remained elevated all along the exercise even during the second bout performed at 50% VO_{2max} . However, a reduction of plasma NEFA concentration was observed when exercise was performed at 70% VO_{2max} compared with 30% VO_{2max} ($p = 0.001$), and the values of plasma NEFA levels returned to basal values. Finally, whatever the exercise intensity, plasma NEFA values remained higher in women than in men (Figure 4). When exercise stopped, blood NEFA concentrations increased similarly in both sexes.

Plasma Catecholamine, Glucose, Insulin, Lactate, GH, Cortisol, ANP, and Leptin Concentrations Measured at Rest and at the End (30 Minutes) of Exercise Bouts. In resting conditions, plasma levels of norepinephrine and epinephrine

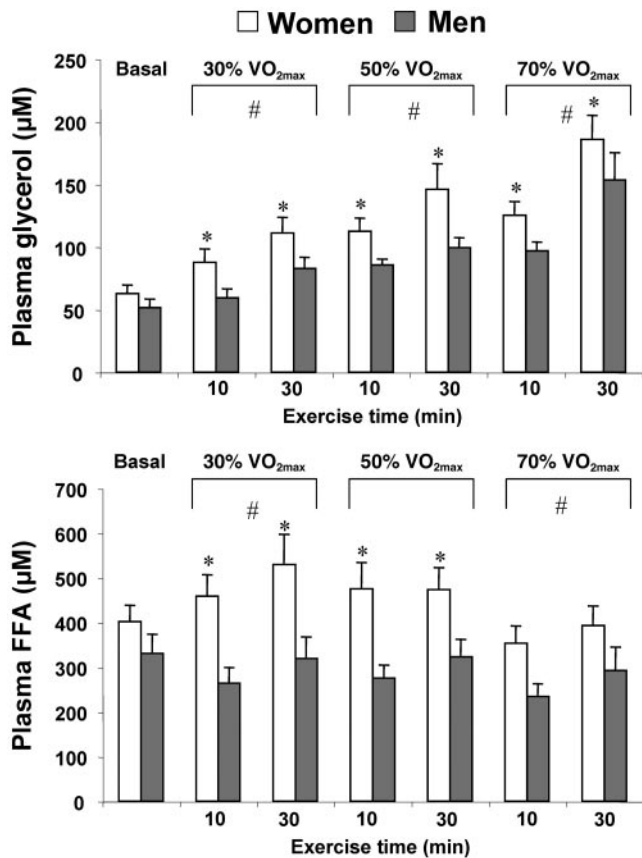


Figure 4: Comparative effects of exercise bouts performed at increasing intensities on glycerol concentrations and plasma NEFAs in overweight men and women. Data are expressed as means \pm SE. * $p < 0.05$, sex-effect difference. # $p < 0.05$, time-effect difference (30 vs. 10 minutes). No significant interaction time-sex term effect at 30%, 50%, or 70% VO_{2max} .

were similar in both groups. Exercise promoted an increase in plasma concentrations of epinephrine and norepinephrine in men and women (Table 2). The rise in norepinephrine levels was higher in men than in women at 50% and 70% VO_{2max} . The increase in plasma epinephrine levels was higher in overweight men than in women whatever the intensity of the exercise bouts (34, 90, and 149 pg/mL vs. 10, 35, and 97 pg/mL at 30%, 50%, and 70% VO_{2max} , respectively). No significant changes in plasma glucose concentration were observed during each exercise bout, in both sexes, whereas plasma insulin levels fell significantly in both groups only during the exercise performed at 50% VO_{2max} . This decrease in plasma insulin was maintained all along the exercise bouts and was not statistically different when compared with the exercise performed at 70% VO_{2max} . At rest, plasma lactate concentration was lower in women than in men. Plasma lactate concentration increased at moderate intensity (50% VO_{2max}) and high intensity (70% VO_{2max}) exercise in both sexes and was higher in men at 50% of VO_{2max} (Table 2).

Plasma GH, ANP, and cortisol concentrations measured at rest were similar in both groups. During exercise, plasma levels of ANP increased similarly in both groups. A major increase in plasma GH was observed in women at 50% and 70% VO_{2max} . As expected, plasma leptin concentrations were higher in women than in men, and exercise did not induce any changes in plasma levels of leptin (Table 2).

Discussion

The main aim of this study was to investigate the factors involved in the regulation of lipid mobilization in SCAT of overweight, healthy, sedentary men and women performing exercise bouts at various intensities. VO_{2max} was similar in both groups after adjustment for fat-free mass (Table 1), and subjects performed all exercise bouts at the same relative workload (percentage of VO_{2max}). The glycerol level in the extracellular space (EGC) was monitored using the in situ microdialysis technique. Glycerol levels in adipose tissue during exercise depend on various factors acting either on lipolytic activity or on adipose tissue blood flow (ATBF). Among these factors, it has been long recognized that catecholamines modulate adipose tissue lipolysis during exercise by stimulation of ARs and through increment of intracellular cAMP level (2). Catecholamines activate cAMP production and lipolysis through β -AR stimulation and inhibit the process through $\alpha 2$ -AR activation (3,18). Fat cell responsiveness to catecholamines depends on the ratio and functional balance between β - and $\alpha 2$ -ARs, which are influenced by sex, anatomical location of the fat depot, and obesity (19). The lipolytic efficiency of epinephrine, which exhibits the highest affinity for the $\alpha 2$ -AR, is decreased in subcutaneous fat cells that express a high number of $\alpha 2$ -ARs (19). Physiological activation of the $\alpha 2$ -adrenergic pathway by epinephrine during exercise partially blunts the lipolytic response in adipose tissue (14).

In addition, it is also known that insulin could reduce cAMP accumulation in the fat cell through activation of a phosphodiesterase-3B. Thus, the reduction of plasma insulin levels occurring during exercise contributes to the physiological control of lipolysis regulation and enhances the lipolytic responsiveness of fat cells (20). Finally, we have shown previously that ANP is a potent lipolytic agent both on isolated adipocytes and when infused directly by a microdialysis probe into SCAT (6). ANP binds to NPR-A and activates a cGMP-dependent pathway in adipocytes. The lipolytic effect of ANP is clearly independent of modulation of cAMP levels (6,7).

Several previous studies have shown sex differences in the adrenergic regulation of lipid mobilization during exercise (21) and have demonstrated that the local adrenergic response involves both ARs (5). In the present study, we found that infusion of propranolol added to phentolamine in the probe reduced by 40% to 50% the increase in EGC

induced by the exercise bout carried out at 70% VO_{2max} in both sexes (when comparing the EGC increase in the probe with phentolamine alone) (Figure 2). This result agrees with previous studies in which propranolol was also found to partially suppress, $\sim 50\%$, exercise-induced glycerol production in SCAT of normal-weight men and women during exercise at two-thirds of their maximum working capacity (2,5). Therefore, it could be proposed that whatever the intensity of the exercise bouts performed in our study, a noticeable proportion of the lipid mobilization is independent of catecholamines (i.e., resistant to β -AR blockade). Thus, in addition to the exercise-induced reduction of insulin secretion, ANP becomes a reasonable candidate to explain the residual lipid mobilization observed during exercise.

The present investigation shows that a low-to-moderate exercise intensity (30% to 50% VO_{2max}) induces a lipid-mobilizing effect in SCAT in overweight men and women. The interstitial levels of glycerol in SCAT were found to be quite similar in overweight men and women, both at rest and during exercise (Figure 1). Isolated fat cells from men and women having the same size range have been shown to exhibit an equal basal lipolytic rate (22). Our results also agree with those showing that the fractional contribution of regional NEFA flux to systemic NEFA flux was similar in both sexes (23). In agreement with values previously reported for obese patients [with a different calibration method, i.e., no-net flux method (15)], we found that the interstitial levels of glycerol were ~ 2 -fold higher in SCAT than in plasma.

In overweight women, it was observed that the blockade of $\alpha 2$ -ARs alone or both $\alpha 2$ - and β -ARs did not influence the increase in EGC when exercise was performed at low and moderate intensities (30% and 50% VO_{2max}). This suggests that catecholamines are not involved in exercise-induced lipid mobilization in the SCAT at these intensities. However, the observation that β -AR blockade was partially efficient when exercise was performed at the high intensity (70% VO_{2max}) level suggests that catecholamines likely contribute to lipid mobilization at the high exercise intensity in women. In contrast, the significant potentiating effect of phentolamine on EGC observed in overweight men suggests that the anti-lipolytic $\alpha 2$ -ARs contribute to the control of lipid mobilization. Because the increase in plasma norepinephrine was observed in both men and women, sex-related differences observed here fit with previously observed responses in normal-weight subjects (5). Accordingly, it was proposed that in men, the SCAT glycerol level was influenced by both β - and $\alpha 2$ -ARs, whereas in women, it was only the β -ARs that appeared to be activated during high exercise level. It was previously suggested that it is only the circulating agents that are involved in the control of lipid mobilization in SCAT (24). The higher increase in epinephrine observed in overweight men, when compared with

overweight women, could explain the potentiating effect on EGC observed when phentolamine was used alone in men because epinephrine is known to possess a higher affinity for $\alpha 2$ -AR (25). In addition, propranolol suppressed the additive effect of phentolamine alone in men, but no difference was found when comparing propranolol plus phentolamine with the control probe; lipid mobilization resistant to propranolol persisted (Figure 2).

Confirming previous results, a higher ethanol outflow-to-inflow ratio was observed in obese patients (11) compared with lean subjects (14). The measurement of ethanol escape through the dialysis probe is a non-quantitative method to estimate changes in ATBF. In agreement with others (5), the stability of ethanol outflow-to-inflow ratio found in the control probe during the two exercise bouts indicated that ATBF did not change. It has been demonstrated previously that β -AR stimulation increased (26) and, in contrast, that α -AR stimulation decreased local ATBF (27). As depicted in Figure 3, under our experimental conditions, phentolamine alone induced a significant increase in ATBF in men and women. Such an increase in blood flow leads to a possible underestimation of lipolysis in this probe at the intensities of 30% and 50% VO_{2max} . Such an effect was not observed when phentolamine and propranolol were combined.

On the basis of these experimental results, it appears that when physical exercise bouts are performed at low-to-moderate intensity, the contribution of the sympathetic nervous activity and circulating catecholamines to the regulation of lipid mobilization is questionable in overweight subjects, particularly in women. Previous studies performed in normal-weight subjects have shown that, even at low or high intensity levels, exercise-induced lipid mobilization in SCAT is only partially reduced by the infusion of propranolol in the probes (4). In the present study performed in women, the lack of blocking effect of propranolol on lipid mobilization in SCAT, during exercise bouts performed at low-to-moderate intensity, confirms that catecholamines are not involved in the control of lipid mobilization.

Several factors could be involved in the initiation of the non-adrenergic-dependent lipid mobilization in SCAT. First of all, a decrease in plasma insulin level may play a crucial role. Because insulin is known to be a potent inhibitor of lipolysis in adipose tissue, it is reasonable to assume that the first event to increase EGC at moderate and high exercise intensity is the reduction in plasma insulin occurring with exercise (Table 2). A second putative factor responsible for non-adrenergic-dependent lipolysis could be ANP, because even at low exercise intensity, plasma ANP level increases. Moreover, a positive relationship was found between plasma ANP and interstitial cGMP levels in SCAT (4). Plasma ANP concentrations increased relative to exercise intensity; the increase was similar in overweight men and

women (Table 2). We have previously shown that ANP promotes a potent increment in intracellular cGMP in isolated fat cells (28). Thus, the cGMP increment in the extracellular space found in the present study could be related to the action of ANP on fat cells and could result from a leakage of cGMP from fat cells of SCAT; the correlation existing between both parameters with respect to the relative workload supports this view (Table 3).

Other relevant endocrine factors could be involved in the exercise-dependent enhancement of lipid mobilization. Plasma GH concentration was increased during exercise. As previously found, GH increase during exercise was higher in women than in men (29). In fact, the lipolytic action of GH usually appears after longer time lags. In vitro studies have shown that GH induced a down-regulation of $G_{\alpha 2}$ from plasma membrane toward a lower density compartment (30). By this mechanism, GH could potentiate cAMP production and lipolysis. A GH effect is very unlikely and cannot be proposed to explain the lipid mobilization observed when α - and β -ARs were blocked by drugs. Plasma cortisol concentration also rose similarly during exercise in men and women. The acute effects of glucocorticoids on lipid mobilization are controversial. It is unexpected that cortisol increases lipolysis over a short-term period. The effects of cortisol on lipolysis have only been demonstrated in fat cells pre-incubated for several hours to days (31). They were associated with circadian rhythms; the morning rise in cortisol was proposed to regulate lipolysis in SCAT (32). Therefore, it is unlikely that the exercise-induced rise in plasma cortisol participates in lipid mobilization during the short-term exercise bouts performed in our study.

Concerning catecholamine release, sexual dimorphisms exist in response to exercise. Hellstrom et al. have shown that for exercise performed at a similar relative workload, plasma norepinephrine, and mainly epinephrine, is increased more in men than in women (5). This result confirms the sex differences found in SNS regulation (29,33). Such sex-related differences in catecholamine responsiveness to exercise were also found in our study on overweight subjects. Despite the lower exercise-induced rise in plasma catecholamines, the overall lipid mobilizing response (assessed by the increase in plasma glycerol levels) was significantly higher in women. Whatever the exercise intensity, a higher increase in plasma glycerol was observed in overweight women than in men, and the level of plasma NEFA remained higher in women than in men (Figure 4). These results show sex-related differences in lipid mobilization in overweight subjects. These increased levels of plasma glycerol and NEFA are probably related to the higher fat mass in overweight women (Table 1). Finally, in both sexes, a relative decrease in plasma NEFA concentration at the higher exercise intensity was observed, whereas glycerol concentration increased. These results fit with previous data

showing that during high intensity exercise, the rate of appearance of NEFA was significantly lower than with decreased exercise intensity (34). Another study (35) showed that the whole lipolytic rate, represented by glycerol appearance, did not differ at two increasing exercise intensities (45% and 65% VO_{2max}). In our study, the increase in plasma glycerol concentration observed at the higher exercise intensity could be related to an exercise-induced splanchnic vasoconstriction that reduces hepatic glycerol uptake.

Some limitations of the present study could exist. First, the $^{133}\text{Xenon}$ (^{133}Xe) dilution technique cannot be applied to evaluate blood flow through a microdialysis probe because it is not possible to predict precisely the volumes of ^{133}Xe diffusion and those of the pharmacological agents in the SCAT (36). When using microdialysis, the volume of adipose tissue explored through the microdialysis probe represents a smaller volume than that explored with ^{133}Xe . The second limitation is related to the lack of a suitable ANP receptor antagonist usable in clinical studies to definitely establish the specific role of ANP.

In conclusion, our study reveals a sex-related difference in the adrenergic control of lipid mobilization during exercise of various intensities. Catecholamines, and more specifically epinephrine, exert an inhibitory effect in vivo on lipid mobilization in male subjects, but the effect is not observed in female subjects. Moreover, our study also demonstrates the physiological relevance of non-adrenergic pathways in the control of lipid mobilization in SCAT during low-to-moderate exercise in overweight humans. There are no significant differences according to sex. The results also strengthen previous in vitro and in vivo studies in which we proposed that the activation of NP receptors could play an important role in the control of lipid mobilization in subcutaneous fat deposits. Finally, it would be of interest to extend our approaches to obese patients with higher BMIs and to study more precisely the involvement of NP receptor activation in the adipose tissue of obese subjects exhibiting low lipid mobilization in SCAT during exercise. It has been shown that the level of NP is lower in obese subjects than in normal-weight subjects (37). Finally, to evaluate the relative role of insulin and ANP during moderate exercise, it would be necessary to suppress the former by maintaining a constant plasma insulin level through an insulin clamp. This point should be the topic of further studies.

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