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Effect of Endurance Training on Adrenergic Control of Lipolysis in Adipose Tissue of Obese Women

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The effect of a 12-wk training program on sc abdominal adipose tissue (SCAAT) was studied in 11 obese women. Before and after the training, biopsies of SCAAT were performed for mRNA levels determination. Using the microdialysis method, involvement of α_2- and β-adrenergic receptor (ARs) in the control of lipolysis in SCAAT was studied using local perfusion of epinephrine alone or supplemented with phentolamine, an α_2-AR antagonist. In addition, the variation in dialysate glycerol concentrations during exercise (50% peak oxygen consumption at 40 min) in a probe perfused with Ringer's solution was compared with that obtained in a probe perfused with Ringer's solution plus phentolamine. Training did not promote changes in the expression of key genes of the lipolytic pathway. The epinephrine-induced rise in the dialysate glycerol concentration was identical before and after training and was similarly potentiated by phentolamine. During exercise, the potentiating effect of phentolamine on the glycerol response was apparent before, but not after, training. The exercise-induced increase in plasma norepinephrine was lower after training ($P = 0.04$). In conclusion, training did not modify either the expression of genes involved in the control of lipolysis or α_2- and β-ARs *in situ* sensitivity to epinephrine in SCAAT. Training reduced the antilipolytic action of catecholamines mediated by α_2-ARs during exercise, probably due to a reduction of exercise-induced catecholamine increase. (*J Clin Endocrinol Metab* 89: 1325–1331, 2004)

THE RISE IN prevalence of obesity and metabolic syndrome in recent decades shows that obesity has become a major threat for public health in developed countries. Nonpharmacological treatments, *i.e.* nutritional intervention and regular physical activity, are the key components of therapy of the metabolic syndrome. Regular physical activity of the aerobic type has been shown to reduce insulin resistance and, consequently, contributes to the cure of metabolic complications of obesity. Among metabolic complications, alterations in adrenergic regulation of lipolysis in adipose tissue have been observed in obese subjects (1–4). These alterations consist in an impairment of β-adrenergic stimulation of lipolysis in sc abdominal adipose tissue (SCAAT) and an increase in the antilipolytic action of catecholamines mediated by α_2-adrenergic receptors (α_2-ARs). The relative contributions of β- and α_2-ARs to the fine tuning of the lipolytic response has been demonstrated by functional *in vitro* studies in isolated human fat cells (5). Studies of lipolysis have shown that the activation of α_2-ARs by epinephrine and norepinephrine impairs the β-adrenergic component of catecholamine-induced lipolysis. In human sc fat cells, where α_2-ARs outnumber β-ARs, the preferential recruitment of the α_2-AR at the lowest catecholamine concentrations inhibits lipolysis (5, 6). The antilipolytic action of catecholamines, particularly that of epinephrine, which exhibits a high affinity for the α_2-AR (7), has been shown to be elevated in isolated sc adipocytes from obese subjects (8). Using microdialysis, it has been shown that α_2-ARs are involved in the regulation of lipolysis during an acute bout of exercise (9). Taking into account that the adipocytes of SCAAT from obese subjects have a high α_2-AR-mediated antilipolytic component *in vitro* (6), it has been demonstrated that the exercise-induced lipolysis in SCAAT is impaired in obese subjects of both genders and that it is the enhanced physiological stimulation of adipocyte α_2-ARs during exercise that contributes to this impairment (4, 10). Furthermore, it has been shown that the α_2-mediated antilipolytic action of catecholamines during exercise can be reduced during a hypocaloric diet (10). The aim of the present study was to investigate whether an alternative intervention, a program of aerobic physical activity, produces changes in the α_2- and β-adrenergic pathways of adrenergic regulation of lipolysis in SCAAT at rest and during exercise. We investigated the adrenergic regulation of lipolysis in SCAAT *in situ* using microdialysis and the expression of key genes involved in lipolysis regulation in biopsy samples of

Abbreviations: AR, Adrenergic receptor; ATBF, adipose tissue blood flow; BMI, body mass index; NEFA, nonesterified fatty acid; SCAAT, sc abdominal adipose tissue; VO_2max, peak oxygen consumption.

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SCAAT using the quantification of mRNA levels by quantitative RT-PCR.

Subjects and Methods

Patients

Eleven obese women (39.2 ± 1.9 yr of age) participated in the study. Mean body weight and body mass index (BMI) were 86.4 ± 2.1 kg and 31.3 ± 0.6 kg/m_2, respectively. All subjects were drug-free, and their weight had remained stable for at least 3 months before the beginning of the study. They all gave written informed consent before the experiments began. The studies were performed according to the Declaration of Helsinki and approved by the ethical committee of the Third Faculty of Medicine (Prague, Czech Republic).

Experimental protocol

The subjects were investigated at 0800 h after an overnight fast while they were in a semirecumbent position, before and after the last day of a 12-wk training program.

Peak oxygen consumption (VO_2max) determination

Five days before each investigation, a maximum exercise test was performed on a bicycle ergometer (Ergoline 800) for each subject to determine the VO_2max (Vmax, Sensor Medics, Yorba Linda, CA). An initial workload of 60 watts was followed by a sequential increase in workload of 30 watts every 3 min until exhaustion. Verbal encouragement was given to attain maximal performance. Heart rate was continuously monitored. The highest VO_2 achieved was taken as the VO_2max, and the workload corresponding to 50% for each subject was calculated.

Microdialysis protocol

At each investigation two microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20 × 0.5 mm with a 20,000 mol wt cut-off were inserted percutaneously after epidermal anesthesia (200 μl of 1% lidocaine, Roger-Bellon, Neuilly-sur-Seine, France) into the abdominal SCAAT at a distance of 10 cm to the right of the umbilicus. The two probes, separated by at least 10 cm, were connected to a microinjection pump (Harvard Apparatus, Les Ulis, France). One probe was perfused with Ringer's solution (139 mmol/liter sodium, 2.7 mmol/liter potassium, 0.9 mmol/liter calcium, and 140.5 mmol/liter chloride), and the second was perfused with Ringer's solution plus 0.1 mmol/liter phentolamine (α-AR antagonist). This nonselective α_1-/α_2-AR antagonist with an efficient α_2-AR antagonist action in human fat cells *in vitro* was the only agent allowed by the ethical committee for use in microdialysis assays in humans. Ethanol (40 mmol/liter) was added to the perfusate to estimate changes in the blood flow, as previously described (11, 12). The ethanol ratio was calculated as: ethanol ratio (%) = (ethanol concentration in outgoing dialysate/ethanol concentration in ingoing perfusate) × 100. The variations in the ethanol ratio were taken as an index of variations of adipose tissue blood flow.

The perfusion rate was set at 2.5 μl/min for the experimental period. Two 10-min fractions of the outgoing dialysate were collected, and then increasing successive concentrations of epinephrine alone (0.01, 0.1, and 1 μmol/liter) were infused in one probe, and epinephrine associated with 100 μmol/liter phentolamine were infused in the second one. Epinephrine at each concentration was infused during 30 min and then was withdrawn from the perfusate. After a recovery period of 90 min, the subjects performed exercise at a load corresponding to 50% of their individual VO_2max for 40 min on a bicycle ergometer. The oxygen consumption and heart rate were monitored (Vmax, Sensor Medics) during the exercise. After the exercise, subjects rested in the semirecumbent position for 30 min. During all protocols, *i.e.* epinephrine perfusions and exercise and recovery periods, 10-min fractions of the dialysate were collected. Water intake was allowed *ad libitum* during the experimental period.

Blood sampling

Before exercise and every 10 min during exercise and recovery, blood samples were collected from an indwelling polyethylene catheter inserted into an antecubital vein for plasma determinations. The catheter was kept patent by a slow infusion of saline. Every 20 min, a supplementary blood sample was collected on 50 μl of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseilles, France) for catecholamine measurement. The plasma was stored at −80 C until analysis.

Adipose tissue biopsies

Four days after the microdialysis experimental protocol described above, a needle biopsy of SCAAT was performed. The biopsy samples were frozen at −80 C and stored for further analysis of mRNA content.

Training program

The training program started after completion of the 2 investigation d. The program consisted of sessions of aerobic exercise 5 d/wk: twice a week an aerobic exercise in the gymnasium supervised by an exercise instructor, and three times a week an individual exercise on a bicycle ergometer (at home). Subjects were instructed to exercise for 45 min at the intensity corresponding to the individually recommended heart rates; the individual heart rate was determined as that corresponding to 50% of their VO_2max as determined during a maximum exercise test. Each participant was provided with a cardiometer (Polar Accurex Plus Cardiometer, Monitor, France) to follow the heart rate during exercise sessions.

Dietary regimen

Throughout the training program the patients were instructed to maintain their habitual diet, which was estimated by a 7-d weighed food record before the beginning of the study. The maintenance of the dietary regimen was controlled twice every 4 wk of the study using a 3-d food record.

Drugs and analytical methods

Phentolamine methanesulfonate (Regitine) was obtained from Ciba-Geigy (Reuil-Malmaison, France). Glycerol in dialysate (10 μl) and that in plasma (20 μl) were analyzed with an ultrasensitive radiometric method (13). Ethanol in dialysate and perfusate (5 μl) was determined with an enzymatic method (14). Plasma glucose was determined with a glucose oxidase technique (Biotrol kit, Merck-Clevenot, Nogent-s-Marne, France), and nonesterified fatty acid (NEFA) was determined by an enzymatic procedure (Wako kit, Unipath, Dardilly, France). Plasma insulin concentrations were measured using RIA kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France). Plasma epinephrine and norepinephrine were assayed in 1-ml aliquots of plasma by HPLC using electrochemical (amperometric) detection.

RNA analysis

After chloroform lipid extraction of adipose tissue biopsies, total RNA was extracted using the RNeasy kit (Qiagen, Chatsworth, CA) and stored at −80 C until analysis. Total RNA concentrations were determined using a fluorometric assay (Ribogreen, Fluoroskan Ascent). RT was performed using 1 μg total RNA, Thermoscript reverse transcriptase (Invitrogen, Carlsbad, CA), and random hexamers as recommended by the manufacturer. Real-time quantitative RT-PCR was performed on GeneAmp 5700 Sequence Detection System using SYBR Green chemistry (PE Applied Biosystems, Courtaboeuf, France). A set of primers was designed for each gene using the software Primer Express 1.5 (PE Applied Biosystems). Amplicons of 65–90 bp with Tm between 79 and 82 C were selected. Ten nanograms of cDNA were used as template for real-time PCR in duplicate. A dissociation curve was generated at the end of the PCR cycles to verify that a single gene product was amplified. For each primer pair a standard curve was obtained using serial dilutions of human adipose tissue cDNA. mRNA levels were assessed using the following primers: for α_2-AR, 5′-gcgagatcaacgaccagaagt-3′ and 5′-cttggcgatctggtagat-3′; for β_2-AR, 5′-ccgaaagttcccgtacgtca-3′ and 5′-cagcccgtgctctgaagaa-3′; for phosphodiesterase 3B, 5′-gacttgcatttgaaatggacagaa-3′ and 5′-atgggcagaccaagatttgc-3′; and for hormone-sensitive lipase, 5′-gtgcaaagacggaggaccactcca-3′ and 5′-gacgtctcggagtttcccctcag-3′. We

used 18S ribosomal RNA as control to normalize gene expression using the Ribosomal RNA Control TaqMan Assay Kit (PR Applied Biosystems).

Statistical analysis

All values are the mean ± SEM. The responses to exercise were analyzed using a paired *t* test and ANOVA when appropriate. During epinephrine infusion and exercise, the changes in dialysate concentrations were analyzed using a paired *t* test on the mean changes over the baseline values. $P < 0.05$ was considered statistically significant.

Results

After 12 wk of training, body weight, BMI, and percentage of fat mass decreased (Table 1). The VO_2max was higher after training (Table 1). Resting heart rate and systolic and diastolic blood pressures remained unchanged (not shown). The maximal power output and the mean load at 50% VO_2max during the exercise test were higher after 12 wk of training (Table 1). At the end of the training period, resting plasma insulin concentrations were unchanged compared with pretraining values (2.6 ± 0.6 *vs.* 3.7 ± 0.6 μU/ml). Training did not modify the resting plasma concentrations of NEFA (460 ± 37 *vs.* 498 ± 58 μmol/liter) and induced an increase in plasma glycerol concentrations (126 ± 12 *vs.* 99 ± 11 μmol/liter; $P < 0.05$). Consequently, the NEFA/glycerol concentrations ratio decreased (3.78 ± 0.36 *vs.* 5.21 ± 0.49; $P < 0.05$). Training induced a reduction in resting plasma glucose concentrations (4.18 ± 0.10 *vs.* 4.57 ± 0.10 mmol/liter; $P < 0.02$), an elevation in plasma norepinephrine levels, and a reduction in epinephrine concentration (Table 2).

TABLE 1. Morphological and physical characteristics and sc abdominal adipose tissue mRNA levels of obese women before and after training

	Before training	After training	*P*
Weight (kg)	86.4 ± 2.1	82.6 ± 2.1	<0.001
BMI (kg/m^2)	31.3 ± 0.6	29.9 ± 0.7	<0.001
% Fat mass	41.1 ± 1.0	38.7 ± 1.0	<0.002
VO_2max	2.04 ± 0.08	2.22 ± 0.10	<0.003
Maximal heart rate	173.9 ± 4.0	174.0 ± 4.1	NS
Maximal power (watts)	164.5 ± 5.8	185.5 ± 7.2	<0.001
Exercise power (watts)	57.5 ± 2.5	72.5 ± 2.8	<0.003
Exercise heart rate	125.8 ± 2.4	130.2 ± 2.5	<0.002
mRNA levels			
α_2-ARs	0.32 ± 0.07	0.32 ± 0.09	NS
β_2-ARs	0.11 ± 0.02	0.14 ± 0.04	NS
Hormone-sensitive lipase	2.20 ± 0.42	2.18 ± 0.40	NS
Phosphodiesterase 3B	1.08 ± 0.13	1.54 ± 0.20	NS

Data are the mean ± SEM. Exercise power and exercise heart rate are the values related to the exercise test at 50% VO_2max that was a part of the microdialysis protocol (see *Subjects and Methods*). Relative amounts of mRNAs were normalized using 18S ribosomal RNA as determined by real-time RT-PCR from nine subjects.

Effect of training on gene expression

mRNA levels for key genes involved in lipolysis regulation in adipose tissue *i.e.* α_2-AR, β_2-AR, hormone-sensitive lipase, and phosphodiesterase 3B, were quantified in SCAAT by real-time RT-PCR in nine subjects before and at the end of the training period (Table 1). The training did not induce changes in gene expression.

Lipolytic response in SCAAT to epinephrine perfusion at rest and to exercise: effect of training

Epinephrine perfusion at rest. Training did not modify the concentration of glycerol in the dialysate at rest. Before training, the resting concentration in dialysate glycerol was significantly increased by the addition of phentolamine to the perfusate (51 ± 6 *vs.* 40 ± 4 μmol/liter; $P < 0.01$). After the training, the resting concentration in dialysate glycerol remained higher in presence of phentolamine (49 ± 8 *vs.* 41 ± 8 μmol/liter; $P < 0.02$). The perfusion of epinephrine through microdialysis probe induced a concentration-dependent increase in the glycerol concentration in dialysate (Fig. 1). At each concentration of epinephrine, the addition of phentolamine potentiated the increase in glycerol concentrations in the dialysate. Neither the response of the dialysate glycerol concentration to epinephrine perfusion nor the potentiating effect of phentolamine on the epinephrine-induced increase in glycerol was modified after the training. Training did not modify the ethanol ratio at rest in both probes (with epinephrine and epinephrine plus phentolamine). Whatever the concentration of epinephrine added to the perfusate, no change in the ethanol ratio was observed during epinephrine perfusion before as well as after the training. The addition of phentolamine did not induce any significant change in the ethanol ratio before or during epinephrine perfusion (not shown).

During exercise. Before training, glycerol concentration in dialysate increased in the control probe during exercise; the increase was significant from the 10th min of exercise and reached 61 ± 14 μmol/liter at the 40th min (Fig. 2). The exercise-induced glycerol increase in the probe with phentolamine was higher than that in the control probe; glycerol increase reached 82 ± 12 μmol/liter at the 40th min. The calculated average area under the curve for glycerol increase over the 40-min exercise period was significantly higher in the probe containing phentolamine than in the control probe ($P < 0.02$).

TABLE 2. Effects of 40-min exercise (0–40 min) on plasma catecholamines concentrations before and after training

Time (min)	Exercise (min)				*P*
	0	20	40	70	
Norepinephrine (pg/ml)					
Before training	108 ± 13	604 ± 86[a]	736 ± 128[a]	129 ± 14	
After training	171 ± 21[b]	575 ± 72[a]	737 ± 108[a]	169 ± 34	0.04
Epinephrine (pg/ml)					
Before training	14 ± 3	41 ± 7[a]	46 ± 9[a]	11 ± 1	
After training	8 ± 1[b]	25 ± 3[a,b]	31 ± 5[a,b]	7 ± 1	0.07

Values are the means ± SEM. *P* in the table indicates the effect of training on the changes in catecholamine concentration.
[a] $P < 0.05$ compared to corresponding basal values measured before exercise.
[b] $P < 0.05$ compared to corresponding values measured before training.

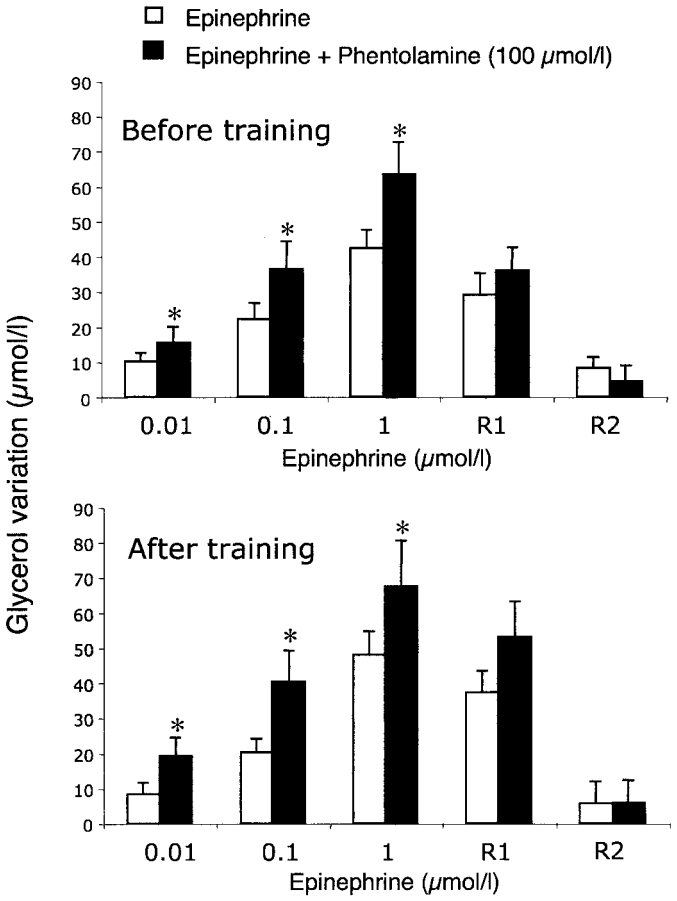

FIG. 1. Glycerol concentration changes in dialysate from sc adipose tissue during the 30-min infusion of graded concentrations of epinephrine alone or associated with phentolamine before and after training (numbers on the x-axis represent the concentrations of epinephrine in the perfusate; R1 and R2 values show changes in glycerol concentrations during two-30 min successive recovery periods). Data are expressed as the mean ± SEM. *, $P < 0.05$ compared with values obtained with epinephrine alone.

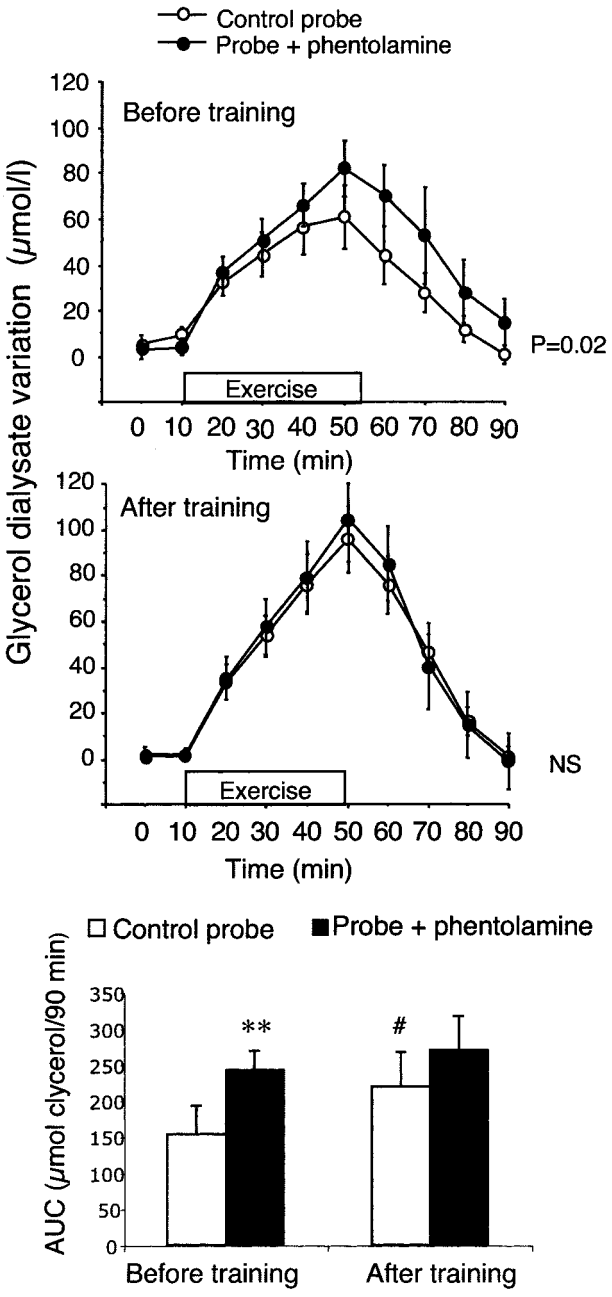

FIG. 2. Time course of glycerol concentration changes in dialysate from sc adipose tissue during the 40-min cycle ergometer exercise before and after training in obese women. Phentolamine was or was not added to the perfusion medium. The *lower part* of the figure is the area under the curve (AUC; micromoles per liter per 40 min) of the variation in glycerol concentration in the dialysate induced by exercise in the control probe or in the probe with phentolamine. Data are expressed as the mean ± SEM. *, $P < 0.05$ compared with control probe; #, $P < 0.05$ compared with before training.

At the end of the training period, the rise of glycerol in the control probe reached 95 ± 14 μmol/liter at the 40th min (Fig. 2). The calculated average area under the curve for glycerol increase over 40 min of exercise tended to be higher than the pretraining value ($P = 0.07$). The dialysate glycerol increase in the probe with phentolamine was not different from that in the control probe; glycerol increase reached 104 ± 18 μmol/liter at the 40th min.

During exercise, the ethanol outflow/inflow ratio decreased ($P < 0.05$) in both probes before as well as after the training. The exercise-induced decrease in the ethanol ratio was not different after compared with before training. There were no differences, before or after the training, in the exercise-induced decrease in the ethanol ratio between the control probe and the probe with phentolamine (Fig. 3).

Exercise-induced changes in plasma NEFA and glycerol levels: effect of training

Plasma NEFA concentrations decreased during the first 15 min of exercise, then progressively increased until the end of the exercise bout, and remained elevated during the recovery period (Fig. 4). No difference was found in the time course of the plasma NEFA concentration changes before and after training. The plasma glycerol level increased 15 min after the beginning of exercise and peaked at the 40th min of exercise. During recovery it decreased to values similar to those found under basal conditions. The calculated average AUC for

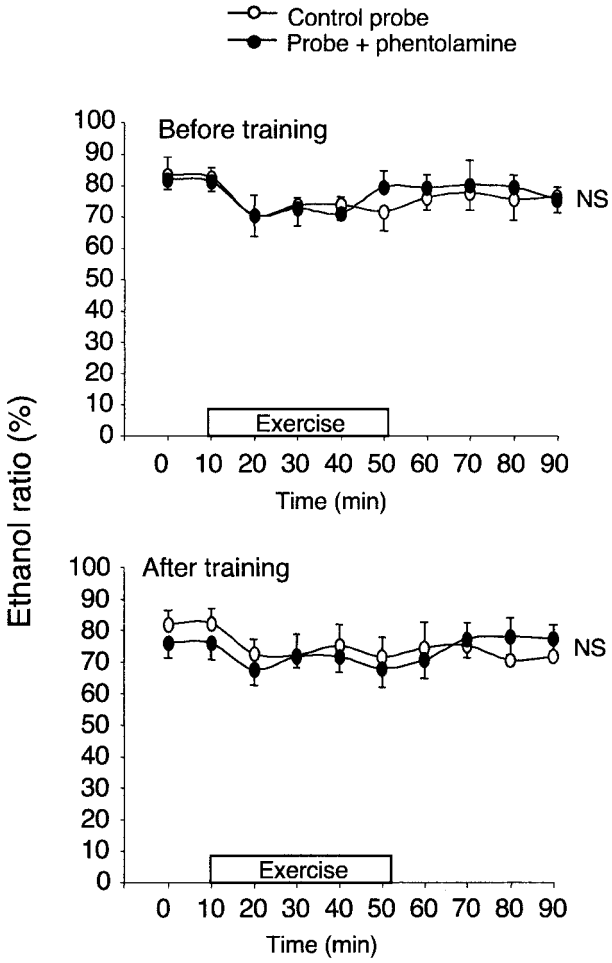

FIG. 3. Change in ethanol ratio in sc adipose tissue during the 45-min cycle ergometer exercise before and after training in obese women. The ethanol ratios (dialysate ethanol/perfusate ethanol × 100) were determined before (rest) and during exercise during cycle ergometer exercise. Data are expressed as the mean ± SEM.

NEFA and glycerol changes over 40 min of exercise were not different before and after training.

Exercise-induced changes in plasma catecholamine, glucose, and insulin levels: effect of training

At rest, plasma norepinephrine values were higher after training than before, but the absolute values during exercise were not different (Table 2). At rest and during exercise, the plasma epinephrine values were lower after than before training. Consequently, the exercise-induced increase in plasma norepinephrine was lower after training ($P = 0.04$), and that of epinephrine tended to be reduced ($P = 0.07$). The time courses of changes in plasma glucose and insulin concentrations were not different before and after training (Fig. 5).

Discussion

The main finding of the study is that in obese women, a medium-term program of aerobic physical activity did not modify the α_2-AR-mediated antilipolytic effect of catecholamines in SCAAT *in situ* or the expression of the α_2-AR gene

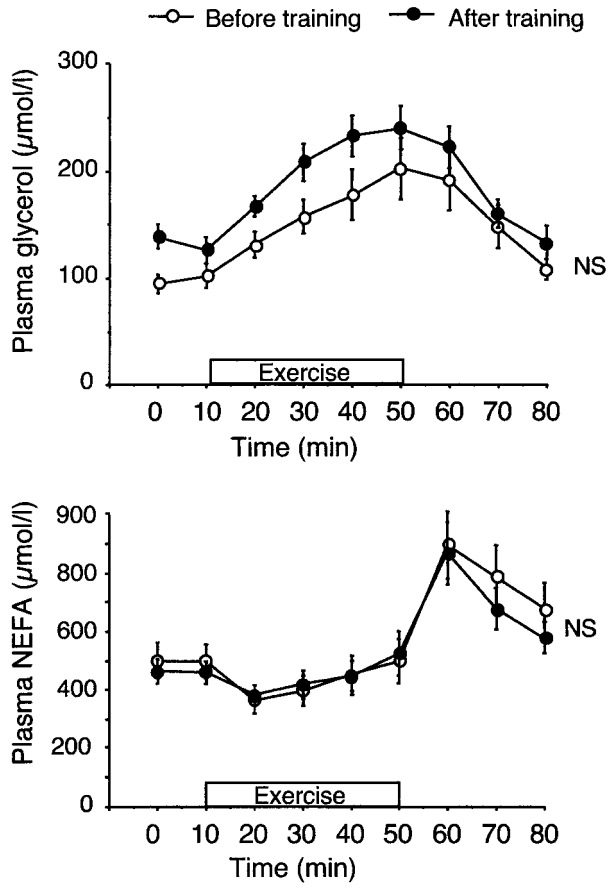

FIG. 4. Time course of glycerol and NEFA plasma concentrations before (rest) and during the 40-min cycle ergometer exercise before and after training in obese women. Data are expressed as the mean ± SEM.

(mRNA levels) in sc adipose tissue. Furthermore, training did not modify either epinephrine-stimulated lipolysis *in situ* in SCAAT or the expression of genes involved in the regulation of lipolysis at receptor (β_2-AR) and postreceptor (hormone-sensitive lipase and phosphodiesterase 3B) levels. The regulation of lipolysis during exercise was modified by the training; the involvement of the α_2-mediated antilipolytic action of catecholamines during exercise was reduced after training.

The contribution of α_2-ARs in the control of lipolysis at rest was evaluated by a local pharmacological challenge (*i.e.* infusion of epinephrine alone and associated with phentolamine directly in the microdialysis probe). An enhancement of the epinephrine-induced increase in lipolysis by addition of the α_2-AR antagonist to the perfusate was observed. It is a functional test to reveal the intrinsic α_2-AR effect related to epinephrine alone. The response is probably related to the number/coupling efficiency of the α_2-ARs existing in fat cells of the sc deposit. Before training, spontaneous glycerol release at rest as well as glycerol release induced by increasing epinephrine concentrations perfused through the probe were potentiated after blockade of α_2-ARs in obese women. This result demonstrates the *in situ* interplay between β- and α_2-ARs in the adipose tissue of obese subjects. The data represent the *in vivo* validation of all previous data collected

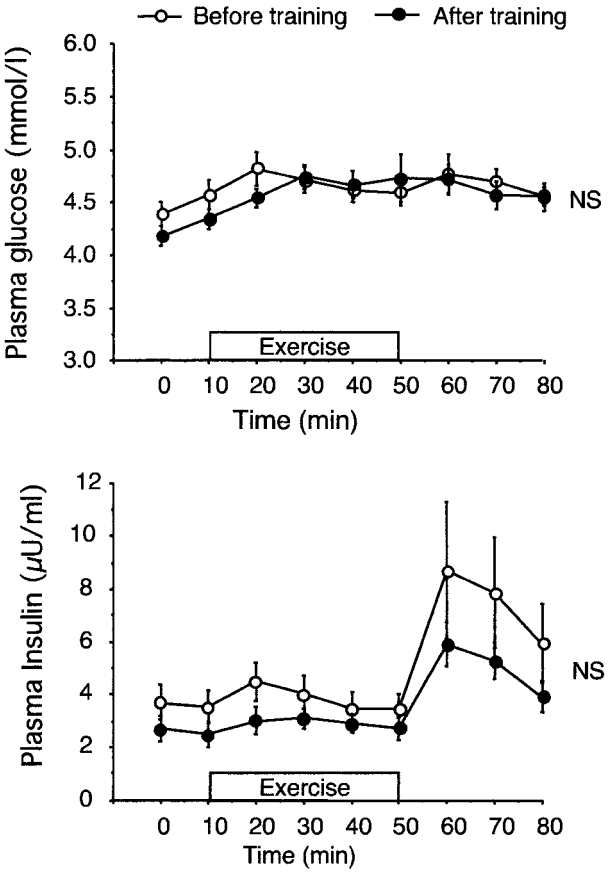

FIG. 5. Time course of glucose and insulin plasma concentrations before and during the 40-min cycle ergometer exercise before and after training in obese women. Data are expressed as the mean ± SEM.

in vitro in human isolated fat cells that have demonstrated the antagonistic action of β- and α_2-ARs (5, 6). Results show that training did not change the α_2-AR response in resting conditions, because the blockade of antilipolytic α_2-ARs similarly enhanced epinephrine-induced glycerol increment in the dialysate before and after training. These results are supported by the lack of change in α_2-ARs mRNA levels consecutive to training.

The findings of the unchanged epinephrine lipolytic action in this study fit with the findings of transversal (15) and longitudinal studies (16) showing that training did not modify catecholamine-induced lipolysis *in situ* at rest.

Despite the lack of the training-induced change in the α_2-mediated antilipolytic activity at rest, we observed a reduction of the involvement of α_2-ARs in the exercise-induced lipid mobilization. Therefore, neuroendocrine regulatory factors might be implied in this training-induced modification. In previous reports (17, 18) it was shown that epinephrine is involved in the control of lipolysis during exercise through activation of antilipolytic α_2-ARs and that the magnitude of α_2-AR-mediated antilipolytic action during exercise is dependent on the magnitude of the exercise-induced increase in epinephrine. In the present study the exercise-induced increase in catecholamines, epinephrine and norepinephrine, in plasma were lower after the training. As the circulating catecholamines are determinant for the exercise-induced lipolytic response (19), this implies a lower α_2-AR-mediated antilipolytic effect. Therefore, the results suggest that the reduction of α_2-mediated antilipolytic effect after training is due to the lower plasma catecholamine response, whereas the training does not modify the adipocyte response to α_2-AR-mediated action. Plasma levels of insulin might be suggested to interfere with the described training-induced changes in lipolysis in SCAAT during exercise. The interference of insulin with the results of this study might be mediated by either training-induced changes in sensitivity to insulin action or changes in plasma insulin levels. In sc adipose tissue, the short-term training does not modify the sensitivity to antilipolytic effect of insulin (20). The lack of a modification of the sensitivity to insulin antilipolytic action in SCAAT has been also found during very low calorie diet (21). The training did not induce a significant decrease in plasma insulin levels in the present study. However, as hyperinsulinemia was shown to increase the relative contribution of α_2-AR-mediated antilipolytic effect of epinephrine, it might be speculated that even the small, nonsignificant, decrease in plasma insulin levels during training could contribute to the changes in α_2-AR-mediated responsiveness found in this study (22).

Adipose tissue blood flow (ATBF) is known to influence glycerol dialysate concentration originating from adipose tissue (23). In the present study the ethanol method was used for evaluation of ATBF. It does not enable the corrections in individual results for adipose tissue blood flow values, but it provides the possibility to assess whether and in which sense the changes occur. At the concentration used, epinephrine alone or associated with phentolamine (α_1/α_2-AR antagonist) did not induce a change in ATBF before or after training. The exercise-induced vasodilatation (reduction of the ethanol ratio) was not modified after training. In the presence of phentolamine, the exercise-induced vasodilatation was not different from that with the control probe, and training did not change the ethanol ratios during exercise. Thus, in the present study the effects of training on glycerol concentration variations were not influenced by ATBF.

In a previous study in obese women submitted to a 12-wk hypocaloric diet, we found a reduction of α_2-AR mRNA in adipose tissue and a reduction of α_2-AR-mediated antilipolytic action *in vivo* after the diet (10). The apparent difference in the results of the present study and those of the latter may be related to a lower weight decrease during training (compared with dietary intervention) and/or the different characters of the intervention (diet *vs.* exercise).

In a previous study carried out in men, we found that exercise-induced lipid mobilization (60 min at a similar relative power, as assessed by the increase in plasma norepinephrine) was largely impaired in SCAAT in obese subjects (4). Another study performed in obese women showed that this impairment was comparatively weaker (10). A gender-related difference was reported in young nonobese subjects (18). The gender difference in fat mobilization and utilization has been previously discussed (24–27). The sex-related difference in exercise-induced lipid mobilization could be related to the lower involvement of the antilipolytic α_2-adrenergic effect on fat cells during exercise in women, which may be a consequence of a lower exercise-induced increase in

epinephrine during exercise of the same relative intensity in women compared with men (18).

This study also raises questions about the relationship existing between the degree of obesity and the β- and α_2-ARs interplay operating in adipose tissue during exercise. In a previous study performed in obese women with a BMI of 34, *i.e.* higher than in the present study (BMI of 31), it was observed that during a similar standardized exercise, the potentiating effect of phentolamine on dialysate glycerol was 46% (10). In the present study the women exhibited a lower BMI, and the potentiating effect of phentolamine was only 26%. Moreover, in nonobese women, the results reported by Hellström *et al.* (18) demonstrated that phentolamine did not produce any additive effect on the increment in the dialysate glycerol concentration during exercise. Together, these observations suggest that the exercise-induced α_2-adrenergic responsiveness is related to fat mass extent; the higher the fat mass, the stronger the inhibitory effect of α_2-ARs during exercise.

In conclusion, the present study demonstrates that medium-term aerobic training does not produce any changes in β_2- and α_2-AR gene expression in adipose tissue of obese women and does not modify the α_2-AR antilipolytic effect *in situ* at rest. During exercise, the lower α_2-AR effect induced by training is not related to changes in α_2-AR gene expression, but may be associated with reduced exercise-induced epinephrine secretion.

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