No Difference in the Lipolytic Response to β-Adrenoceptor Stimulation In Situ But a Delayed Increase in Adipose Tissue Blood Flow in Moderately Obese Compared With Lean Men in the Postexercise Period

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This study was undertaken to determine the effect of previous exercise on adipose tissue responsiveness to β-adrenoceptor stimulation and on adipose tissue blood flow (ATBF). Eight lean and 8 obese men (body mass index [BMI], 23.6 ± 2.1 [SD] v 29.0 ± 1.9 kg · m^{-2}) were investigated with abdominal subcutaneous microdialysis and ^{133}Xe clearance. A stepwise isoprenaline infusion (10^{-8}, 10^{-7}, and 10^{-6} mol · L^{-1}) was administered in situ in the microdialysis catheter before and 2 hours after a submaximal exercise bout (90 minutes of cycling at 55% of maximal O_2 uptake). No differences in the response (increase in interstitial glycerol v preinfusion level) to isoprenaline infusions were found between the 2 groups. In both groups, there was no difference in the response to postexercise versus preexercise infusion. When the vasodilating agent hydralazine (0.125 g · L^{-1}) was infused into the microdialysis catheter to control for the vascular effects of isoprenaline, an interaction effect between exercise and isoprenaline dose was found. Analyses showed an attenuated response to the high isoprenaline dose after exercise (lean, 251 ± 42 [SE] μmol · L^{-1}; obese, 288 ± 77 μmol · L^{-1}) versus before exercise (lean, 352 ± 62 μmol · L^{-1}, P = .045 v after; obese, 380 ± 94 μmol · L^{-1}, P = .021 v after), indicating a desensitization of lipolysis to β-adrenoceptor stimulation. ATBF and arterial plasma glycerol increased after exercise in both groups, but the increase was delayed in obese subjects. Arterial plasma insulin was higher in the obese versus lean subjects at all times, and decreased during recovery in both groups. In conclusion, abdominal subcutaneous adipose tissue responsiveness to β-stimulation is not enhanced postexercise in lean and obese men, whereas previous exercise increases ATBF. Furthermore, the data suggest slower lipid mobilization postexercise and resistance to the antilipolytic effect of insulin in the obese.
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THE SYMPATHOADRENAL SYSTEM is of major importance for the stimulation of adipose tissue lipolysis. A sustained increase in the plasma free fatty acid (FFA) concentration is observed for several hours after the end of prolonged dynamic exercise,[1,2] resulting in elevated rates of both FFA oxidation and FFA reesterification.[2-4] Plasma concentrations of epinephrine and norepinephrine increase during exercise, but no sustained increase is found after exercise.[2,5] However, the plasma norepinephrine concentration is an insensitive indicator of sympathetic nervous activity in the tissues, since the clearance rate of catecholamines is high.[6]

Previous studies ex vivo have shown an increased lipolytic response to catecholamine stimulation in human gluteal adipocytes[7] and in suprailiac fat cells[8] removed immediately after an exercise bout. Thus, the adipose tissue sensitivity to catecholamines may increase during exercise, but it is not known if this effect is sustained in the postexercise period and if it differs between lean and obese subjects. To our knowledge, there is no study on the lipolytic response to catecholamines in situ in the postexercise period. The changes in responsiveness may originate both at the adrenoceptor level, with an alteration in the number of adrenoceptors or affinity for the agonist, and the postadrenoceptor level, with a modification of the relationship between adrenoceptor occupancy and the physiological response.[9] On the other hand, prolonged exposure of an effector tissue to an agonist may result in desensitization with a gradual decrease in response.[9]

Since adrenoceptors are expressed by both adipocytes and vascular cells, catecholamine stimulation results in both metabolic and vascular effects. Adipose tissue blood flow (ATBF) may be of significance for the rate of FFA mobilization. Bülow[10] found an increase in ATBF during prolonged exercise (3 to 6 periods of 50 minutes' cycling at about 50% of maximal O_2 uptake with 10 minutes' rest between each period) in lean subjects using the ^{133}Xe clearance technique, whereas no change in ATBF was found during 30 minutes of cycling using

the ethanol clearance method.[11] Several studies have demonstrated a decreased ATBF per unit adipose tissue weight in obese versus lean subjects during rest.[12-14] However, little is known about ATBF following an exercise bout.

The present study was designed to test the hypothesis that the lipolytic sensitivity to catecholamines increases after exercise. The purpose was to determine the effect of in situ β-adrenoceptor stimulation, using the microdialysis technique, on the interstitial glycerol concentration in abdominal subcutaneous adipose tissue before and after exercise in both lean and obese men. To control for the possible vascular effects of β-adrenoceptor stimulation, infusions of the vasodilating agent hydralazine in separate catheters together with β-agonist were also performed. Also, postexercise ATBF was determined in both lean and obese men.

SUBJECTS AND METHODS

Subjects

Eight lean and 8 moderately obese healthy men, aged 20 to 33 years participated in this study (Table 1). Obesity was defined as a body mass index (BMI) of at least 27.0 kg · m^{-2}. All subjects were physically active but were not engaged in endurance training. After a medical

Table 1. Physical Characteristics and Exercise Data of the Subjects

Characteristic	Lean Subjects (n = 8)	Obese Subjects (n = 8)	P
Age (yr)	22.8 ± 2.8	26.1 ± 3.4	.049
Weight (kg)	76 ± 7.6	92.3 ± 13.0	.009
Height (m)	1.80 ± 0.08	1.78 ± 0.11	.701
BMI (kg · m^{-2})	23.6 ± 2.1	29.0 ± 1.9	<.001
Work HR	138 ± 9	136 ± 2	.542

NOTE. Data are the mean ± SD.

examination, they were fully informed about all procedures before written consent was obtained. The protocol was approved by the Ethics Committee of the University of Gothenburg.

Before the experiments started, all subjects were familiarized with bicycle exercise at a constant pedaling rate. All testing and experimental exercise procedures were performed on a cycle ergometer (Bodyguard 990; Øglænd, Stavanger, Norway). About 2 weeks before the experiments, the relation between the heart rate (HR) and workload for each subject was determined. Based on this relationship, an appropriate workload was predicted to yield a HR corresponding to about 55% of maximal O_2 uptake.[15]

Experimental Protocol

The experiments were performed in a quiet laboratory with room temperature kept at 27°C. The subjects reported to the laboratory at 7:00 AM after an overnight fast. They were transported to avoid unnecessary physical activity prior to the experiments. The subjects were told not to exercise for 2 days before an experiment, and otherwise not to make any changes in their dietary or exercise habits. Tobacco and alcohol were not allowed 24 hours before the experiments.

At the start of the experiments, the subjects voided and body weight was measured. Thereafter, the subjects rested supine in bed. A catheter was inserted percutaneously into a radial artery during local analgesia. A HR recorder (Polar Sport Tester; Polar Electro OY, Kempele, Finland) was fastened to the chest to record the HR every minute throughout the experiment.

Four microdialysis catheters (30 × 0.3 mm, Cuprophan B4 AH, 3,000 molecular weight cutoff; Cobe, Denver, CO) were placed in the abdominal subcutaneous tissue. Two of the catheters were placed on the right side and the other 2 on the left side of the umbilicus, and always more than 3 cm apart. The nylon tubing inlet of the catheter was connected to a microinjection pump (CMA/100; Carnegie Microdialy-

sis, Stockholm, Sweden). After the insertion, each probe was continually perfused at a rate of 3.0 μL · min^{-1}.

The exercise bout started at 10:55 AM (Fig 1). During exercise, the subject cycled continuously for 90 minutes at the predetermined fixed workload corresponding to about 55% of maximal O_2 uptake and a constant pedaling rate of 75 rpm. The rationale for the choice of exercise protocol was to ensure high rates of FFA mobilization and oxidation during exercise,[16] as well as elevated rates after exercise.[2,4] The recovery period started at 12:25 PM, and the subjects rested in bed until 5:25 PM (5 hours postexercise; Fig 1). They were not allowed to sleep during this period. They were allowed to drink water freely but received no food during the experiments.

Microdialysis

The perfusion fluid consisted of saline with 2.5 mmol · L^{-1} glucose (to prevent drainage of interstitial glucose),[17,18] 25 μmol · L^{-1} glycerol (to prevent drainage of interstitial glycerol), and ^{3}H-glycerol (2,000 cpm · 10 μL^{-1}; Fig 1). In 2 of the catheters (CON+H and ISO+H), 0.125 g · L^{-1} hydralazine (Hydralazine Hydrochloride; Sigma-Aldrich, Stockholm, Sweden) was added to the perfusate with the aim to produce maximal vasodilatation, thereby attenuating changes in local blood perfusion, including vascular effects of isoprenaline. This hydralazine concentration has been shown to increase and clamp ATBF as estimated by the ethanol clearance technique.[19]

After 30 to 60 minutes of equilibration in the morning, dialysates were collected in 10-minute intervals from the outlet of the tubing, and the first sample was collected at 8:20 AM. A maximum delay of 12 minutes was expected between an ongoing change in the interstitial tissue and the dialysate for glycerol when samples were collected every 10 minutes.[20]

At 8:30 AM, a stepwise infusion (10^{-8}, 10^{-7}, and 10^{-6} mol · L^{-1}) of the nonselective β-adrenoceptor agonist isoprenaline (Isoprenalin; Nycomed Pharma, Oslo, Norway) was started in 2 of the catheters, one without (ISO) and one with hydralazine (ISO+H; Fig 1). Each dose was given for 45 minutes. The collection of samples started after 15 minutes, and 3 samples were collected at each stage. The other two catheters (CON and CON+H) served as control catheters where no isoprenaline was added to the perfusion fluid. During exercise and the first 2 hours postexercise, all 4 catheters were again perfused with isoprenaline-free solution (Fig 1).

Two hours after exercise, the stepwise isoprenaline infusion was repeated in the same catheters as before exercise (ISO and ISO+H). After this last infusion was completed, 10^{-4} mol · L^{-1} of the nonselective β-adrenoceptor blocker propranolol (Inderal; Zeneca, Oslo, Nor-
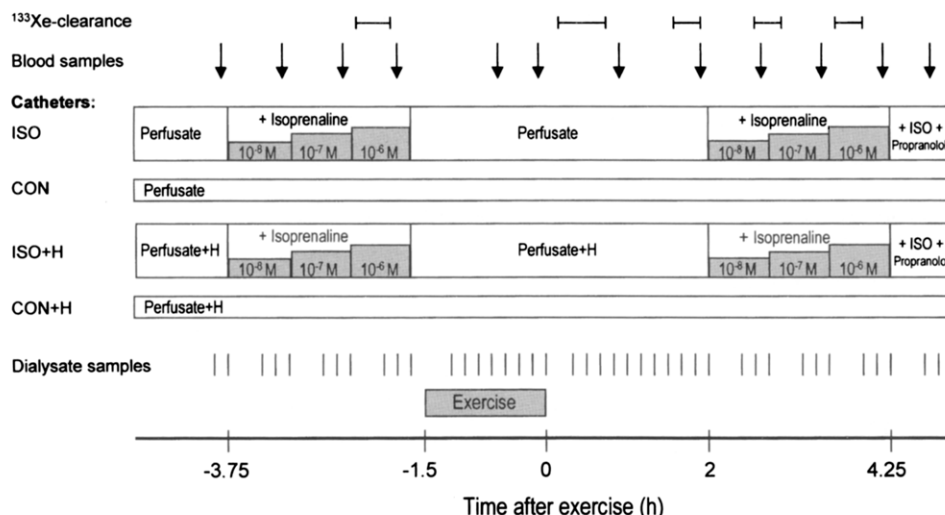
Fig 1. Experimental design of the study.

way) was added to the solution with the highest isoprenaline concentration (Fig 1).

Blood Flow Measurements

ATBF was determined by the ^{133}Xe clearance method.[12] Briefly, 6 to 9 MBq ^{133}Xe (Mallinckrodt, Petten, The Netherlands) in 0.1 mL sterile saline was injected into the subcutaneous adipose tissue, one injection at each side of the umbilicus. The depot was injected at least 5 mm under the skin with a special injection device at an angle of 45° slowly over a 2-minute period. ^{133}Xe disappearance was monitored continuously with a 2 × 2-inch NaI(T1) detector (Canberra Industries, Meriden, CT) covered by a cylindrical collimator and coupled to a multichannel analyzer (ND 62; Nuclear Data, Schaumburg, IL). The detector was placed 30 to 50 cm from the ^{133}Xe depot. Only an energy interval corresponding to the photopeak of 81 keV was registered. Counts were accumulated during consecutive 30-second intervals and plotted as a function of time. Subcutaneous ATBF was then calculated as ATBF = $\kappa \cdot \lambda \cdot 100$ (mL $\cdot$ 100 g$^{-1} \cdot$ min^{-1}), where λ is the tissue to blood partition coefficient for ^{133}Xe at equilibrium, and κ is the rate constant of the washout curve. A straight line was fitted through the experimental points, and κ was estimated as (ln y$_1$ − ln y$_2$) $\cdot T^{-1}$, where y$_1$ and y$_2$ are the counting rates at 2 occasions, and T is the time in minutes between these registrations. The time interval between 2 registrations was at least 15 minutes. In the calculations, a mean value of 10 mL $\cdot$ g^{-1} was used for the tissue to blood partition coefficient. Comparative studies indicate that this value may be used as an estimate for subcutaneous blood flow studies in both lean and obese subjects.[21] ATBF was determined before exercise and at intervals in the postexercise period (Fig 1).

Blood Sampling and Chemical Analyses

Blood samples were drawn according to the following schedule (Fig 1): 5 minutes before the start of the preexercise isoprenaline infusion, 5 minutes before the end of each stage of the infusion, after 1 and 1.5 hours of exercise, after 0.83 and 1.83 hours of recovery, and then again in the last 5 minutes of each stage of the postexercise isoprenaline infusion. The last sample was collected 4.75 hours after exercise, during the propranolol infusion.

Arterial blood samples to be analyzed for norepinephrine and epinephrine were transferred to tubes containing EGTA, reduced glutathione, and isotonic saline. After centrifugation, they were stored at −80°C until analysis by a HPLC method.[22] The other blood samples were kept on ice until centrifugation within 15 minutes in a refrigerated centrifuge at 3,000× g and 4°C for 10 minutes (Beckman GS-15R; Beckman Instrument, Fullerton, CA). The plasma was frozen at −20°C until analysis. Glycerol concentrations in tissue dialysates and plasma were determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP in the presence of glycerol kinase.[23] Enzymatic methods were used to determine FFA concentrations (Wako Chemicals, Neuss, Germany). Insulin analyses were performed with a radioimmunoassay method (Insulin RIA 100; Pharmacia, Uppsala, Sweden).

Calculations

The relative recovery of interstitial glycerol was calculated with the internal reference technique.[24] ^{3}H-glycerol (DuPont NEN Products, Brussels, Belgium) corresponding to an activity of 2,000 cpm $\cdot$ 10 μL^{-1} was added to the perfusion fluid. Radioactivity in the second sample in each catheter was measured in a liquid scintillation counter. The relative recovery of tissue glycerol in the microdialysate (R) was calculated according to the formula, R = Fol = (P$_c$ − D$_c$) $\cdot$ P$_c^{-1}$, where Fol is the fractional outflow of labeled glycerol, P$_c$ is the counts in the perfusate, and D$_c$ is the counts in the dialysate.[24,25] Interstitial glycerol was then calculated as [(G$_D$ − G$_P$) $\cdot$ R^{-1}] + G$_P$, where G$_D$ is the glycerol concentration in the microdialysate and G$_P$ is the glycerol concentration in the perfusate.

The mean of the last 2 dialysate samples prior to the preexercise and postexercise isoprenaline infusion is taken to represent preexercise and postexercise basal values for the interstitial glycerol concentration, respectively. Also, the mean values of the 3 samples during each stage of the isoprenaline infusion, during the propranolol infusion, and corresponding time points for the control catheters are used.

Statistical Methods

All statistical calculations were performed using the SPSS software package (SPSS, Chicago, IL). The overall significance of differences in various parameters was analyzed using a repeated-measures ANOVA with group as a between-subject factor and time, exercise, hydralazine, etc., as within-subject factors (factors depending on the parameter tested). Huyn-Feldt corrections for violations of the underlying assumptions of independence across repetition were used. The corrected df are presented in parentheses with the F ratios. Significant main effects between groups were followed up with paired comparisons using t tests for independent samples. To compare exercise or postexercise values with preexercise values within each group, ANOVA or paired-samples t tests were used. A P value less than .05 was considered statistically significant. Results are presented as the mean ± SE unless otherwise specified.

RESULTS

HR

The mean HR was higher in the obese group than in the lean group in both the preexercise and postexercise period (Fig 2). During exercise, the HR increased to the same level in the 2 groups. In both groups, HR was elevated during the postexercise versus preexercise period.

Arterial Plasma Hormones

No group differences were found in the arterial plasma catecholamine concentration. In both groups, plasma norepinephrine increased about 8-fold during exercise (both groups, respectively, $P < .0005$ v preexercise; Fig 3). At the next sample 50 minutes after exercise, norepinephrine declined to resting levels again. The same pattern was found for plasma epinephrine, which also increased significantly during exercise (lean $P = .003$ and obese $P = .03$ v preexercise; Fig 3).
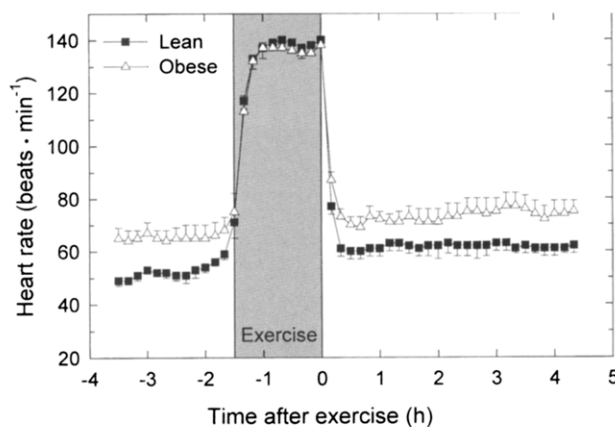

Fig 2. Time plot of HR in lean (■) and obese (△) subjects before, during, and after prolonged submaximal cycling (mean ± SE, n = 8 in both groups). There were significant differences in HR between groups before and after exercise; NS between groups during exercise. Elevated HR postexercise v preexercise in both groups.
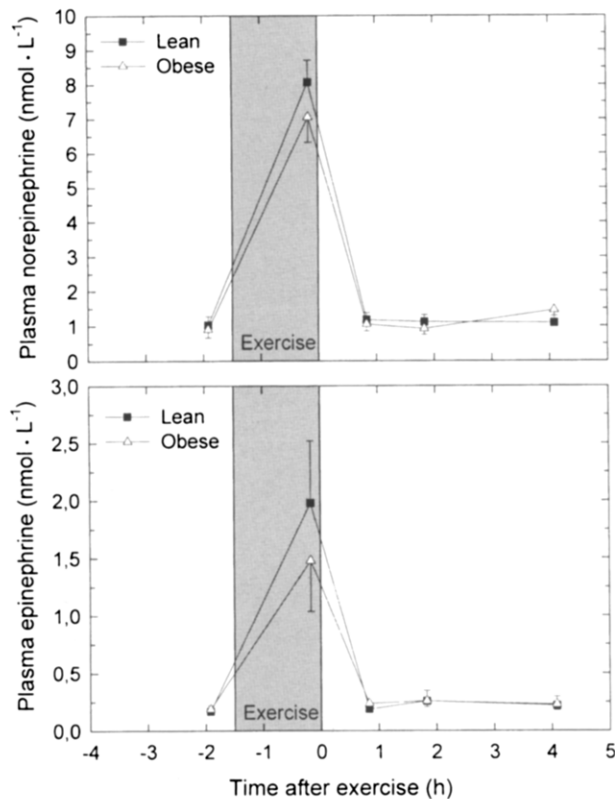
Fig 3. Time plot of arterial plasma norepinephrine and epinephrine in lean (■) and obese (△) subjects before, during, and after prolonged submaximal cycling (mean ± SE, n = 8 in both groups). *v respective preexercise value, P < .05. NS between groups for both parameters.

The plasma insulin concentration of the obese group was about twice that of the lean group during the whole experiment (Fig 4). In both groups, plasma insulin decreased significantly during exercise (lean $P < .0005$ and obese $P = .003$ v last preexercise value). After an increase during the first hour postexercise, plasma insulin remained stable at a lower level
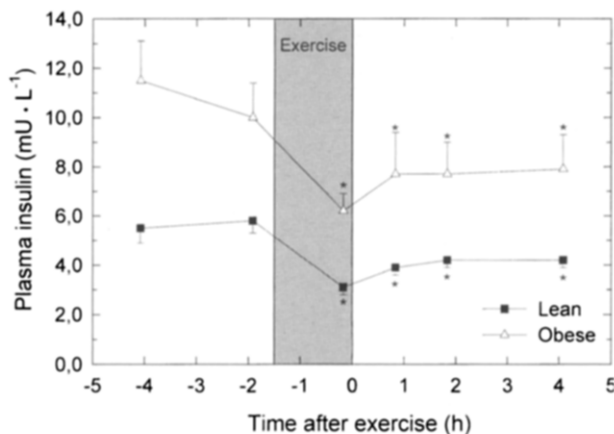

Fig 4. Time plot of arterial plasma insulin in lean (■) and obese (△) subjects before, during, and after prolonged submaximal cycling (mean ± SE, n = 8 in both groups). *v respective last preexercise value, P < .05. Significant overall group difference.

during the entire postexercise period compared with preexercise values.

Arterial Plasma FFA, Plasma Glycerol, and R Values

There was no overall difference in plasma FFA concentrations during the day between the 2 groups (Fig 5). Relative to basal levels, plasma FFA concentrations nearly doubled during exercise. After exercise, plasma FFAs continued to increase during the first 2 to 3 hours and remained elevated above preexercise levels for the rest of the experiment.

No overall difference in the plasma glycerol concentration was observed between the groups (Fig 5). Plasma glycerol increased severalfold during exercise compared with preexercise values. Postexercise glycerol levels were significantly higher than the preexercise levels in both groups. However, this increase was delayed in the obese (lean v obese during the first 2 hours postexercise, $P < .0005$).

The respiratory exchange (R) value in the lean group before exercise was .84 ± .01 (data not shown). After exercise, the R value decreased to .78 to .79, and for all time points, the R value was significantly lower than the preexercise value. In the obese group, the R value before exercise was .81 ± .01. No differences between the groups were found preexercise or postexercise.
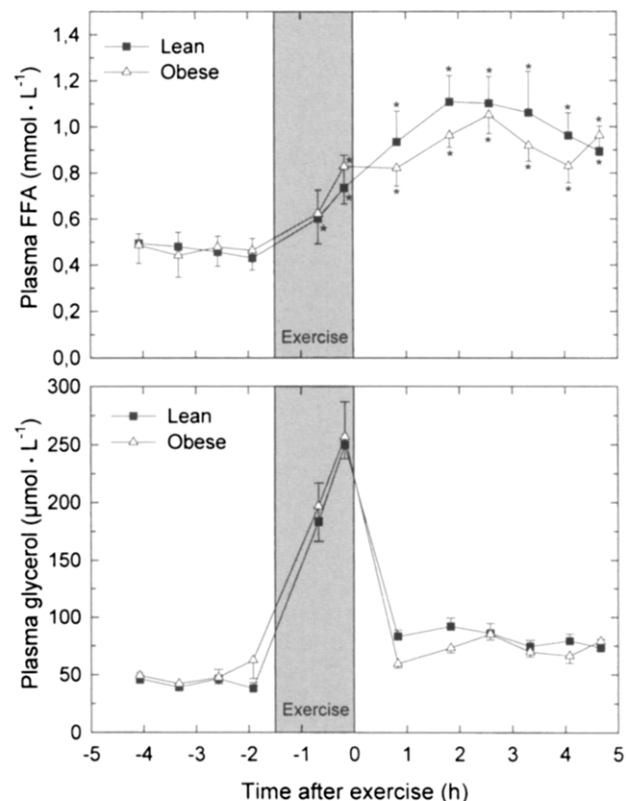

Fig 5. Time plot of arterial plasma FFA and glycerol concentrations in lean (■) and obese (△) subjects before, during, and after prolonged submaximal cycling (mean ± SE, n = 8 in both groups). *Postexercise value v respective last preexercise value, P < .05. Elevated plasma glycerol postexercise v preexercise in both groups. NS between groups for both parameters.

Effect of Exercise on Adipose Tissue Responsiveness to β-Adrenoceptor Stimulation

Relative recoveries. The mean relative recoveries of glycerol in the different catheters were .25 to .36 in the lean group, and no effect of hydralazine was found on the relative recovery. In the obese group, the mean relative recoveries of glycerol in the different catheters were .22 to .34 (NS v lean).

Nonstimulated interstitial glycerol concentration. The nonstimulated interstitial glycerol concentrations were not different between the 2 groups during rest before or after exercise (Fig 6A and B). Neither was any interaction effect found between group and exercise. The interstitial glycerol concentrations calculated for the control catheters were about 2 to 4 times higher versus the arterial plasma during rest before exercise in both groups. The concentrations increased significantly during exercise, and rapidly returned to basal values again after exercise. Statistical analyses of the interstitial-arterial (i-a) glycerol differences for the control catheters showed similar results (Fig 6C and D).

Response to isoprenaline infusion. Mean basal interstitial glycerol concentrations before the first isoprenaline infusion were 130 ± 47 $\mu mol \cdot L^{-1}$ (ISO) and 117 ± 14 $\mu mol \cdot L^{-1}$ (ISO+H, NS v ISO) in the lean group. Corresponding values in the obese were 145 ± 31 $\mu mol \cdot L^{-1}$ (ISO, NS v lean) and 123 ± 25 $\mu mol \cdot L^{-1}$ (ISO+H, NS v lean). There were no differences between preexercise and postexercise basal values except for the ISO+H catheter in the lean group, where an increased concentration was observed postexercise (167 ± 23 $\mu mol \cdot L^{-1}, P = .035$ v preexercise).

The 2 groups did not differ with respect to the changes in interstitial glycerol during the stages of preexercise and postexercise isoprenaline infusion, during exercise, or during propranolol infusion (Fig 7A and B).

In the ISO catheter, only the highest isoprenaline concentration significantly elevated interstitial glycerol over the basal levels. There were no differences in the response (increase in intercellular glycerol) to postexercise versus preexercise isoprenaline infusion in this catheter (Fig 7A and B). Statistical

analyses of changes in i-a glycerol differences for the ISO catheter showed similar results (Fig 7C and D).

Effect of vasodilatation with hydralazine. There was a significant overall suppressive effect of hydralazine on interstitial glycerol (all subjects, $F(1,8) = 9.19, P = .016$; Fig 6A and B) and on i-a glycerol differences (all subjects, $F(1,8) = 9.18, P = .016$; Fig 6C and D). In the presence of hydralazine, an enhanced response to isoprenaline was found (all subjects, $F(1,14) = 9.34, P = .009$; Fig 7A and B). The changes in i-a glycerol differences during isoprenaline infusion were also increased in the presence of hydralazine (all subjects, $F(1,14) = 9.33, P = .009$; Fig 7C and D).

In the catheter where hydralazine was included in the perfusion fluid, the response to isoprenaline was attenuated after exercise (Fig 7A and B). An interaction effect between exercise and isoprenaline dose was observed following ISO+H perfusion (all subjects, $F(1.70,23.77) = 6.84, P = .006$). Paired comparisons between the corresponding stages of postexercise and preexercise infusions showed an attenuated response during the highest isoprenaline dose postexercise (lean, 251 ± 42 $\mu mol \cdot L^{-1}$; obese, 288 ± 77 $\mu mol \cdot L^{-1}$) compared with preexercise (lean, 352 ± 62 $\mu mol \cdot L^{-1}, P = .045$ v post; obese, 380 ± 94 $\mu mol \cdot L^{-1}, P = .021$ v post). Statistical analyses of changes in i-a glycerol differences for the ISO+H catheter showed similar results (Fig 7C and D).

Effect of propranolol. When propranolol was added to the perfusion fluid, interstitial glycerol concentrations and i-a glycerol differences were effectively decreased to nonstimulated levels again in both groups (Fig 7).

ATBF

A significant overall difference in ATBF was found between the 2 groups ($F(1,14) = 5.19, P = .039$), and also an interaction effect between group and time ($F(2.47,34.61) = 3.13, P = .046$; Fig 8).

Before exercise, there was no difference in ATBF between the lean (4.0 ± 0.4 $mL \cdot 100$ $g^{-1} \cdot min^{-1}$) and obese subjects (3.3 ± 0.9 $mL \cdot 100$ $g^{-1} \cdot min^{-1}$). In both groups, there was no
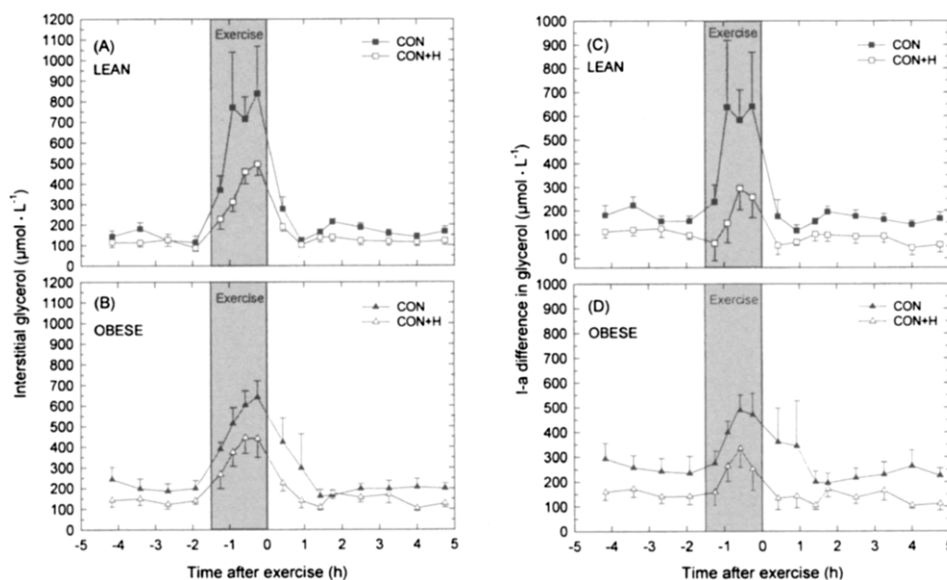
Fig 6. Time plot of calculated interstitial glycerol concentration in lean (A: CON, n = 5; CON + H, n = 6) and obese subjects (B: CON, n = 6; CON + H, n = 7). Bars show the SE. Corresponding i-a glycerol differences are shown in C and D. Closed symbols, catheters without hydralazine; open symbols, catheters with hydralazine added. Significant increase during exercise. Suppressive effect of hydralazine. NS between groups.
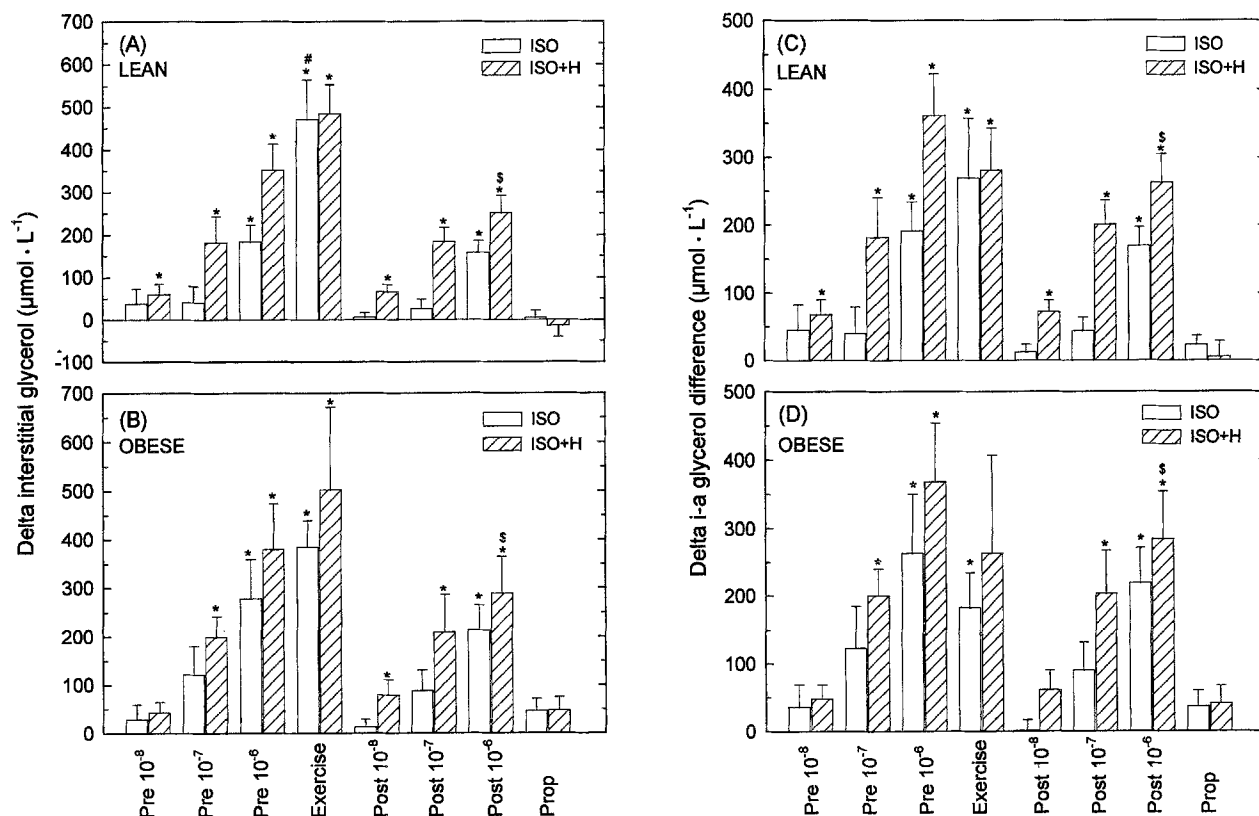
Fig 7. Plot of changes in interstitial glycerol concentration during isoprenaline and propranolol infusions and exercise in lean (A: ISO, n = 8; ISO + H, n = 8) and obese subjects (B: ISO, n = 7; ISO + H, n = 8). Bars show the SE. Corresponding changes in i-a glycerol differences are shown in C and D. (□) Catheters without hydralazine; (▨) catheters with hydralazine added. Pre 10^{-8}, pre 10^{-7}, and pre 10^{-6} show changes during infusion of 10^{-8}, 10^{-7}, and 10^{-6} mol · L^{-1} isoprenaline, respectively, v preexercise basal value; post 10^{-8}, post 10^{-7}, and post 10^{-6} show corresponding changes during postexercise infusion v postexercise basal value; exercise shows changes from preexercise basal value to the end of the exercise bout; prop shows changes during propranolol infusion v postexercise basal value. *Change v corresponding basal value, $P < .05$. #Exercise v pre 10^{-6} response, $P < .05$. $Postexercise response v corresponding preexercise response, $P < .05$.

change in ATBF directly after exercise compared with preexercise values. In the lean, ATBF was increased to significantly higher values at the next measurement 1 hour later. ATBF increased significantly during the postexercise period also in the obese group, but this increase was slower than in the lean group,

and appeared statistically significant after a time lag of 3.5 hours.

DISCUSSION

In the present study, abdominal subcutaneous lipolysis was studied during rest preexercise and postexercise in both non-stimulated conditions and during β-adrenoceptor stimulation in situ. The results do not support the hypothesis of an increased lipolytic sensitivity to catecholamines in the recovery period after exercise. The main findings are as follows: (1) When local blood flow was controlled by hydralazine, the abdominal subcutaneous adipose tissue response to β-adrenoceptor stimulation (measured as an increase in interstitial glycerol concentration) was attenuated during the high-dose isoprenaline infusion postexercise versus preexercise. With no control for changes in blood flow, no differences in the response to preexercise and postexercise isoprenaline infusions were observed; (2) There were no differences between lean and obese subjects in the response to β-adrenoceptor stimulation. Since arterial plasma insulin was elevated in the obese compared with the lean group, this indicates resistance to the antilipolytic effect of insulin in the obese; and (3) ATBF increased in the postexercise period, but more slowly in obese subjects. In accordance, a lower
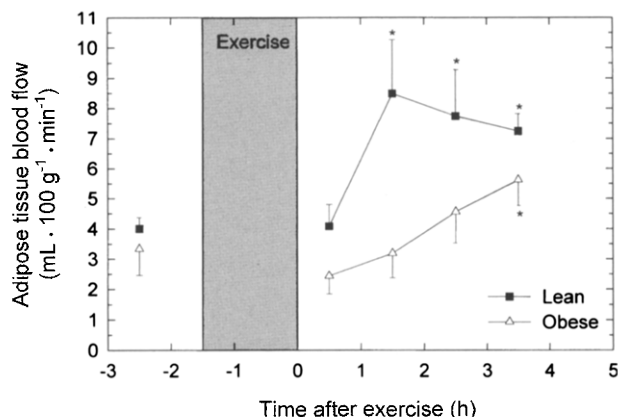

Fig 8. Time plot of ATBF in lean (■) and obese (△) subjects before and after prolonged submaximal cycling (mean ± SE, n = 8 in both groups). *Postexercise value v respective preexercise value, $P < .05$. Significant overall group difference.

plasma glycerol concentration was found in obese subjects during the first 2 hours after exercise.

The present results show that the increased lipolytic response to catecholamine stimulation immediately after an exercise bout observed in vitro[7,8] is not sustained in vivo during the recovery period, since no increase in adipose tissue responsiveness to β-adrenoceptor stimulation was found after exercise. However, it should be noted that only one fat depot was investigated in the present study and the microdialysis was performed in abdominal subcutaneous adipose tissue, whereas the in vitro experiments were performed on gluteal and suprailiac adipose cells, respectively. Also, the exercise bout used in the present study (90 minutes at about 55% of maximal O_2 uptake) was of lower intensity than the exercise bouts in the studies cited (30 minutes at two thirds of maximal O_2 uptake, and 90 minutes at 88% of maximal HR, respectively), since it was desirable to ensure a high lipolytic rate during exercise[16] and an elevated rate of FA oxidation after exercise.[4] However, the release of FFA from abdominal subcutaneous tissue corresponds closely with systemic FFA concentrations in a variety of situations,[26] indicating that the response of the tissue is at least qualitatively representative of adipose tissue throughout the body. Also, it is known that prolonged exposure of an effector tissue to an adrenoceptor agonist may result in desensitization.[9] This study shows that even during exercise of moderate intensity, a desensitization of the β-adrenoceptors on adipose cells may occur during the postexercise period.

Isoprenaline acts on both $β_1$- and vasodilating vascular $β_2$-adrenoceptors, and may induce changes in both the lipolytic rate and blood flow. When changes in local blood flow were attenuated by infusion of the vasodilator hydralazine, an interaction effect between exercise and dose on the lipolytic response was shown, with an attenuated response to the highest isoprenaline dose postexercise. This may indicate that high local levels of catecholamines during exercise produce desensitization of the β-adrenoceptors on adipose cells. Moreover, the unaltered response to isoprenaline infusion in the ISO catheter may indicate also that the blood flow response was desensitized during the postexercise infusion. A decrease of the blood flow response may obscure the decrease of the lipolytic response by attenuating the washout of interstitial glycerol. Arner et al[27] also noted a desensitization to isoprenaline in human subcutaneous tissue, but the possible role of β-adrenoceptor–mediated changes in blood flow was not assessed. From the present results, it is suggested that tachyphylaxis occurs at the β-adrenoceptors on adipose cells and blood vessels, making it difficult to interpret data from in situ experiments with microdialysis unless blood flow is controlled, eg, by adding a vasodilating agent such as hydralazine. From the protocol used in this study, it is not possible to determine if the desensitization to isoprenaline is due to changes at the adrenoceptor or postadrenoceptor level.

The effects of isoprenaline on both lipolysis and blood flow may also explain why significant increases in interstitial glycerol were detected only at the highest isoprenaline dose in the ISO catheters. A stimulation of both adipose tissue and vascular β-adrenoceptors may reduce each respective effect on interstitial glycerol. However, if a desensitization of vascular β-adrenoceptors is the more pronounced effect, interstitial glycerol will increase. A decrease of both the abdominal subcutaneous

lipolytic response and the ATBF response during repeated intravenous epinephrine stimulation (three 35-minute infusions with a 30-minute break between) has been shown in resting humans.[28] In accordance with the present results, Klein et al[29] reported that the increase in whole-body glycerol release from the baseline during a 60-minute intravenous epinephrine infusion was attenuated 90 minutes after exercise (1 hour of cycling at 70% of maximal O_2 uptake) compared with rest in trained subjects. In the present study, isoprenaline was given in situ, avoiding systemic effects such as stimulation of insulin release.[30]

It should be kept in mind that the subjects received no food during the experiment ($\sim$10 hours), which was undertaken after an overnight fast. With the present protocol, a possible effect of fasting cannot be determined. After a prolonged submaximal exercise bout, glycogen stores are reduced, and the importance of fat as substrate is increased. Even though the subjects received no food, no increase in the abdominal lipolytic response to isoprenaline was found after exercise, which strengthens our conclusion.

The glycerol concentration in interstitial fluid was about 2 to 4 times higher than in arterial plasma during nonstimulated rest before exercise. This is in agreement with previous studies,[11,13] and indicates ongoing lipolysis. When hydralazine was included in the perfusion liquid, interstitial glycerol was decreased in the control condition, due to increased washout of glycerol in the vicinity of the catheter. This confirms the importance of measurements, or control of, blood flow together with interstitial glycerol for investigation of adipose tissue lipolysis with the microdialysis technique.[13,31]

The abdominal subcutaneous adipose tissue response to β-adrenoceptor stimulation appeared similar in lean and moderately obese men. Adrenergic stimulation has been reported to be less effective in a subgroup of obese subjects due to altered $β_2$-adrenoceptor function.[32] Therefore, it has been suggested that a reduced sympathoadrenal activity may be a factor in the development of obesity. Similarly, lower plasma glycerol and FFA responses to an intravenous isoprenaline infusion in obese versus lean subjects have been observed.[33] However, it was not possible to determine if this was due to differences in the lipolytic response or the ATBF response or systemic effects of the isoprenaline infusion. Our data do not support the hypothesis that obesity may be due to an insufficient lipolytic response to adrenergic agents, since no differences in the response to isoprenaline were found between the groups. Instead, the higher plasma insulin concentrations in the obese subjects indicate an impaired antilipolytic effect of insulin in obesity, as previously suggested.[13]

It is not likely that possible differences in the workload explain the lack of differences in the response to isoprenaline between groups. Although maximal O_2 uptake was not measured and, instead, an appropriate workload corresponding to about 55% of maximal O_2 uptake was estimated based on the relation between HR and workload for each subject, respectively,[15] an influence of possible differences in the exercise intensities between subjects is small, since the comparison is made late in the postexercise period and the exercise bout is submaximal. For the comparison of the response to isoprenaline

after versus before exercise within each group, each subject was his own control.

The results of the present study confirm previous findings of increased plasma FFA and glycerol concentrations for several hours after prolonged exercise of moderate intensity. The elevated plasma FFA concentration is followed by an increase in the rate of FFA oxidation.[1] From the present results, the increased fat mobilization after prolonged exercise cannot be explained by an increase in the sensitivity to catecholamines. Rather, a prolonged depression of the arterial plasma insulin concentration may be of importance for the postexercise increase in lipolysis.

ATBF is also of importance for regulation of the lipolytic rate and mobilization of FFA. Both an increased[10] and unchanged[11] ATBF have been found during exercise. Bülow[10] found that the increase in ATBF during very prolonged exercise was sustained during the first hour postexercise. We are not aware of any other studies on ATBF in the recovery period postexercise. In the present study, ATBF was not different from preexercise levels immediately after the end of the exercise bout, but increased during the recovery period. An essentially constant mean ATBF in humans during 7 hours of fasting has been reported,[14] making it less likely that ATBF changed during the day due to the postabsorptive state. However, from the present protocol, we cannot examine the role of fasting. The increased ATBF in the postexercise period documented in the present study is probably of major importance for the mobilization of glycerol and FFA from adipose tissue during this period.

Preexercise resting ATBF was similar in both groups. A higher ATBF during rest in lean compared with markedly obese

subjects has been reported.[13,14] The increase in ATBF in the postexercise period observed in the lean group occurred at a slower rate in the obese. One may hypothesize that a slow ATBF response in the obese subjects may delay lipid mobilization in response to regular exercise. In support of a delayed washout of interstitial glycerol, we noted lower plasma glycerol during the first 2 hours postexercise in the obese subjects. A blunted ATBF response also has been found in response to an oral glucose tolerance test in markedly obese versus lean subjects.[13] Interestingly, Blaak et al[14] found that the β-adrenoceptor–mediated ATBF response was attenuated in obesity.

In conclusion, the present data demonstrate no increase in the abdominal subcutaneous lipolytic sensitivity to catecholamines after exercise. No increase in the abdominal subcutaneous adipose tissue response to in situ β-adrenoceptor stimulation was found postexercise in lean and obese men. Hyperinsulinemia prevailed in the obese, but still no difference in the adipose tissue lipolytic response to β-adrenoceptor stimulation was detected between the subject groups, indicating resistance to the antilipolytic effect of insulin in obesity. In addition, the postexercise increase in ATBF and plasma glycerol was delayed, indicating slower lipid mobilization from adipose tissue in obese subjects.

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