Adipose tissue lipolysis is increased during a repeated bout of aerobic exercise

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Stich, V., I. de Glisezinski, M. Berlan, J. Bulow, J. Galitzky, I. Harant, H. Suljkovicova, M. Lafontan, D. Riviere, and F. Crampes. Adipose tissue lipolysis is increased during a repeated bout of aerobic exercise. J Appl Physiol 88: 1277–1283, 2000.—The goal of the study was to examine whether lipid mobilization from adipose tissue undergoes changes during repeated bouts of prolonged aerobic exercise. Microdialysis of the subcutaneous adipose tissue was used for the assessment of lipolysis; glycerol concentration was measured in the dialysate leaving the adipose tissue. Seven male subjects performed two repeated bouts of 60-min exercise at 50% of their maximal aerobic power, separated by a 60-min recovery period. The exercise-induced increases in extracellular glycerol concentrations in adipose tissue and in plasma glycerol concentrations were significantly higher during the second exercise bout compared with the first (P < 0.05). The responses of plasma nonesterified fatty acids and plasma epinephrine were higher during the second exercise bout, whereas the response of norepinephrine was unchanged and that of growth hormone lower. Plasma insulin levels were lower during the second exercise bout. The results suggest that adipose tissue lipolysis during aerobic exercise of moderate intensity is enhanced when an exercise bout is preceded by exercise of the same intensity and duration performed 60 min before. This response pattern is associated with an increase in the exercise-induced rise of epinephrine and with lower plasma insulin values during the repeated exercise bout.

Microdialysis; norepinephrine; epinephrine; growth hormone

ADIPOSE TISSUE LIPOLYSIS IS increased during exercise. The major stimulus for the enhanced lipolysis seems to be circulating catecholamines in combination with a low insulin concentration. The increased lipolysis and nonesterified fatty acid (NEFA) mobilization result in an increased concentration of circulating NEFA, which is the major determinant of the rate of NEFA uptake in exercising muscles. The rate of lipolysis, and thus NEFA mobilization, during exercise has been found to be influenced by the nutritional state before exercise (22, 26), by intake of carbohydrates during exercise (12), and by the training status (28). The contribution of NEFA to total energy consumption has been shown to be dependent on the relative power output and the duration of the exercise bout (16). However, we have limited knowledge about the influence of prior exercise on lipolysis and substrate utilization during a subsequent exercise bout. It has been found that short, intensive exercise does not alter the fractional contribution of lipid and carbohydrate during a subsequent moderate [40% of maximum O2 consumption (V0max)] exercise bout (13). On the contrary, it was demonstrated that 30-min exercise at 70% of V0max induced higher fat utilization during another exercise period of the same intensity and duration performed 60 min after the first exercise bout (39). The question as to whether a previous exercise bout can modify the mobilization of endogenous substrates and substrate choice is important, not only for active sportsmen but also for any physically active people.

The aim of the present study was to investigate the lipolytic rate in subcutaneous abdominal adipose tissue during two bouts of exercise of 60-min duration at 50% of V0max with a 60-min rest period between. The microdialysis method, which was used previously for assessment of lipolysis in situ during exercise (1, 12), was used in this study. The results demonstrate that exercise-induced lipolysis is increased by an earlier bout of prolonged exercise.

METHODS

Subjects

Seven nontrained men (mean age: 22.3 ± 1.5 yr) were selected for this study. All were drug free. Their mean body weight was 73.7 ± 5.3 kg (range: 70–82 kg) and had been stable for at least 3 mo before the beginning of the study. Their body mass index was 23.0 ± 1.6 kg/m2 (range: 21.1–25.3 kg/m2). All subjects had given their informed consent before the study, and the investigation protocol was approved by the Ethics Committee of Prague Faculty of Medicine.

Experimental Protocols

The subjects were investigated at 8 AM after an overnight fast. They refrained from any strenuous exercise for 48 h

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before the experiment, and they were instructed to maintain their habitual diet. In a supine position, the microdialysis probes were inserted into abdominal subcutaneous tissue, and perfusion of the in situ microdialysis probes was started (see Microdialysis). Then an indwelling polyethylene catheter was introduced into an antecubital vein and perfused with saline solution to keep patency. The catheter was used for blood sampling throughout the study protocol. Then the subjects were placed in a semirecumbent position and stayed at rest (Rest1) for 180 min. During this period, the microdialysis probes were calibrated, and baseline microdialysis and blood samples were collected (see Microdialysis and Blood sampling, respectively). Afterward, the subjects started the first bout of exercise (Ex1) on an electromagnetically braked bicycle ergometer (Ergometrics 800s Ergoline, Jaeger, Germany) at a power output corresponding to 50% of their V\textsubscript{O2,max}. The exercise duration was 60 min. Then Ex1 subjects remained resting for 60 min (Rest2) in a semirecumbent position and thereafter started their second 60-min exercise bout (Ex2). After Ex2, the subjects stayed for another 60 min, resting in a semirecumbent position.

During both exercise bouts, heart rate was monitored continuously (Polar Accurex Plus Cardiometer, Monitor, France) and oxygen uptake (\(V\textsubscript{O2}\)) was measured (Vmax, continuously (Polar Accurex Plus Cardiometer, Monitor, France) and oxygen uptake (V\textsubscript{O2}) was measured (Vmax, continuously (Polar Accurex Plus Cardiometer, Monitor, France)) at the 10th, 25th, and 50th min of exercise for 6 min. \(V\textsubscript{O2,max}\). One week before the investigation period, \(V\textsubscript{O2,max}\) was assessed by using a graded test on an electromagnetically braked bicycle ergometer. The initial power output was 50 W and was increased by 25 W every 3 min until exhaustion. \(V\textsubscript{O2}\) was measured by using a Vmax apparatus during the test, and the highest \(V\textsubscript{O2}\) value was considered as \(V\textsubscript{O2,max}\). The mean \(V\textsubscript{O2,max}\) of the group was 46.4 ± 5.3 ml O\textsubscript{2} · kg\textsuperscript{-1} · min\textsuperscript{-1} (3.44 ± 0.21 l/min), and the maximum heart rate was 188.9 ± 7.4 beats/min.

Microdialysis. Lipolysis in adipose tissue was assessed with a microdialysis technique that has been described previously (1). A microdialysis probe (Carnegie Medecin, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-mol wt cutoff was inserted percutaneously after epidermal anesthesia (200 µl of 1% lidocaine, Roger-Bellon, France) into the abdominal subcutaneous adipose tissue at a distance of 100 mm immediately to the right of the umbilicus. The probe was connected to a microinjection pump (Harvard Apparatus, South Natick, MA) and perfused with sterile Ringer solution (154 mmol/l sodium, 4 mmol/l potassium, 2.5 mmol/l calcium, 160 mmol/l chloride) at a flow rate 2.5 µl/min. Ethanol (1.7 g/l) was added to the perfusate to estimate changes in the adipose blood flow, as previously described (2, 3). The glycerol and ethanol concentrations were measured in the outgoing dialysate. Dialysate collection was started 40 min after probe insertion. First, the recovery ratio was determined for each probe by using a modification of the “zero-flow” calibration procedure at various perfusion rates, which was recently applied for interstitial glycerol concentration in muscle and adipose tissue (31) and previously described by our group (2, 3). Briefly, the probes were perfused at a rate 0.5 µl/min and then 2.5 µl/min for 30 min at each rate and were dialysate collected. Glycerol concentrations in the two dialysate samples were plotted (after log transformation) against perfusion at the rates of 0.5 and 2.5 µl/min. With the use of a straight-line fit, the glycerol concentration at zero flow, corresponding to the interstitial glycerol concentration, was calculated. Values given in Results were in agreement with those determined by using a higher number of perfusion rates in lean subjects (2, 3).

Thereafter, during the entire duration of the study, the probe was perfused at a rate of 2.5 µl/min, and 15-min fractions of outgoing dialysate were collected. All fractions were kept on ice during the experiment. At the end of the study, an aliquot of dialysate was taken from each tube for an immediate assay of ethanol. The remaining dialysate in each tube was kept frozen at −80°C until glycerol analysis.

Adipose tissue blood flow measurements. Ethanol washout method. The ratio of ethanol concentration in the samples of outgoing dialysate to that in the ingoing perfusate was calculated (outflow-to-inflow ratio) and taken as an index of the adipose tissue blood flow (ATBF) changes during the course of the experiment as previously described (12, 15).

Blood sampling. Blood samples were drawn from the intravenous catheter 10 min before and again immediately after Ex1. Thereafter, blood sampling was performed in 15-min intervals. Blood was collected in 50 µl of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseille, France) to prevent the oxidation of catecholamines and was immediately centrifuged in a cold-refrigerated centrifuge, and the plasma was stored at −80°C until analysis.

Analytic methods. Glycerol in dialysate (10 µl) and in plasma (20 µl) was measured with an ultrasensitive radiometric method (7), and the intra-assay and interassay variabilities were 5.0 and 9.2%, respectively. Ethanol in dialysate and perfusate (5 µl) was determined with an enzymatic method (5), and the intra-assay and interassay variabilities were 3.0 and 4.5%, respectively. Plasma glucose and NEFAs were determined with a glucose-oxidase technique (Biotrol, Paris, France) and an enzymatic procedure (Wako, Unipath, Dardilly, France), respectively. Plasma insulin concentrations were measured by using RIA kits (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) and growth hormone (GH) (ICN Pharmaceuticals, Orsay, France). Plasma lactate and cortisol concentrations were determined with enzymatic procedures (Sigma Chemical, l’Isle d’Abeau Chesne, France, and Merck Clevonet, Chelles, France, respectively). Plasma epinephrine and norepinephrine were assayed in 1-ml plasma aliquots by high-performance liquid chromatography by using electrochemical detection. The detection limit was 20 pg/sample. Day-to-day variability was 4%, and within-run variability was 3%.

Statistical analysis.

All the values are means ± SE. Paired t-test and ANOVA with repeated measures over time were used for statistical comparisons. When appropriate, plasma and extracellular responses were calculated as the total changes over baseline values [equal to areas under the curves (AUC)] from time (t) = 0 tot = 60 min and from t = 120 min tot = 180 min. AUC were calculated by using a trapezoidal method. Significance values are quoted in Tables 1–3 and Figs. 1–3. P < 0.05 was
considered to be statistically significant. Statistical analysis was performed with a statistical software package (Statview II, Abacus Concepts, Berkeley, CA).

RESULTS

General Observations

There were no differences in the VO₂ between the two exercise bouts (25–30 min: Ex1, 1.60 ± 0.08 vs. Ex2, 1.58 ± 0.07 l/min; 55–60 min: Ex1, 1.64 ± 0.06 vs. Ex2, 1.60 ± 0.06 l/min) and in average heart rate at the end (55–60 min) of exercise (Ex1, 136 ± 3 vs. Ex2, 141 ± 4 beats/min). The power output was lower at the end of Ex2 compared with Ex1 (Ex2, 80.0 ± 3.5 vs. Ex1, 97.1 ± 3.1 W; P < 0.05). Respiratory ratio was lower at the end of Ex2 compared with Ex1 (Ex1, 0.87 ± 0.03 vs. Ex2, 0.84 ± 0.02; P < 0.05).

Microdialysis of Subcutaneous Adipose Tissue

The mean concentration of glycerol in dialysate at Rest1 was 61.3 ± 10.3 µM. With the use of the calibration procedure described in Microdialysis, the recovery ratio and the actual concentration of glycerol in the extracellular compartment were calculated for each probe. The average concentration of extracellular glycerol at rest was 214.4 ± 22.6 µM. This value was three to five times higher than the resting glycerol concentration in plasma, indicating that there was a net release of glycerol from adipose tissue.

Ex1 induced a significant increase in extracellular glycerol concentration from the 15th min of exercise (P < 0.05) until the highest value (728 ± 159 µM) was reached at the 60th min of Ex1. During the subsequent recovery period, Rest2, the extracellular concentration decreased to a value of 221 ± 44 µM, not different from that at Rest1. Ex2 again elicited an increase in extracellular glycerol concentrations, and the value reached at the 60th min of Ex2 was significantly higher than that at the 60th min of Ex1 (1,126 ± 298 vs. 728 ± 159 µM; P < 0.01; Fig. 1A). When the exercise-induced responses of extracellular glycerol were assessed by using the calculation of AUC (Table 1), the response to Ex2 was markedly higher than that during Ex1.

ATBF

Table 2 presents the results of the evaluation of ATBF using both the 133Xe washout and ethanol washout methods. ATBF using 133Xe washout calculated for the last 30 min of each experimental period and the average ethanol outflow-to-inflow ratio calculated over the last 30 min of each period were taken as indexes of ATBF (see Adipose tissue blood flow measurements in METHODS for details). Whether measured by 133Xe washout (in a subset of 5 subjects) or by ethanol washout (in all 7 subjects), no significant variations in subcutaneous ATBF were found during the course of the study; i.e., no differences were found when each of the two rest periods were compared with the subsequent exercise bout or the two exercise bouts with one another.
Plasma NEFA and Glycerol Levels

During the baseline period, plasma concentrations of NEFA and glycerol were 576.7 ± 126.5 and 41.0 ± 7.1 µM, respectively. No significant difference between values at Rest1 and Rest2 were found for glycerol, whereas NEFA levels were higher at Rest2 than at Rest1 (882.3 ± 92.5 vs. 576.7 ± 126.5 µM; P, 0.05).

Exercise induced significant increases in plasma NEFA and glycerol except for NEFA during Ex1 (Fig. 1B). For both plasma glycerol and plasma NEFA, when assessed by the AUC, the exercise-induced increases were higher during Ex2 compared with Ex1 (Table 1).

Plasma Catecholamine Concentrations

At Rest1, plasma norepinephrine and epinephrine concentrations were 155.6 ± 39.8 and 37.0 ± 9.3 pg/ml, respectively. Ex1 induced a significant increase in plasma concentrations for both catecholamines (Fig. 2). During the subsequent Rest2 period, both catecholamines decreased to values not significantly different from those at Rest1. Ex2 induced an increase in both norepinephrine and epinephrine concentrations. Norepinephrine increased at the 60th min of Ex2 to values not different from those at the corresponding time during Ex1, whereas the epinephrine concentration was higher at the 60th min of Ex2 (P < 0.01) compared with Ex1. When assessed by the AUC method, the rise in epinephrine was significantly higher during Ex2 compared with Ex1 (P < 0.01), whereas no difference in the exercise-induced response was found for norepinephrine (Table 1).

Plasma Glucose and Insulin

During the Rest1 period, the plasma concentrations of glucose and insulin were 4.66 ± 0.24 mM and 4.15 ± 0.79 µU/ml, respectively. No significant variations in blood glucose were found during Ex1, whereas the glycemia decreased significantly during Ex2 (Rest2, 4.85 ± 0.23 vs. 60-min Ex2, 4.33 ± 0.20 mM; P < 0.05). The insulin plasma concentration at the end of Rest2 was lower compared with that at Rest1 (2.99 ± 0.79 vs. 4.15 ± 0.57 µU/ml; P < 0.06), although the difference did not reach statistical significance. Both exercise bouts elicited significant declines in insulin. The exercise-induced declines in plasma insulin were not different during the Ex1 and Ex2 periods; however, the levels of insulin throughout Ex2 were lower (P < 0.05 to P < 0.001) compared with levels at the corresponding times during Ex1 (Fig. 3).

Plasma Lactate, GH, and Cortisol Concentrations

The values of plasma concentrations of lactate, GH, and cortisol during the whole study protocol are shown in Table 3. Plasma lactate concentration rose significantly during each exercise bout but stayed under 2 mmol/l. The increase was lower during Ex2 compared with Ex1 (P, 0.05). Resting GH plasma concentration was higher during Rest2 than Rest1. The GH concentration increased in...
response to the two exercise bouts. Evaluation using AUC (Table 1) indicated a blunted exercise-induced increase in Ex2 compared with Ex1.

For plasma cortisol concentrations, no variations were found through the Ex1 and Rest2 periods. During Ex2, a significant increase in cortisol was induced (Table 3).

### DISCUSSION

In the present study, we investigated lipid mobilization from adipose tissue and underlying metabolic and hormonal responses during two successive bouts of moderate-intensity aerobic exercise of the same duration and the same relative intensity, separated by a 60-min recovery period.

Striking differences between the patterns of exercise-induced responses during the two exercise bouts were observed, and the results suggest markedly higher lipid mobilization during the repeated exercise bout. The extracellular concentration of glycerol measured in situ in subcutaneous abdominal adipose tissue increased markedly during both bouts of exercise, but the increase in Ex2 was two to threefold higher compared with that in Ex1.

Extracellular glycerol concentrations are influenced by local blood flow in adipose tissue: the higher blood flow contributes to the lowering of the extracellular glycerol levels (14). In this study using $^{133}$Xe washout as well as ethanol washout, no significant variations in the local ATBF could be demonstrated. The unchanged blood flow during the whole experiment implies that changes in extracellular glycerol concentrations can be interpreted as reflecting changes in lipolysis.

The response pattern of the plasma glycerol concentration was similar to that of extracellular glycerol, but concentrations in the plasma remained two to three times lower than those in the extracellular compartment throughout the study protocol. The rise in NEFA during Ex2, which was in contrast to unchanged levels during Ex1, was in agreement with the overall image of higher lipolysis during Ex2. The higher availability of circulating NEFA for exercising muscles led to greater fat utilization during Ex2 (expressed by a decrease in the respiratory exchange ratio).

Lipid mobilization from adipose tissue is mainly controlled by catecholamines and insulin. Catecholamines stimulate lipolysis through the $\beta$-adrenergic pathway and inhibit it through the $\alpha_2$-adrenergic pathway, and insulin inhibits lipolysis. In the present study, the response of epinephrine and norepinephrine was strikingly different during Ex2 in comparison to Ex1. Whereas the increase in norepinephrine was similar during the two bouts, the rise in epinephrine was two to three times higher during Ex2. The enhancement of the glycerol response during Ex2 is thus associated with the enhanced epinephrine and unchanged norepinephrine responses. This observation seems to demonstrate, in physiological conditions, the role of epinephrine in the control of lipolysis in human adipose tissue. Pharmacological stimulation of lipolysis with epinephrine was previously demonstrated in situ with microdialysis by using either an intravenous infusion of epinephrine (35) or a local perfusion of epinephrine through a microdialysis probe (32).

However, besides epinephrine, several other factors might contribute to the higher response of extracellular glycerol to repeated exercise. The insulin levels showed a significant decrease during both bouts of exercise, as described previously in many reports (for review see Ref. 17). However, the insulin levels in plasma were significantly lower throughout Ex2. The decrease in plasma insulin during exercise was shown to facilitate NEFA mobilization from adipose tissue (21, 37), and, consequently, the lower plasma insulin levels during Ex2 could contribute to the enhanced response during Ex2.

GH has also been shown to regulate lipolysis in adipocytes (20, 33). GH exerts a delayed lipolytic effect (33), and it cannot be excluded that the higher response of extracellular glycerol in Ex2 might be influenced by increased levels of GH during Ex1. The levels of GH reached during Ex1 were close to those reported in the study by Møller et al. (33) to induce a significant rise in NEFA and glycerol, peaking 120 min after GH administration.

Moreover, the blunted response of GH during Ex2 might be in accordance with the markedly higher levels of NEFA during Ex2 compared with Ex1, as NEFAs were suggested by some authors to inhibit GH plasma levels during exercise (11). It has been shown recently that the GH response to exercise is blunted in upper body obese women in comparison to lower body obesity, and the difference might be associated with the higher exercise-induced increase in NEFA in upper body obesity (24). In contrast to our study, a higher GH response to repeated bouts of exercise was reported recently (25). The longer duration and lower intensity of exercise in the present study might be the reasons for the discordant results.

Besides the exercise-induced changes in the secretion of hormones regulating lipolysis, the enhanced lipolytic response could be due to enhanced responsive-
ness of adipose tissue to the action of lipolytic hormones (sensitization of the adipocyte) induced by the preceding exercise bout. In fact, an exercise-induced increase in adipose tissue responsiveness to the lipolytic action of epinephrine and isoprenaline was found in vitro (36). However, the finding was not confirmed in an in vivo study (29). The design of this study and the fact that the response of several hormones regulating lipolysis was modified during Ex2 prevent us from making any conclusions about a possible change in adipose tissue responsiveness by Ex1.

Although the main purpose of this study was to assess lipolysis during the repeated exercise bouts, the findings of the modified responses of some hormones to Ex2 deserve comment. Several factors contributing to the higher epinephrine response during Ex2 may be suggested. The blood glucose level during exercise was shown to influence the epinephrine (and much less norepinephrine) response to exercise (18, 19). Maintained hyperglycemia during exercise was shown to suppress the catecholamine, GH, and glycerol responses to exercise (30). The oral glucose supplementation during exercise blunted in situ lipolytic and plasma epinephrine responses to exercise (12). In the present study, blood glucose levels declined during Ex2 but not during Ex1. As even the small decreases in plasma glucose enhance epinephrine secretion (27), this differential evolution of blood glucose could contribute to the higher epinephrine response during Ex2.

Another mechanism involved in the higher epinephrine response might be found in the lower insulinaemia before Ex2 compared with Ex1; indeed, Galbo et al. (17, 19) suggested that the availability of insulin in the period preceding exercise might be an important determinant of the catecholamine response.

Furthermore, the lower plasma insulin levels during Ex2 might be due to higher α2-mediated inhibition of insulin secretion by epinephrine (23).

Partial depletion of glycogen, which was likely to be produced by Ex1 performed in the fasted state, could play a role in the differences in the hormonal and lipolytic responses during Ex2. The pattern of differences in hormonal and metabolic responses between Ex1 and Ex2 in this study is similar to that which was observed between the glycogen-depleted state produced by a high-fat diet (19, 22) or very-low-calorie diet (26) and the state under a normal (or low-fat) diet. The glycogen depletion influences the lipid metabolism per se (6, 38) or through its effect on blood glucose and hormonal response to exercise (18, 19, 22).

The present results relate to moderate-intensity aerobic exercise, and the phenomenon of the enhancement of lipolysis could be different in exercises of higher intensities during which the lipolytic rate, as well as the relationship between lipolysis and free fatty acid kinetics, changes with increasing exercise intensity (4, 8, 34).

In conclusion, with the use of a microdialysis method, this study demonstrates that repetition of moderate-endurance exercise elicits a markedly higher increase in the extracellular glycerol concentrations in subcutaneous abdominal adipose tissue, suggesting a higher lipid mobilization during the repeated exercise bouts. A possible mechanism underlying the enhanced lipolytic response might be a higher epinephrine response and lower plasma levels of insulin during the repeated exercise.

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