Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise

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HODGETTS, VANESSA, SIMON W. COPPACK, KEITH N. FRAYN, AND T. DEREK R. HOCKADAY. Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. J. Appl. Physiol. 71(2): 445-451, 1991.—To investigate possible factors that limit fat utilization during exercise, arteriovenous differences of plasma nonesterified fatty acids (NEFA) and glycerol were measured across the subcutaneous adipose tissue of the anterior abdominal wall in nine subjects who exercised for 60 min at 50-70% of their maximal O2 consumption. The large gradient of NEFA concentration from adipose tissue venous to arterial plasma increased throughout the exercise period. Maximal plasma NEFA concentrations in adipose venous drainage were reached postexercise (median 3,800 pmol/l), with a median NEFA-to-albumin molar ratio of 5.7. Fractional reesterification of fatty acids within the tissue (assessed from the ratio of NEFA to glycerol release) was 20-30% in the basal state and declined during exercise. After exercise there was apparently negative reesterification, implying release of NEFA retained in adipose tissue during exercise. Although these findings challenge current views on the regulation of NEFA release, they are in agreement with the concept of supply of fatty acids from adipose tissue as the major factor limiting fat oxidation during sustained exercise.

METHODS

Subjects. Nine healthy subjects (2 males, 7 females) aged 20-26 yr were studied. Their weights ranged from 55 to 78 kg (median 75 kg), and their body mass indexes (weight/height2) from 19 to 26 kg/m2 (median 25). Their maximal O2 consumption (V\textsubscript{O2, max}) values, measured as described below, were 3.0-4.1 l/min (median 3.4) or 39-55 ml·min\(^{-1}\)·kg body wt\(^{-1}\) (median 51). All were regular sports players at university team level.

Protocol. The protocol (Fig. 1) was chosen on the basis of preliminary experiments, for the following reasons. The exercise was found to produce a marked elevation of fatty acid concentrations within 1 h, which was the maximal time for which subjects could comfortably exercise with a hand in a heated box. The alternation of higher- and lower-intensity work loads allowed the subject to adopt a more relaxed posture while adipose tissue venous acids in muscle is potentially limited by several factors, including skeletal muscle capillary density, mitochondrial size and number, and carnitine availability (8, 20). Uptake of fatty acids by working human leg muscle is linear with the fatty acid inflow rate during exercise, as it is at rest (17), although it occurs with a lower fractional extraction, perhaps suggesting some saturation (2, 31).

The concentration of nonesterified fatty acids (NEFA) in systemic plasma rarely exceeds 2 mmol/l, even during extreme endurance exercise, representing a molar ratio to albumin of ~3:1. Because there are two or three high-affinity binding sites for fatty acids on albumin (30), a rise above this ratio produces a marked elevation of unbound fatty acids (29). It has therefore been suggested that the adipose tissue stores are supplying fatty acids to the limit set by the plasma transport capacity, and the limitation on usage must be imposed at the muscle end (8).

We have recently developed a technique for sampling the venous blood that drains the subcutaneous adipose tissue of the anterior abdominal wall in humans (11, 14). It might be possible to distinguish between limitations at the level of fatty acid release or fatty acid utilization by looking at the level of fatty acids in the venous drainage from adipose tissue stores; a comparison with glycerol release will give information about reesterification rates, and a comparison between levels in adipose tissue drainage and systemic plasma might show where the rate-limiting step lies. Here, using this technique, we report studies of fat mobilization during exercise.
samples were taken during the lower-intensity work load periods.

The \( \text{VO}_2 \text{max} \) of each subject was assessed on an initial visit by use of an incremental procedure (work rate increasing by 15 W/min) on an electrically braked bicycle ergometer (Siemens Ergomed 740).

The subjects fasted overnight before the day of the experiment. On the subject's arrival in the laboratory at 8:00 a.m., a 10-cm 22-gauge cannula (Secalon Hydrocath, Viggo, Swindon, UK) was introduced over a guide wire into one of the superficial veins draining the subcutaneous adipose tissue of the anterior abdominal wall. As described previously (11, 14), this procedure was used to collect samples of the venous drainage from the subcutaneous abdominal adipose depot, with only a small contribution from the metabolism of overlying skin. A 16-gauge cannula was also introduced retrogradely into a dorsal vein draining one hand, and this hand was kept in a box warmed to between 60 and 70°C throughout the experiment to provide samples of arterialized blood (25). Both cannulas were kept patent by slow infusions of saline (NaCl, 150 mmol/l).

At least 30 min after cannulation, baseline blood samples were taken while the subject rested on a bed (“−40” on Fig. 1). Two further sets of baseline samples were taken at 20-min intervals before the subject mounted the bicycle ergometer. “Mean basal” levels referred to in RESULTS are the mean results from these three sets of resting samples.

Electrocardiogram (ECG) electrodes were positioned when the subject was seated on the bicycle ergometer, and the ECG was monitored during exercise. (“Basal” heart rates were thus not recorded.) The exercise consisted of two 5-min warm-up periods at 50 and 100 W, respectively (timing taken as 0−5 and 5−10 min). The subject then exercised for alternating 5 min periods at work loads corresponding to 70% and 60% of the \( \text{VO}_2 \text{max} \) for 30 min (10−40 min). For the next 30 min (40−70 min), the subject worked for alternating 5-min periods at 60 and 50% of \( \text{VO}_2 \text{max} \). Blood samples were taken 7, 17, 27, 37, 47, 57, and 67 min after starting the warm-up (i.e., after 2 min of each low-intensity period). At 70 min the subject began a 5-min “warm-down” period at 50 W. Samples were taken at 72 min, and then, with the subject on the bed, at 77, 87, 100, and 120 min. Samples consisted of 4 ml of blood from each of the two cannulas, taken simultaneously if possible. The blood was drawn into heparinized syringes, and hematocrit was measured in a microcentrifuge. The samples were kept at 4°C before preparation of plasma, which was stored at −20°C. Extra arterialized blood was taken at the four times when plasma catecholamine concentrations were measured (Fig. 1); the plasma was stored at −70°C.

**Analytic methods.** Plasma lactate and glycerol concentrations were measured on portions of plasma deproteinized with perchloric acid by use of methods described previously (14), adapted to an Instrumentation Laboratory Multistat microcentrifugal analyzer. Plasma glucose and NEFA concentrations were measured by enzymatic methods, as described previously (14). Plasma albumin concentrations were determined by a bromocresol green-binding technique (Sigma Chemical, Poole, UK) on the Multistat analyzer. Plasma cortisol (Coat-a-Count kit, DPC, Los Angeles, CA), growth hormone (kit from CIS, High Wycombe, UK), and insulin concentrations were determined by radioimmunoassay. Plasma epinephrine and norepinephrine concentrations were determined by liquid chromatography with electrochemical detection after extraction from plasma with ion exchange resin and alumina (15).

**Use of arterialized blood.** ArterIALIZATION was checked periodically by measurement of \( \text{O}_2 \) saturation with a Corning 2500 Co-oximeter (Ciba Corning Diagnostics, Halstead, Essex, UK). No samples with \( \text{O}_2 \) saturation <95% were accepted. The median saturation in resting samples was 95% and that during exercise was 96%. The only arteriovenous differences considered in this study are for NEFA and glycerol. Abumrad et al. (1) found “slight but insignificant” differences between true arterial and arterialized blood for both NEFA and glycerol (mean differences 39 and 10 \( \mu \text{mol/l} \), respectively, during the basal period). Brooks et al. (5), with poorer arterialization than in our study as judged from \( \text{O}_2 \) saturation, found an even smaller and nonsignificant difference for glycerol concentrations between true arterial and arterialized blood. These differences are very small compared with the arteriovenous differences for NEFA and glycerol across the subcutaneous adipose tissue (see Fig. 5).
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and are unlikely to affect the interpretation of our results. In our more recent experiments in resting subjects (S. W. Coppock et al., unpublished observations), we have used either arterialized or true arterial blood, and we find indistinguishable metabolic patterns.

Calculations. The molar ratio of albumin to NEFA was calculated with the assumption of a relative molecular mass of 66 kDa for human albumin.

Fractional reesterification rates of fatty acids within adipose tissue, described and discussed previously (11, 12, 14), were calculated as follows. Concentrations of both NEFA and glycerol in plasma were converted to equivalent whole blood concentrations (10). It was assumed that NEFA are confined to the plasma compartment, and the following formula was used

\[ B = P \times (100 - H)/100 \]

where \( B \) and \( P \) are the blood and plasma NEFA concentrations, respectively, and \( H \) is the hematocrit (%). For glycerol, the relationship between plasma and blood concentrations is extremely close \((r = 0.99 \text{ on the basis of 73 samples in which both were measured})\) and independent of hematocrit over a wide range of concentrations (10); the formula used was

\[ B = (P + 2.8)/1.08 \]

where \( B \) and \( P \) are the blood and plasma glycerol concentrations, respectively, in \( \mu \text{mol/l} \). Fractional reesterification was calculated on the assumption that lipolysis liberates three fatty acid molecules for each glycerol and that no glycerol is reutilized within the tissue; these assumptions have been discussed previously by us (12) and by others (32). Fractional reesterification was expressed as the percentage of fatty acids released in lipolysis that are reesterified without leaving the tissue, using the formula

\[ \text{percentage reesterification} = 100 \times (1 - \frac{V-A_{\text{NEFA}}}{3} \times \frac{V-A_{\text{Glyc}}}{V_{\text{Glyc}}}) \]

where \( V-A_{\text{NEFA}} \) is the venoarterial concentration difference for release of NEFA across the adipose tissue (expressed in whole blood) and \( V-A_{\text{Glyc}} \) is that for glycerol release. It should be noted that this calculation is independent of the rate of blood flow through the tissue.

Results are presented as means \( \pm \) SE. The significance of differences at different times was assessed with one-way analysis of variance (ANOVA); significance values quoted in the text and figure legends are based on post hoc paired \( t \) tests for those variables showing significant variation with time.

RESULTS

General findings. Subjectively, the exercise protocol was found to be strenuous, probably because the subjects had fasted overnight and were to some extent stressed by the presence of the cannulas, the need to cooperate during blood sampling, and the need to keep one hand in the heating box. Heart rates rose rapidly during the warm-up period (immediately before warm-up, 94 \( \pm \) 5 beats/min; at 2 min, 165 \( \pm \) 5 beats/min; \( P < 0.001 \) ) and remained elevated for the duration of the exercise period (at end, 177 \( \pm \) 3 beats/min; \( P < 0.001 \) ).

Arterialized glucose, lactate, and hormone concentrations. Arterialized plasma glucose concentrations were variable from subject to subject during the exercise period (Fig. 2A), and variation with time was not significant. Arterialized plasma lactate concentrations (Fig. 2C) rose sharply during the early part of the exercise period (basal, 1.38 \( \pm \) 0.20; at 17 min, 6.49 \( \pm \) 1.21 mmol/l; \( P < 0.01 \) ) but fell during the period at lower work loads.

Plasma insulin concentrations (Fig. 2B) fell steadily during exercise (basal, 11 \( \pm \) 2; at 67 min, 6 \( \pm \) 1 mU/l; \( P < 0.01 \) ). At the end of exercise, in contrast, they rose in some subjects (at 77 min, 14 \( \pm \) 3 mU/l; \( P < 0.02 \) vs. 67
Before falling in parallel with the plasma glucose concentration.

Plasma cortisol and growth hormone concentrations (Fig. 3) both rose sharply during the exercise period. Growth hormone concentrations were high initially (at 0 min, 12 ± 5 mU/l), presumably reflecting the anticipation of exercise. They peaked at the end of the first 30-min period (higher work loads; at 37 min, 58 ± 21 mU/l; P < 0.01 vs. basal) before declining steadily. Cortisol concentrations, in contrast, rose steadily throughout, reaching a peak just after the end of exercise (at 0 min, 290 ± 50; at 77 min, 860 ± 120 nmol/l; P < 0.01).

Plasma norepinephrine concentrations (Table 1) increased during the warm-up period and rose further by the end of exercise before declining during the final rest period. Plasma epinephrine concentrations did not change significantly (Table 1).

**FIG. 4.** Arterialized plasma albumin concentrations. Values are means ± SE for 9 subjects. Dashed lines are as in Fig. 1. Adipose venous plasma albumin concentrations were superimposable on arterialized values and for clarity are not shown. Variation in concentration with time was significant (P < 0.01 by ANOVA). Concentrations were significantly different from mean baseline from 17 to 72 min.

Plasma albumin, NEFA, and glycerol concentrations. Plasma albumin concentrations rose during the exercise period (Fig. 4; mean basal, 38 ± 2 g/l; at 67 min, 44 ± 1 g/l, P = 0.02). There was no arteriovenous difference for albumin across the adipose tissue. The mean arterial albumin concentration over all samples was 42.5 g/l, and the mean adipose venous albumin concentration was 42.7, with a mean difference of 0.3 ± 0.2 (SE) g/l (not significant).

In the baseline period, there was release of both NEFA and glycerol from the adipose tissue, as indicated by the venoarterial (V-A) differences (Fig. 5, A and B). For both compounds, the venous levels were two to four times the arterialized levels in the fasting state.

ArterIALIZED plasma NEFA concentrations fell slightly during the early part of the exercise (mean basal, 533 ± 79 µmol/l; at 17 min, 385 ± 59 µmol/l, P = 0.01) despite continued NEFA release from adipose tissue, as indicated by an increasing V-A difference. After the end of exercise, both arterialized and adipose-venous plasma NEFA concentrations rose sharply (mean arterialized at 87 min, 1,685 ± 365 µmol/l, P < 0.001 vs. 67 min). In some cases, very high levels were reached. The median (and range) of peak values observed in individual subjects were 1,850 (970–2,160) for the arterial and 3,800 (2,100–4,540) µmol/l for the adipose venous.

In contrast, arterIALIZED plasma glycerol concentrations rose throughout the exercise period (mean basal, 57 ± 7 µmol/l; at 67 min, 361 ± 27 µmol/l; P < 0.001), and again there was an increasing V-A difference, indicating continued release from adipose tissue. After exercise, glycerol concentrations in both arterial and adipose venous plasma fell rapidly; there was no postexercise peak as observed for NEFA.

The molar plasma NEFA-to-albumin ratios are shown in Table 2; the peak values in arterial plasma were almost

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**TABLE 1.** Plasma catecholamine concentrations

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<tr>
<th>Time</th>
<th>Epinephrine</th>
<th>Norepinephrine</th>
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<tbody>
<tr>
<td>Basal</td>
<td>0.30±0.04</td>
<td>1.8±0.3</td>
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<tr>
<td>&quot;Warm-up&quot;</td>
<td>0.42±0.05</td>
<td>3.7±0.3*</td>
</tr>
<tr>
<td>End of exercise</td>
<td>0.70±0.21</td>
<td>8.7±1.4*</td>
</tr>
<tr>
<td>&quot;Warm-down&quot;</td>
<td>0.62±0.33</td>
<td>4.3±0.7*</td>
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Values are means ± SE in nmol/l for 9 subjects; statistics were prepared on log-transformed data. Details of exercise protocol and timing of samples are given in Fig. 1. * P = 0.01 vs. basal by paired t test, † P ≤ 0.05.
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FIG. 5. Plasma concentrations of nonesterified fatty acids (NEFA, A) and glycerol (B) in arterialized (▲) and adipose venous (■) plasma and calculated fractional reesterification rates of fatty acids (C). Values are means ± SE for 9 subjects. Dashed vertical lines are as in Fig. 1; horizontal dotted line is at zero reesterification. For both NEFA and glycerol, there were significant variations with time in arterIALIZED and venous concentrations and in arteriovenous difference (all P < 0.001 by ANOVA). Variation in fractional reesterification with time was significant (P < 0.001 by ANOVA). Reesterification values were significantly different from mean baseline from 72 to 82 min. Concentrations were significantly different from mean baseline at 7, 17, and from 72 to 100 min for arterial NEFA and from 7 to 100 min for glycerol.

The fractional reesterification rates of fatty acids within the adipose tissue, calculated as described under METHODS, are shown in Fig. 5C. They were 20–30% in the fasting state and then steadily declined throughout exercise to reach values near zero at the end of that period. After exercise, the calculated values for fractional reesterification became transiently very negative; the meaning of these values is discussed below.

DISCUSSION

This is, so far as we are aware, the first direct study of fat mobilization from adipose tissue in humans during exercise. Although arteriovenous differences have been measured across canine subcutaneous adipose tissue during exercise, the depot studied seemed not to be representative of “average” adipose tissue (6). We have shown previously that the depot studied with the technique employed here behaves, at rest, similarly to the bulk of adipose tissue (11, 12, 14). The use of this technique has enabled us to make some unique observations about the regulation of fatty acid release.

The hormonal and nervous environment during strenuous exercise is clearly strongly favorable to lipolysis. Concentrations of catecholamines are elevated (norepinephrine reflecting increased sympathetic activity), as are those of cortisol and growth hormone, both longer-term activators of lipolysis (13). The importance of adrenergic influences on lipolysis during exercise has been shown in the microdialysis measurements of Arner et al. (3); local propranolol treatment abolished the rise in adipose tissue glycerol during bicycle exercise in normal subjects. Growth hormone exerts its action on fat mobilization in vivo over a period of ~2 h (26), and so its reinforcement of lipolysis would be most important during sustained exercise. Insulin concentrations fell during the exercise despite rising plasma glucose concentrations, presumably reflecting adrenergic inhibition of insulin secretion. At the end of exercise, when the adrenergic inhibition was rapidly removed, insulin concentrations rose following the peak in glucose concentrations. We did not measure plasma glucagon concentrations. Other work suggests that these would not have responded much during the 60-min exercise period (4, 23, 31), glucagon becoming important only in more prolonged exercise (2).

The arterialized plasma NEFA concentrations decreased marginally during the early part of exercise. Because there was clearly continuing release of NEFA from adipose tissue, this fall presumably reflects the rapid uptake of NEFA by the working muscle. Wolfe et al. (32) showed that the rate of appearance of NEFA rises steadily throughout moderate-intensity exercise (40% VO₂max for 2 h), although in their studies this was accompanied by a rise in the arterialized NEFA concentration. Jones et al. (22) found a decreasing rate of appearance of

<table>
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<tr>
<th>TABLE 2. NEFA-to-albumin molar ratios in plasma</th>
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<tr>
<td>Arterialized</td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>Basal</td>
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<tr>
<td>67 min*</td>
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<tr>
<td>120 min</td>
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<td>Highest observed</td>
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<td>Median</td>
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Values are means ± SE for 9 subjects except “highest observed,” which are peak ratios in individual subjects. * Samples taken just before end of exercise (see Fig. 1).
palmitate during very heavy work (70% \( V_{O_{2\max}} \) for 40 min), but this was accompanied by a marked decrease in plasma palmitate and a much greater rise in lactate concentration than in our study. Glycerol concentrations rose during exercise in our study as well as in those of others (22, 32), the disparity presumably reflecting the fact that glycerol is not taken up by muscle, and its utilization may not be increased during exercise.

At the end of exercise, there were rapid increases in plasma concentrations of both glucose and NEFA. A postexercise NEFA peak has been observed in many previous studies (4, 17, 18, 31, 32). These increases in arterial concentration presumably reflect the effects of a continuing high rate of production with a sudden decrease in utilization. For NEFA, however, the picture is more complicated. Glycerol concentrations did not show a corresponding postexercise peak, and there was a disparity between V-A differences for glycerol and NEFA immediately postexercise; that for NEFA remained approximately unchanged, whereas that for glycerol decreased rapidly, indicating decreasing lipolysis. There was consequently apparent a highly negative reesterification rate. The most consistent explanation of these findings would seem to be that NEFA accumulated within the adipose tissue during the exercise period because of a limitation of transport into the systemic plasma, whereas glycerol, because of its smaller size and water solubility, was able to escape into the general circulation. Jones et al. (22) found evidence for lipolysis “which did not lead to influx of free fatty acids into the plasma” during heavy exercise, although they also discussed the possibility that this reflected lipolysis of intramuscular triacylglycerol stores. An inhibition of the release of fatty acids from adipose tissue has been suggested as an explanation for discrepancies between glycerol and NEFA concentrations in severely injured patients (reviewed in Ref. 13). It is not clear why the efflux of NEFA should have been limited. Adipose tissue blood flow in humans and dogs usually increases during exercise (7), although adipose tissue perfusion can be constricted by high sympathetic tone (27) and by a high NEFA-to-albumin ratio (9, 24). The apparent fractional reesterification rate averaged over the period of exercise was <20%; however, if some fatty acids were retained in the tissue, the true “metabolic” reesterification rate may have been somewhat lower. After exercise, with rapid changes in lipolysis and blood flow, non-steady-state effects appear to have dominated, with wash-out of the “entrapped” fatty acids and an apparently negative reesterification rate.

These results show several features of the regulation of fat mobilization during exercise. First, suggestions of a high fractional reesterification rate of fatty acids within the adipose tissue as a limiting factor for NEFA release (6, 8, 24) were not borne out in our studies. Rather, the fractional reesterification rate declined somewhat, despite factors that are often thought to increase it: high plasma lactate concentrations (16) and a high extracellular NEFA to albumin ratio (24, 28). Wolfe et al. (32) also found, by isotopic methods, an “intracellular” fractional reesterification rate of 20% at rest that declined during sustained exercise. Release of NEFA continued in our studies despite extracellular NEFA concentrations >4 mmol/l and NEFA-to-albumin ratios >6:1 in some subjects.

Nevertheless, the picture emerging from our studies is one in which the supply of NEFA from adipose tissue is indeed limiting for their utilization by muscle. It is difficult otherwise to account for the large, and increasing, gradient between adipose-venous and arterialized plasma and the sudden rise in arterialized concentration at the end of exercise. This limitation of NEFA release might reflect a limited activity of the hormone-sensitive lipase, reinforced by a limitation of adipose tissue fatty acid export capacity, causing accumulation of NEFA within the tissue.

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Some of the findings of this study have been presented in abstract form (13).

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