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Free fatty acid kinetics in the late phase of postexercise recovery: importance of resting fatty acid metabolism and exercise-induced energy deficit

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Abstract

Free fatty acid (FFA) availability increases several-fold during exercise and remains significantly elevated for at least 3 to 6 hours after exercise cessation. Little, however, is known regarding the duration of the postexercise rise in FFA flux. In the present study, we used stable isotope–labeled palmitate infusion to examine fatty acid metabolism in 27 healthy untrained men and women (age, 29 ± 7 years; body mass index, 25 ± 4 kg/m^2) between 13 to 16 hours and 21 to 24 hours after a single bout of moderate-intensity endurance exercise (1-2 hours at 60% of peak oxygen consumption), performed in the evening, and after a time-matched resting trial. Postabsorptive FFA rate of appearance (Ra) and FFA concentration in plasma were significantly greater after exercise than rest throughout the recovery period ($P < .015$), but the exercise-induced increases declined from approximately 40% at 13 to 16 hours to approximately 10% at 21 to 24 hours postexercise ($P = .001$). The magnitude of the exercise-induced increase in plasma FFA concentration was proportional to the increase in FFA Ra. Correlation analysis demonstrated that exercise-induced changes in plasma FFA Ra at 13 to 16 hours are (1) negatively associated with resting plasma FFA Ra and (2) positively associated with the net energy expenditure of exercise and the exercise-induced changes in whole-body fat oxidation rate (all P values < .05). In multivariate stepwise linear regression analysis, baseline plasma FFA Ra ($P \leq .008$) and net energy expenditure of exercise ($P \leq .005$) independently predicted the exercise-induced change in plasma FFA Ra at 13 to 16 hours. We conclude that the exercise-induced increase in FFA mobilization is (1) long-lived, persisting for 12 to 24 hours after exercise, with a progressive decline with time; (2) greater in subjects with low than high resting plasma FFA availability; and (3) greater after exercise with high than low energy demand.

1. Introduction

Fatty acids are an important oxidative fuel for humans, both at rest and during exercise [1]. Free fatty acid (FFA) release from adipose tissue is well regulated by the coordinated action of many endocrine, paracrine, and other factors [2,3], allowing appropriate availability of fatty acids to meet the energy requirements of tissues. Thus, adipose tissue lipolytic rate and the release of FFA into the circulation rise approximately 5-fold above resting values during moderate-intensity exercise to meet the increased fuel demand [4,5]. It is well established that the exercise-induced lipolytic surge persists for at least 3 to 6 hours into recovery [6-10]. In addition, it has been suggested that increased plasma FFA concentrations [10] and increased adipose tissue FFA release rates [7] in the immediate postexercise period (3-6 hours later) are directly related to the intensity and/or duration of prior exercise. However, beyond the immediate postexercise period, it is not known for how long and to what extent lipolysis remains stimulated.

There is evidence of increased plasma FFA concentrations for as long as 12 to 16 hours after exercise [11-17], yet not all studies measuring plasma FFA concentration as an index of lipolytic activity find them increased by at least 12 hours into recovery [18-23]. The reasons for this discrepancy are not

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known but could be related to differences in the type of exercise performed. Furthermore, exercise is a physiologic condition where FFA concentration in plasma may not accurately represent FFA flux rates [24]. In addition, there is evidence that baseline FFA availability at rest may affect the exercise-induced changes in plasma FFA concentration. Experimental elevation of plasma FFA concentration via lipid infusion at rest, immediately before strenuous exercise, markedly blunted (by more than 50% compared with isotonic sodium chloride solution infusion) the increase in plasma FFA concentration during exercise [25]. Furthermore, studies in animals have presented evidence of feedback down-regulation of adipose tissue FFA release by increased arterial FFA concentration [26]. These observations suggest the existence of a biological ceiling in vivo, similar to the stimulation of human adipocyte lipolysis by various lipolytic agents in vitro [27]. To elucidate the effect of exercise energy expenditure and baseline FFA metabolism on the plasma FFA concentration and kinetics response during the late phase of recovery from exercise, we evaluated FFA concentrations and kinetics on the day after a single bout of moderate-intensity endurance exercise of varying duration and again after an equivalent period of rest in healthy but untrained subjects.

2. Materials and methods

2.1. Subjects and preliminary testing

Twenty-seven men and women (age, 28.9 ± 7.2 years; body mass index, 24.7 ± 4.0 kg/m^2; means ± SD) volunteered for the study; several of them participated in our previous studies examining postexercise lipoprotein metabolism [28-30]. All subjects were considered to be in good health after completing a medical evaluation, which included a history and physical examination and standard blood tests. All were normoglycemic and normolipidemic; none consumed tobacco products or took medications known to affect lipid metabolism. Subjects' body composition (fat mass and fat-free mass) was assessed by dual-energy x-ray absorptiometry (Delphi-W densitometer; Hologic, Waltham, MA), and peak oxygen consumption ($\dot{V}O_{2peak}$) was determined on a bicycle ergometer as previously described [28-30]. Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center Advisory Committee at Washington University School of Medicine in St Louis, MO.

2.2. Experimental protocol

Each subject completed 2 time-matched stable isotope–labeled tracer infusion studies within 4 weeks in randomized order: one after resting and one after cycling on the preceding afternoon. Subjects were instructed to adhere to their regular diet and to refrain from exercise for a minimum of 3 days before being admitted to the General Clinical Research Center the afternoon before each isotope infusion study (rest and exercise). For the exercise study, subjects cycled on a semirecumbent cycle ergometer (EC-C400R Ergometer; Cateye Fitness, Source Distributors, Dallas, TX) for 60 or 120 minutes between 5:00 and 7:00 PM. The workload was set to elicit a $\dot{V}O_2$ equivalent to 60% of $\dot{V}O_{2peak}$; $\dot{V}O_2$ was measured (TrueOne 2400 Metabolic Measurement System; ParvoMedics, Salt Lake City, UT) at regular intervals during exercise, and the workload was adjusted as necessary to maintain the desired $\dot{V}O_2$ (within ±5%). For the resting study, subjects lied in bed or sat in a chair. After completion of the exercise or the equivalent period of rest, subjects took a shower and then rested in a chair. At approximately 7:30 PM, they consumed a standard meal containing approximately 15 kcal/kg body weight (~55% of total energy from carbohydrate, 30% from fat, and 15% from protein), and then fasted (except for water) and rested in bed until completion of the study the next day.

At 5:30 AM the following morning, 1 catheter was inserted into a forearm vein to administer stable isotope–labeled tracers; and a second catheter was inserted into a vein in the contralateral hand, which was heated to 55°C with a thermostatically controlled box, to obtain arterialized blood samples [31]. Catheters were kept open with slow, controlled infusion of 0.9% NaCl solution (30 mL/h). At 7:00 AM (time = 0; ~12 hours after the cessation of exercise or the equivalent period of rest on the previous evening), blood samples were obtained for the determination of background palmitate tracer-to-tracee ratio (TTR) in plasma; and a constant infusion of [2,2-$^2H_2$]palmitate (0.03 μmol/[kg·min]), dissolved in 25% human albumin solution, was started and maintained for 12 hours. Additional blood samples were collected at 60, 90, 120, 180, and 240 minutes and again at 9, 10, 11, and 12 hours to determine palmitate TTR and FFA concentrations in plasma. Resting metabolic rate (RMR) and whole-body fat oxidation rate were measured by using indirect calorimetry (Deltatrac Metabolic Monitor; SensorMedics, Yorba Linda, CA) between 2.0 and 2.5 hours after beginning the isotope infusion [32].

2.3. Sample collection and analyses

Blood samples were collected in chilled tubes containing sodium EDTA. Samples were placed on ice, and plasma was separated by centrifugation within 30 minutes of collection and stored at −80°C until final analyses were performed. Plasma insulin concentration was measured by radioimmunoassay (RIA; Linco Research, St Louis, MO). Plasma FFA concentrations were quantified by gas chromatography (HP 5890 Series II GC; Hewlett-Packard, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard [33]. Plasma free palmitate TTR was determined by analyzing the methyl ester derivative with gas chromatography–mass spectrometry (Agilent Technologies/HP 6890 Series GC System–5973 Mass Selective Detector, Hewlett-Packard) [33].

2.4. Calculations

Palmitate rate of appearance (Ra) in plasma was calculated by dividing the palmitate tracer infusion rate by the average plasma palmitate TTR value between 1 to 4 hours and 9 to 12 hours into the infusion (ie, 13-16 and 21-24 hours postexercise, respectively) during both physiologic and isotopic steady state [34-37]; total FFA Ra (in micromoles per minute) was derived by dividing palmitate Ra by the proportional contribution of palmitate to total plasma FFA concentration [37]. Exercise-induced changes were calculated as differences from respective time-matched resting values.

The gross energy expenditure of exercise was determined from respiratory measurements during exercise; net energy expenditure of exercise was calculated by subtracting the RMR during the equivalent period of rest from the corresponding gross energy expenditure during the exercise session.

2.5. Statistical analysis

All data sets were normally distributed according to the Kolmogorov-Smirnov test and are presented as means ± SD. Differences between exercise and rest and between time into recovery (13-16 and 21-24 hours) were evaluated by using analysis of variance with repeated measurements for trial and time. Relationships between exercise-induced changes in FFA metabolism and other parameters of interest were assessed with linear correlation and multiple regression analyses. A P value less than or equal to .05 was considered statistically significant. All analyses were carried out with SPSS 16 for Windows (SPSS, Chicago, IL).

3. Results

Average heart rate during exercise was 134 ± 10 beats per minute (70% ± 4% of age-predicted maximum heart rate). Oxygen consumption remained constant during exercise at 1.74 ± 0.60 L/min, corresponding to 60% ± 6% of subjects' $\dot{V}O_{2peak}$. Absolute power output during the exercise session ranged from 60 to 194 W (113 ± 36 W), and net energy expenditure ranged from 244 to 2021 kcal (665 ± 442 kcal).

Plasma insulin concentration tended to be lower after exercise than rest (P = .088) and was significantly lower (P < .001), during both the exercise and resting trials, at 21 to 24 hours (rest, 3.2 ± 2.2 mU/L; exercise, 2.8 ± 1.2 mU/L) than at 13 to 16 hours (rest, 5.5 ± 3.0 mU/L; exercise: 5.1 ± 2.9 mU/L). Resting metabolic rate tended to be greater at 13 to 16 hours after exercise than rest (1.11 ± 0.18 and 1.07 ± 0.17 kcal/min, respectively; P = .051), and whole-body fat oxidation rate was approximately 20% greater after exercise than rest (61 ± 23 and 52 ± 19 mg/min, respectively; P = .020).

Free fatty acid Ra and FFA concentration in plasma were significantly greater after exercise than rest (P < .015), and both were greater (P< .001) at 21 to 24 hours than at 13 to 16 hours after exercise or rest (Fig. 1). The magnitude of the differences between exercise and rest in FFA Ra and FFA concentration in plasma was significantly greater at 13 to 16 hours than at 21 to 24 hours (~40% and ~10%, respectively; P = .001). Exercise-induced changes in plasma FFA Ra and FFA concentration (expressed as percentage increase above resting values) were strongly and positively correlated with each other both at 13 to 16 hours, when the magnitude of change was almost entirely proportional, and at 21 to 24 hours (Fig. 2).

Exercise-induced changes in plasma FFA Ra at 13 to 16 hours, when expressed in both absolute (in micromoles per minute) and relative (percentage) terms, were negatively associated with baseline plasma FFA Ra during the resting trial and positively associated with the net energy expenditure of exercise (Fig. 3); these relationships were no longer

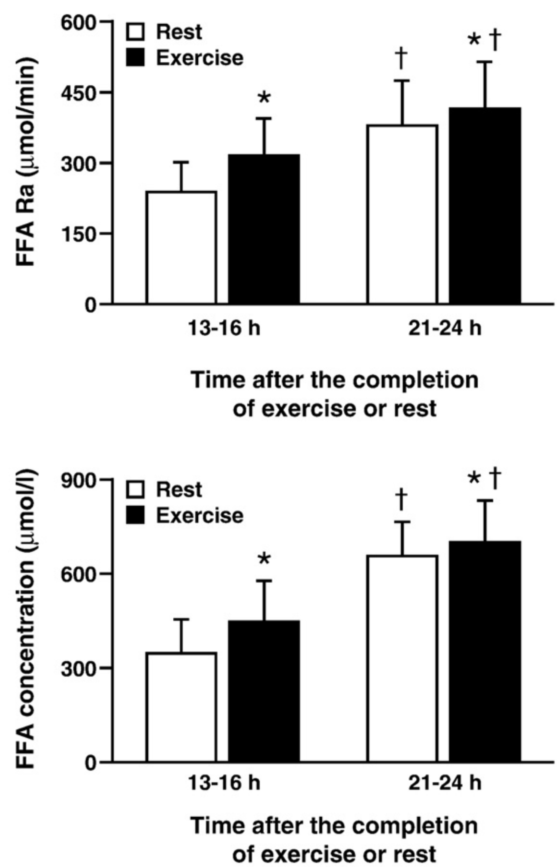

Fig. 1. Free fatty acid Ra (top) and FFA concentration (bottom) in plasma 13 to 16 hours and 21 to 24 hours after a single bout of exercise or an equivalent period of rest. Values are means ± SD. *Value after exercise is significantly different from time-matched value after rest (P < .015). †Value at 21 to 24 hours is significantly different from trial-matched value at 13 to 16 hours (P < .001).

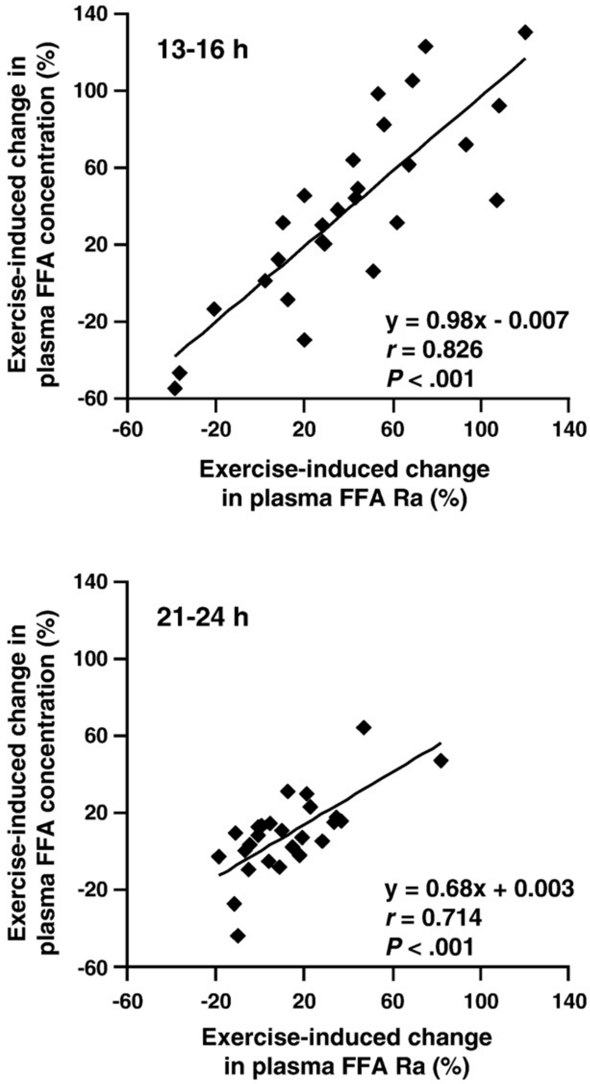

Fig. 2. Relationship between exercise-induced changes in FFA Ra and FFA concentration in plasma at 13 to 16 hours (top) and 21 to 24 hours (bottom).

apparent by 21 to 24 hours (all P values > .1). At 13 to 16 hours, changes in FFA Ra were positively associated with changes in whole-body fat oxidation rate, when expressed in both absolute (r = 0.567, P = .002) and relative (r = 0.535, P = .004) terms, but did not correlate with respective changes in plasma insulin concentration (P values > .8) and RMR (P values > .5).

In multivariate stepwise linear regression analysis, baseline plasma FFA Ra ($P \leq .008$) and net energy expenditure of exercise ($P \leq .005$) independently predicted the exercise-induced change in plasma FFA Ra at 13 to 16 hours, when expressed in both absolute (in micromoles per minute) and relative (percentage) terms, together accounting for 48% and 65% of the total variance, respectively. Sex, age, fat mass, and fat-free mass (in kilograms and percentage of body weight), VO_{2peak} (in liters per minute and milliliters per kilogram per minute), power output, baseline and exercise-induced changes (absolute and relative) in insulin concentration, RMR, and whole-body fat oxidation rate did not enter the prediction models.

4. Discussion

Exercise is a very potent lipolytic stimulus to meet the increased energy requirements due to muscle work. During exercise, whole-body lipolytic rate and plasma FFA availability increase by approximately 5-fold above resting values [4,5]. Here we show that the exercise-induced lipolytic surge is (1) long-lived but diminishes with time until it is nearly vanished by 24 hours after the exercise and (2) dependent on baseline (ie, resting) fatty acid metabolism and the acute exercise-induced energy expenditure. Our findings are consistent with observations during the immediate postexercise recovery. First, increased plasma FFA concentrations [10] and increased adipose tissue FFA release [7] some 3 to 6 hours after exercise cessation are directly related to the intensity and/or duration of prior exercise. Higher-intensity exercise results in greater adipose tissue FFA release rates and plasma FFA concentrations in the immediate postexercise period (3-6 hours) than lower-intensity exercise [7,10], and longer-duration exercise elicits greater increases in plasma FFA concentrations during the immediate (~6 hours) [10] and late (the next day) [38] phases of the recovery than shorter-duration exercise, likely because of the greater energy deficit induced by higher-intensity and longer-duration exercise. In fact, manipulating the intensity of exercise while keeping total energy expenditure constant does not modify the FFA concentration response to exercise some 16 hours later [39]. Second, experimental elevation of plasma FFA concentrations at rest, before commencing exercise, results in a markedly blunted increase (~75% compared with ~200%) in plasma FFA concentrations at the end of exercise [25]. Our findings indicate that these phenomena extend until late into the recovery from exercise, probably because of a gradual return to resting values after exercise.

Our findings help explain the inconsistent results from previous studies in which the prolonged effect of exercise on plasma FFA concentration was evaluated. Some investigators found increased fasting plasma FFA concentrations the morning after a single bout of moderate-intensity exercise lasting 1 to 2 hours compared with a time-matched resting trial [11-17], whereas others found no differences [18-23]. For example, in healthy young men, the same exercise bout (90 minutes at 60%-65% of VO_{2peak}; total energy cost ~1100 kcal) did not alter fasting plasma FFA concentrations the next day in subjects whose average resting FFA concentrations were approximately 0.8 mmol/L [38] but caused an approximately 50% increase in subjects whose average resting FFA concentrations were approximately 0.4 mmol/L [15]. Our data suggest that what might be considered discrepant finding is in fact a normal physiologic response to

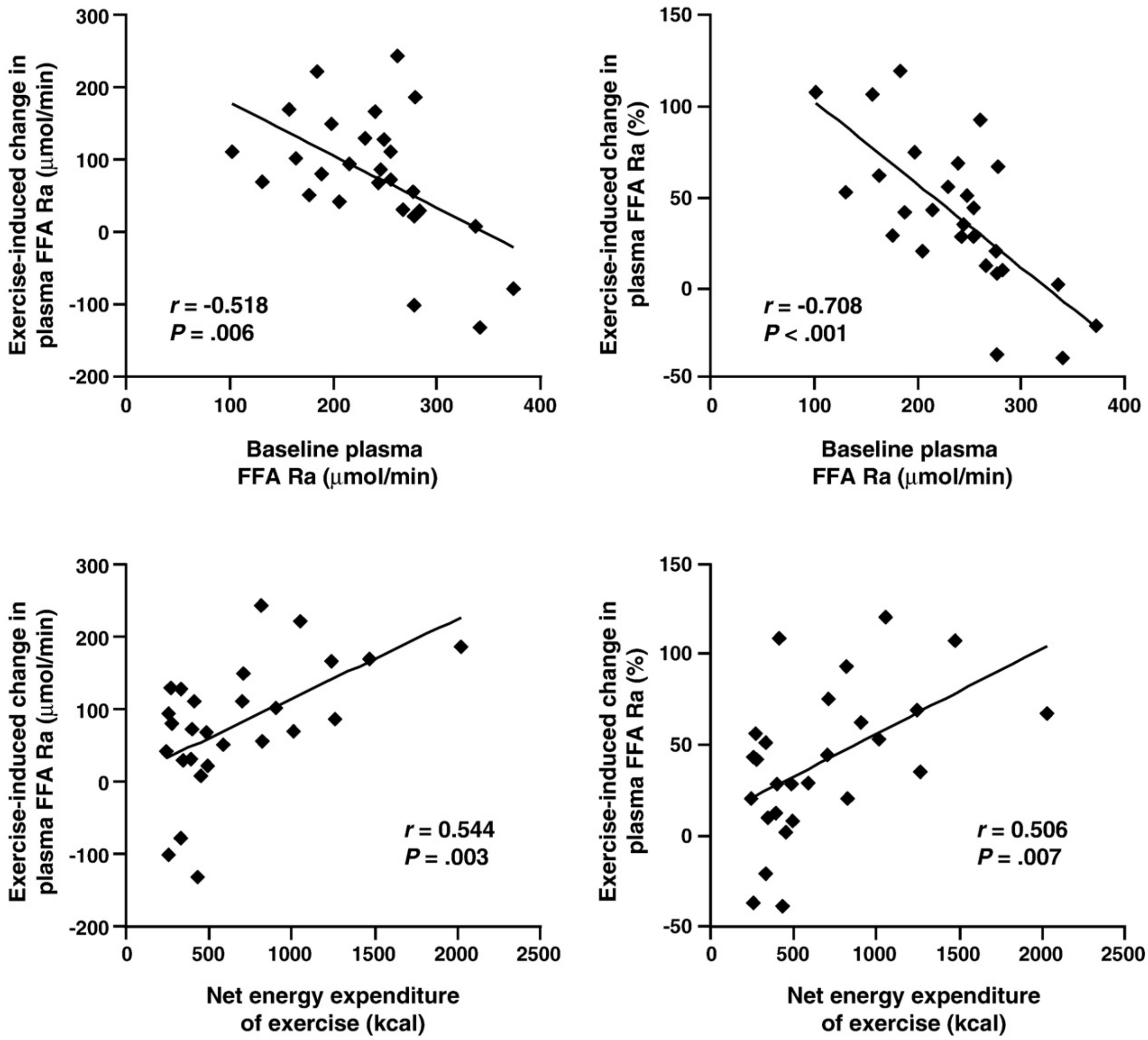

Fig. 3. Relationships between absolute (left) and relative (right) exercise-induced changes in FFA Ra in plasma at 13 to 16 hours, and baseline FFA Ra during the resting trial (top) and net energy expenditure of exercise (bottom).

different resting FFA availability. In addition, our results may help explain differences in the postexercise lipid metabolism response between men and women. For instance, we have recently reported a significant increase in plasma FFA availability (by ~55%) on the morning after a single 1-hour exercise bout in men [29] but not in women [28], who had approximately 50% higher resting FFA availability than men [28,29]. On the other hand, the exercise-induced increase in FFA Ra during the immediate postexercise recovery period (3 hours after exercise cessation) was reported to be comparable in men and women who had similar resting FFA Ra [40]. In the present study, data for men and women fell onto the same regression lines, indicating that previously observed differences in the FFA metabolism response to exercise in men and women are most likely secondary to sex differences in FFA metabolism at rest [28,29,36,41,42] and differences in net energy expenditure of exercise when men and women perform exercise at the same relative intensity [28,29,40,43].

The results from our study along with the data reported in the literature indicate that the increase in plasma FFA availability at rest several hours after exercise in trained compared with untrained subjects [44,45] likely represents an acute effect of exercise only, rather than a cumulative effect in response to repeated exercise sessions (ie, training). For example, in cross-sectional studies, it was found that postabsorptive FFA Ra in plasma is 50% to 100% higher in endurance-trained than in untrained subjects when measurements are made the day after a typical training session [44,45], but not different when exercise is withheld for 48 hours [46,47]. Likewise, longitudinal studies report that several weeks of endurance training in previously sedentary subjects increase postabsorptive FFA Ra and FFA concentration in plasma by 40% to 50% compared with pretraining values when measured within 1 day from the last training session [48], but not 36 to 72 hours later [49-54]. Furthermore, detraining for approximately 1 week leads to a significant reduction in fasting plasma FFA concentration by 40% to 50% in endurance-trained individuals compared with the day after their last training session [55,56]; and this effect is already evident after approximately 60 hours without exercise [55]. The acute lipolytic effect of exercise is likely not due to the negative energy balance induced by exercise. Supplementary food intake to compensate for the exercise-induced energy deficit does not abolish the exercise-induced increase in basal plasma FFA concentration approximately 14 hours postexercise [57]. Furthermore, reducing dietary energy intake through calorie restriction to match the energy expended during exercise does not lead to a significant change in fasting plasma FFA concentration the next morning [13].

In accordance with the results from previous studies in which subjects performed similar exercise as in our study and measurements were made 12 to 24 hours postexercise [15,39,58], we observed that exercise brought about a minor increase in RMR (~4%) and an approximately 20% increase in whole-body fat oxidation rate. Unlike FFA Ra, the postexercise increases in RMR and fat oxidation rate are probably due to the exercise-induced energy deficit. In studies that did not adjust for the exercise-induced energy deficit (ie, negative energy balance after exercise; as is the case in our study), RMR and lipid oxidation rates were increased during the immediate (~3 hours) postexercise period [40,43] and the late phase of the recovery (12-48 hours) from exercise [15,39,58]. However, these responses were absent in studies where the exercise-induced energy deficit was compensated with additional energy intake [59-61]. In addition, although RMR has been suggested to be a major determinant of basal FFA Ra [41], our results clearly demonstrate a dissociation of this relationship in response to exercise and suggest that the increase in FFA mobilization late into the recovery from exercise is not merely the result of increased energy demand. Similar observations, that is, increased plasma FFA availability without simultaneous changes in RMR and lipid oxidation, have been made previously by us [29] and other investigators [12]. Therefore, after exercise, there appears to be no direct link between the availability of plasma FFA and RMR and fat oxidation.

The mechanisms responsible for the increase in plasma FFA availability the morning after a single bout of exercise remain obscure. It is unlikely that the accelerated FFA flux at least 13 hours after exercise observed in the present study is related to changes in fatty acid metabolism occurring during exercise because complete blockade of the normal lipolytic response during exercise by pharmacologic means (acipimox) does not abolish the exercise-induced increase in fasting plasma FFA concentration approximately 15 hours later [16]. Increased plasma catecholamine and decreased plasma insulin concentrations are thought to be the most important hormonal signals mediating the lipolytic surge during exercise [62]. However, catecholamine concentrations fall sharply immediately after exercise cessation, return to preexercise levels within approximately 2 hours of recovery, and do not change thereafter [10,63]. In addition, the greater FFA Ra and FFA concentrations in trained athletes (the day after a typical training session) compared with untrained subjects have been observed without any differences in resting catecholamine and insulin concentrations [45]. Likewise, increased fasting plasma FFA Ra [29] and FFA concentrations [13,15,16] the morning after a single bout of exercise have been shown without any accompanying changes in plasma insulin concentration. This is consistent with the absence of a relationship between exercise-induced changes in plasma insulin and those in plasma FFA Ra in the present study. It has been suggested that the "slower" exercise-induced increase in growth hormone concentration, albeit not involved in the lipolytic response during exercise, is an important factor mediating the postexercise (~4 hours into recovery) increase in adipose tissue lipolysis [6,63]. Enhanced adipose tissue blood flow is unlikely to be responsible for the late exercise-induced increase in plasma FFA availability because, although it is acutely increased during exercise and remains elevated for approximately 4 hours after its cessation [8], there is no evidence that this effect persists into the late phase of postexercise recovery (12-16 hours later) [21]. The mechanisms for the increase in FFA availability late into the recovery from exercise warrant further investigation.

In summary, exercise is a potent stimulus for the mobilization of FFA, not only during but for up to 24 hours after exercise, in a manner that depends directly on the energy expenditure of exercise and inversely on resting plasma FFA availability. The blunted exercise-induced FFA release during the late phase of the recovery in subjects with high fasting plasma FFA concentration probably prevents fatty acid cytotoxicity at a time when energy requirements are not substantially increased.

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