Fatty acid kinetic responses to running above or below lactate threshold

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Kanaley, Jill A., Carl D. Mottram, Paul D. Scanlon, and Michael D. Jensen. Fatty acid kinetic responses to running above or below lactate threshold. *J. Appl. Physiol.* 79(2): 439–447, 1995.—During running exercise above the lactate threshold (LT), it is unknown whether free fatty acid (FFA) mobilization can meet the energy demands for fatty acid oxidation. This study was performed to determine whether FFA availability is reduced during running exercise above compared with below the LT and to assess whether the level of endurance training influences FFA mobilization. Twelve marathon runners and 12 moderately trained runners ran at a workload that was either above or below their individual LT. Fatty acid oxidation (indirect calorimetry) and FFA release ([1-^{14}C]palmitate) were measured at baseline, throughout exercise, and at recovery. The plasma FFA rate of appearance increased during exercise in both groups, running above or below the LT, but the total FFA availability for 30 min of exercise was greater ($P < 0.01$) in the below LT group (marathon, 23 ± 2 mmol; moderate, 21 ± 2 mmol) than in the above LT group (18 ± 3 and 13 ± 3 mmol, respectively). Total fatty acid oxidation (indirect calorimetry) greatly exceeded circulating FFA availability, regardless of training or exercise group ($P < 0.01$). No statistically significant exercise intensity or training differences in fatty acid oxidation were found (above LT: marathon, 71 ± 12, moderate, 64 ± 17 mmol/30 min; below LT: marathon 91 ± 12, moderate, 60 ± 5 mmol/30 min). In conclusion, during exercise above or below LT, circulating FFA cannot meet the oxidative needs and intramuscular triglyceride stores must be utilized. Further marathon training does not enhance effective adipose tissue lipolysis during exercise compared with moderate endurance training.

[1-^{14}C]palmitate; indirect calorimetry; free fatty acids

SHIFTS IN ENERGY SUBSTRATE mobilization and utilization (16, 31) occur as exercise intensity increases, and these shifts are associated with the lactate threshold (LT). Work intensities above the LT result in progressive increases in anaerobic glycolysis, which accompanies aerobic metabolism to sustain adequate levels of ATP regeneration (7, 34). Investigators (23, 35) have examined substrate oxidation using indirect calorimetry at work rates relative to the LT; however, assessing the potential role of substrate mobilization to meet the energy demands of high-intensity exercise has less commonly been attempted. Free fatty acid (FFA) rate of appearance (Ra) increases slightly in moderately trained subjects during light exercise, whereas during heavy bicycle exercise FFA Ra decreases (23, 29). Associated with the reduced FFA Ra during high-intensity exercise (29), a decrease in fatty acid oxidation was observed. If reduced FFA Ra is a limiting factor for fatty acid oxidation, this could result in an even greater dependence on carbohydrate oxidation to meet energy needs (3). Although decreases in fatty acid oxidation are observed at work rates above the LT, it is unclear whether reduced FFA availability is a limiting factor because fatty acid oxidation may exceed FFA availability during high-intensity exercise (29).

Endurance training results in shifts in the LT and is associated with an increased capacity for fatty acid oxidation (13, 14, 32, 36). Animal studies have demonstrated that exercise training increases the lipolytic response of adipose tissue to catecholamines (4). However, during submaximal exercise at the same absolute intensity, plasma FFA concentrations are lower in trained than in untrained individuals (25, 36). Trained individuals oxidize fatty acids more readily without increasing leg extraction of plasma FFA (17), suggesting increased oxidation of intramuscular triglyceride fatty acids compared with that in untrained individuals (14). Whether highly trained individuals are able to mobilize both adipose tissue FFA and/or intramuscular fatty acids to a greater extent than moderately trained individuals above and below the LT is unknown, however.

The purpose of this investigation was to determine the potential contribution of circulating FFA to total fatty acid oxidation during running above and below the LT and to establish whether highly trained endurance runners mobilize and oxidize more FFA than do moderately trained runners. It was hypothesized that, while running above the LT, adipose tissue FFA availability and fatty acid oxidation would be suppressed compared with those below the LT. Also, it was hypothesized that FFA availability would meet the fatty acid oxidative demands above the LT in the highly trained runners but not in the moderately trained runners and that both marathon and moderately trained runners would have an adequate FFA mobilization relative to fatty acid oxidation when exercising below the LT.

METHODS

Subjects

Informed written consent was obtained from 24 male runners, 12 marathon and 12 moderately trained. The subjects ranged in age from 20 to 39 yr. The marathon runners were defined as individuals who had competed in a minimum of two marathons with race times below 3 h and had run a minimum of 72 km/wk for the past 2 yr. The moderately trained runners were defined as individuals who had not competed in marathons within the past 2 yr, ran primarily 10-km races, and ran <32 km/wk. The training characteristics are presented in Table 1.

Materials and Assays

[1-^{14}C]palmitate (Amersham, Arlington Heights, IL) was prepared for intravenous infusions, as previously described

TABLE 1. *Physical and training characteristics*

	Marathon Trained	Moderately Trained
Age, yr	31.5±1.6	33.1±1.9
Height, cm	181.6±1.9	175.3±1.5
Weight, kg	72.5±2.4	81.4±1.9*
Fat mass, kg	9.2±1.4	17.0±1.5*
Fat-free mass, kg	59.9±1.5	60.0±1.2
Percent fat	13±2	22±2*
Body cell mass, kg	36.1±1.0	40.3±1.2
Training characteristics		
Years of training	11.5±2.1	5.7±0.9*
Kilometers per week	91.4±11.5	21.6±3.4*
Marathon time, min	173±5	
Number of marathons	9±3	
10-km Time, min	35.6±1.4	46.6±1.7*

Values are means ± SE; n = 12 runners/group. Fat-free mass and fat mass were measured by dual-energy X-ray absorptiometry. Percent fat was calculated as (fat mass/weight) × 100. * Significantly different from marathon-trained group, $P < 0.01$.

(19). Plasma palmitate and FFA concentrations and specific activities (SAs) were measured with high-performance liquid chromatography using [$^2H_{31}$]palmitate as an internal standard (22). Plasma insulin (12), glucagon (8), cortisol (cortisol MAIA kit, Serono, Braintree, MA), and growth hormone concentrations (27) were determined by radioimmunoassay. Arterial plasma lactate and glucose concentrations were measured with a lactate/glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma bicarbonate was determined by measuring arterial pH, and the partial pressure of arterial carbon dioxide was determined with an Instrument Laboratory 1302 blood gas analyzer (Lexington, KY). Plasma β-hydroxybutyrate and acetoacetate were measured with the methods of Cahill et al. (5). Body composition was determined by total body water (18), bromide space (37), and dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI). Bromide space was used to determine extracellular water, in turn allowing intracellular water and body cell mass to be estimated (6). A dietary history was taken before the overnight stay, and each subject consumed >200 g carbohydrate daily for at least 2 wk before the study.

Exercise Protocols

The subjects came to the exercise laboratory on three separate occasions for *1*) a peak aerobic capacity test from which the LT and ventilatory threshold (VT) were determined; *2*) a practice run to confirm these threshold levels; and *3*) a FFA turnover study, at which time the subjects ran either above or below the LT and VT.

During the peak aerobic capacity test, a radial artery catheter was placed for blood sampling by using local anesthesia. The treadmill exercise protocol was initiated at 110 m/min at 0% grade, and the speed was increased by 30 m/min every 2 min thereafter until a speed of 240 m/min was attained in the marathon runners (215 m/min in the moderately trained runners). At this time, the treadmill grade was increased 1% each minute until the subject reached volitional fatigue. Throughout the exercise test, the subjects' expired air was sampled breath by breath with a Perkin-Elmer mass spectrometer (1). Volume was determined from the digital integration of flow over time (Fleisch no. 3 pneumotachograph and Validyne MP45). Heart rate was monitored throughout the exercise test. Blood samples were drawn in the last 15 s of each minute for the measurement of plasma lactate concentrations. The results of this test were used to establish each individual's peak aerobic capacity, LT, and VT. From these variables, the work rates for the prolonged run were determined. Six subjects in each group were randomly assigned to run at a work rate that was either above or below their individual LT and VT for the FFA turnover study. Those individuals exercising above the LT were assigned a work rate that elicited an oxygen consumption ($\dot{V}O_2$) just above their VT and a lactate level that was greater than the breakpoint. Those individuals running below the LT exercised at a work rate that elicited an $\dot{V}O_2$ 10% below their VT and a lactate levels below their individual breakpoint.

On the second visit, two 10-min practice runs were performed to ensure appropriate selection of the work rates and to familiarize the subjects with the protocol of the study day. In the last 2 min of the 10-min period, $\dot{V}O_2$ was measured and blood was obtained by venipuncture to measure blood lactate concentrations. If necessary, adjustments to the work rates were made, and the same procedure was repeated for the second 10-min run.

The third visit, the FFA turnover study, required the subjects to spend one night in the Mayo Clinic General Clinical Research Center. Subjects arrived at the General Clinical Research Center at 1700 and were fed a standard evening meal. At 1900, an 18-gauge catheter was placed into a superficial midforearm vein and kept patent with an infusion of 0.9% NaCl. Blood and breath samples were obtained to provide a background for SA before the tracer infusion. An oral dose of tritiated water and bromide was administered for the determination of total body water and extracellular water space, respectively. Blood samples were taken every hour for the next 4 h.

The following morning at 0700 the subjects were moved to the exercise lab and remained seated for the next hour. A [1-^{14}C]palmitate (~0.3 μCi/min) infusion was started with a Harvard syringe pump (model 22, Harvard Apparatus, South Natick, MA) at 0730. The tracer infusion rate was determined by counting (in quadruplicate) 50-μl aliquots of each individual's infusate and correcting it to the calibrated infusion rate of 0.100 ml/min. Because of the short circulating half-life of FFA (21), it was not necessary to give a priming dose and, unlike experiments with stable isotopes, it was not necessary to increase the tracer infusion rate during exercise. A radial artery catheter was placed for sampling arterial blood. Baseline blood samples were drawn at 5-min intervals between 0800 and 0815 for the measurement of plasma FFA concentration and SA. Concomitantly, two 2-min standing breath samples were taken. Subjects commenced running at 0825 at their assigned work rates. Those running below the LT ran for 1 h, and those running above the LT ran as long as possible. Water was available to the subjects ad libitum. To monitor work rates and calculate substrate oxidation, indirect calorimetry via breath-to-breath analysis was performed during the last 2 min of each 10-min interval throughout exercise. Blood samples were taken simultaneously with breath samples. Plasma lactate was monitored during exercise to ensure that the appropriate work rate was selected. After exercise, blood and breath samples were taken every 10 min while the subjects sat quietly for 30 min. Heart rate was monitored, and arterial blood gases were measured at the same time as breath and blood samples. Breath samples for $^{14}CO_2$ SA were collected at the same time points as the indirect calorimetry measurements.

Analysis and Calculations

The LT was determined as the first breakpoint in lactate concentrations above baseline values. The lactate response during exercise was plotted and divided into linear compo-

nents. The breakpoint was the intersection of the linear components generated, and the $\dot{V}O_2$ (in ml/min) and the velocity at the breakpoint were established. The VT was selected, as previously described (24). The VT was identified as a nonlinear increase in ventilation ($\dot{V}E$) when plotted against $\dot{V}O_2$ or a simultaneous breakpoint increase in $\dot{V}E/\dot{V}O_2$ and the partial pressure of oxygen in end-tidal expired air. Also sought was a steady partial pressure of carbon dioxide in end-tidal expired air while $\dot{V}E$/carbon dioxide production ($\dot{V}CO_2$) remained constant. The point just before the specific increase in the $\dot{V}E$, $\dot{V}E/\dot{V}O_2$, and the partial pressure of oxygen in end-tidal expired air and where the other criteria were satisfied was considered the VT.

By using the [1-^{14}C]palmitate tracer, the FFA Ra was calculated with non-steady-state equations (21). Baseline FFA flux was averaged over the four resting blood samples. To compare the above and below LT groups, only those data points in the first 30 min of exercise were used in the calculations. For the below LT group, a separate analysis was also done to compare fatty acid kinetics over the 60-min period.

To calculate substrate oxidation, the mean of two 1-min breath samples was used from each sampling period. The total fatty acid and carbohydrate oxidations (in μmol/min) were calculated by using indirect calorimetry and correcting for urine nitrogen excretion (9). The oxidation values were also corrected for changes in the bicarbonate pool during exercise. By using a literature value for the bicarbonate pool, total bicarbonate space was calculated. The change in the bicarbonate space (bicarbonate pool × change in serum bicarbonate) from one sampling point to the next was then calculated and converted to milliliters of carbon dioxide. This difference was then subtracted from the $\dot{V}CO_2$ at each time before calculating oxidation.

The quantities of FFAs that entered the systemic circulation (isotopic determination) and of fatty acids oxidized (indirect calorimetry) during the exercise and recovery time intervals were calculated by using the total area under the curve of FFA Ra and fatty acid oxidation, respectively. The area under the FFA Ra curve over time (exercise and/or recovery) should represent the total FFA available in the systemic circulation for oxidation and will subsequently be referred to as FFA availability. FFA availability was expressed as total micromoles or millimoles because fat-free mass was comparable between groups. Statistical comparisons of FFA availability and fatty acid oxidation between groups (marathon vs. moderately trained) and exercise intensity (above vs. below LT) were performed with a 2 × 2 analysis of variance with repeated measures (Statistical Analysis Software, Cary, NC). To assess the pattern of response between the two groups and two exercise intensities, FFA Ra or plasma substrate and/or hormone concentrations vs. time were analyzed with regression analysis, and the slopes were compared with a 2 × 2 analysis of variance. All values are expressed as means ± SE. Throughout the remainder of the paper, exercise group refers to the exercise intensity either above or below the LT and training group refers to marathon or moderately trained subjects.

RESULTS

Descriptive and Training Characteristics

All subjects completed this study, but technical problems with the infusion pump during one exercise test resulted in the FFA data from one subject being omitted from the analysis. The two groups of subjects (moderate vs. marathon runners) were similar in age and height (Table 1). The marathon runners had a significantly lower body weight and fat mass ($P < 0.01$) but similar fat-free mass. Peak $\dot{V}O_2$ was significantly less in moderately trained runners than in marathon runners ($P < 0.01$). The average work duration for running above the LT was 43.8 ± 3.3 and 42.8 ± 3.8 min for the marathon and moderately trained groups, respectively.

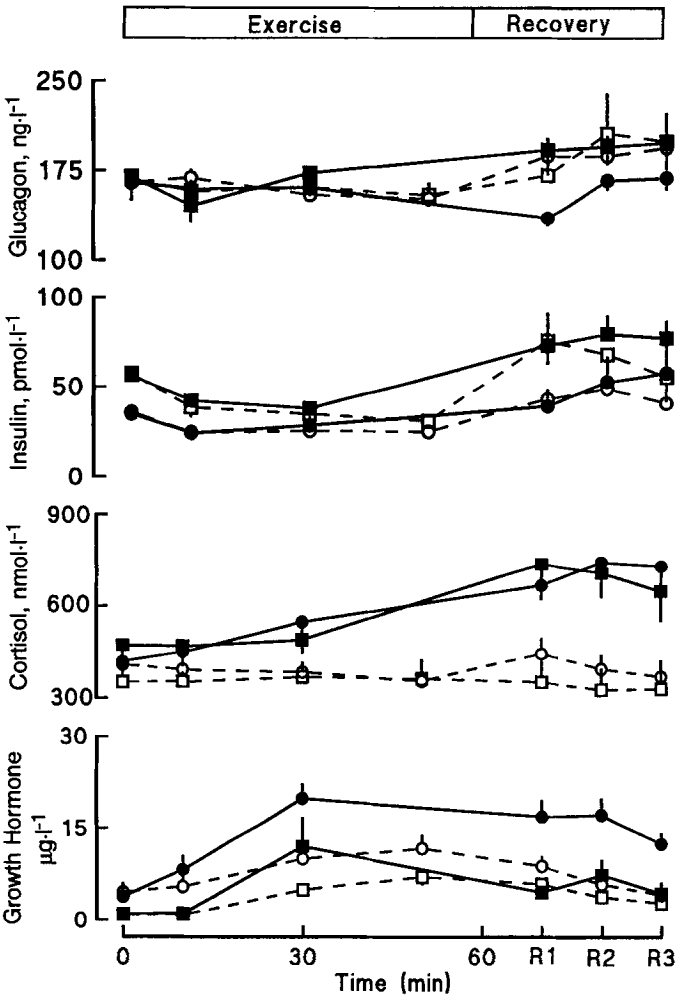

FIG. 1. Plasma glucagon, insulin, cortisol, and growth hormone concentrations at baseline and during exercise and recovery (R1, R2, and R3). ●, Above lactate threshold (LT), marathon runners; ■, above LT, moderately trained runners; ○, below LT, marathon runners; □, below LT, moderately trained runners. Values are means ± SE.

Plasma Hormone, Glucose, and Substrate Concentrations

Baseline plasma insulin concentrations were less in marathon runners than in moderately trained runners (35 ± 3 and 57 ± 3 pmol/l, respectively; $P < 0.05$), but no between-group differences in the insulin response to exercise were observed either above or below the LT (Fig. 1). All groups showed a decreasing trend in insulin concentrations during exercise ($P < 0.01$), with a return to baseline levels during recovery (Fig. 1). Baseline growth hormone concentrations were lower in the moderately trained than in the marathon runners (1.0 ± 0.3 and 4.1 ± 1.5 ng/l, respectively; $P < 0.01$). Plasma growth hormone concentrations increased in all groups by 30 min of exercise ($P < 0.01$; Fig. 1),

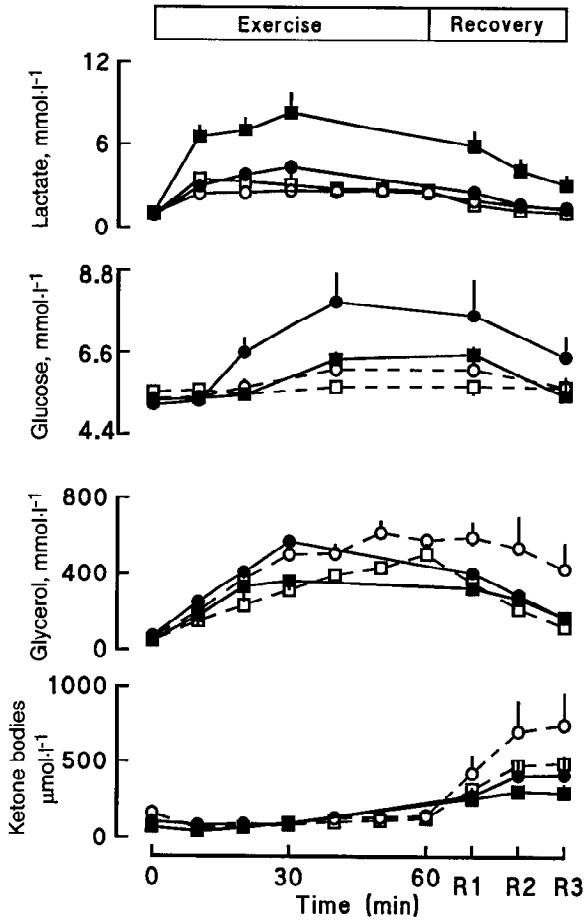

FIG. 2. Plasma lactate, glucose, glycerol, and total ketone body concentrations at baseline and during exercise and recovery. ●, Above LT, marathon runners; ■, above LT, moderately trained runners; ○, below LT, marathon runners; □, below LT, moderately trained runners. Values are means ± SE. Zero time point values are mean of 3 baseline measurements.

and a significant time by exercise group interaction revealed that the increase was greater in the above LT group ($P < 0.05$). Baseline plasma cortisol and glucagon concentrations were not different between the exercise or training groups, and the changes observed during exercise were not statistically significant (Fig. 1). No group differences were observed in the pattern of hormone responses during recovery.

The average plasma lactate concentrations were greater in subjects running above the LT than below the LT ($P < 0.01$; Fig. 2, Table 2) and were also greater in moderately trained runners than marathon runners whether they were running above or below the LT ($P < 0.01$). Because the lactate concentration at the breakpoint for the LT was less in marathon than in moderately trained runners, plasma lactate concentrations in the marathon runners above the LT were not substantially different from those in the moderately trained runners below the LT (Fig. 2). Plasma glucose concentrations were similar between groups at baseline and increased during exercise ($P < 0.05$; Fig. 2) and more so in the marathon runners above the LT ($P < 0.05$). No differences in baseline plasma ketone body concentrations between marathon and moderately trained runners were observed (Fig. 2). Plasma ketone bodies increased slightly during exercise ($P < 0.05$) and dramatically during recovery ($P < 0.01$). Plasma glycerol concentrations increased during exercise ($P < 0.01$; Fig. 2), and the pattern of response was not significantly different between groups.

Fatty Acid Kinetics

Baseline. Baseline FFA flux was not statistically different between the above and below LT groups or between the marathon and moderately trained runners (marathon, 619 ± 82 and 451 ± 76 μmol/min; moderate, 482 ± 67 and 503 ± 32 μmol/min, respectively). Baseline fatty acid oxidation, by indirect calorimetry, was less than the baseline FFA flux in both exercise groups (above LT, 394 ± 129 μmol/min; below LT, 448 ± 108 μmol/min; $P < 0.01$).

Exercise. Plasma FFA SA and concentration between exercise and training groups are shown in Fig. 3. The pattern of change in plasma FFA concentration was similar in each group. Figure 4 depicts the plasma FFA Ra and fatty acid oxidation responses throughout 30 or 60 min of exercise between the groups. Total FFA availability and fatty acid oxidation by exercise and training group are illustrated in Fig. 5. During exercise, FFA Ra increased above baseline in both groups and at both exercise intensities ($P < 0.01$). After 30

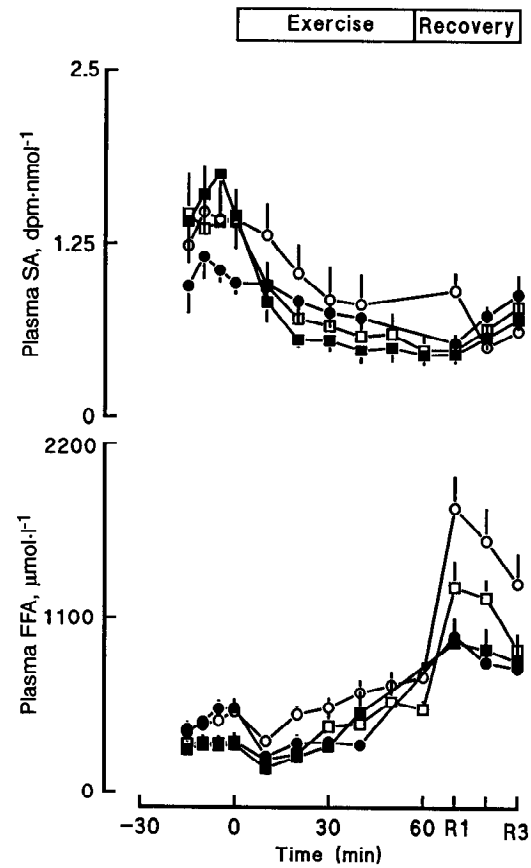

FIG. 3. Plasma specific activity (SA) and free fatty acid (FFA) concentration at rest and during exercise and recovery. ●, Above lactate threshold (LT), marathon runners; ■, above LT, moderately trained runners; ○, below LT, marathon runners; □, below LT, moderately trained runners.

TABLE 2. *Exercise test characteristics*

	Above Lactate Threshold		Below Lactate Threshold	
	Marathon	Moderate	Marathon	Moderate
Maximum test				
Peak $\dot{V}O_2$, ml·kg^{-1}·min^{-1}	65.6±3.5	51.6±3.7*	64.1±3.2	52.0±2.9*
Lactate threshold				
Breakpoint (mmol/l)	3.1±0.3	4.3±0.3*	3.6±0.5	4.4±0.4*
$\dot{V}O_2$, ml/min	3,695±252	3,040±286	3,248±1.2	3,283±239
%Peak $\dot{V}O_2$	77.5±4.7	67.0±3.6	73.3±3.6	71.4±2.3
Ventilatory threshold				
$\dot{V}O_2$, ml/min	3,657±149	3,275±331	3,481±89	3,275±228
%Peak $\dot{V}O_2$	77.6±4.7	68.6±5.3	76.4±1.6	73.6±4.9
FFA turnover study				
$\dot{V}O_2$, ml/min	4,072±250	3,544±265	3,286±82	3,081±123
%Peak $\dot{V}O_2$	85.5±4.1	79.2±1.3	72.3±2.8†	70.3±1.3†
RQ	0.92±0.02	0.92±0.02	0.88±0.02	0.91±0.01
Lactate, mmol/l	4.2±0.4	6.6±0.9*	2.2±0.3†	3.6±0.3*†

Values are means ± SE. $\dot{V}O_2$, O_2 consumption; FFA, free fatty acid; RQ, respiratory quotient. * Significantly different from marathon runners, $P < 0.01$. † Significantly different from above lactate threshold values, $P < 0.001$.

min of running above the LT, FFA Ra had increased to 879 ± 175 and 732 ± 145 μmol/min in the marathon and moderately trained groups, respectively, whereas at the end of 60 min of running below the LT, FFA Ra increased to 1,553 ± 144 and 1,548 ± 326 μmol/min, respectively. During 30 min of exercise above the LT, FFA availability (expressed as the area under the curve) was 17.6 ± 3.1 and 12.8 ± 2.3 mmol in the marathon and moderately trained runners, respectively, whereas FFA availability was significantly greater in the below LT group (23.1 ± 1.8 mmol and 20.7 ± 2.3 mmol, respectively; $P < 0.01$). Exercise training (marathon vs. moderately trained) did not result in significant differences in total FFA availability during exercise either above or below LT.

No statistically significant exercise intensity or training differences in fatty acid oxidation over 30 min of exercise were found (above LT: marathon, 71 ± 12 mmol, moderate, 64 ± 17 mmol; below LT: marathon, 91 ± 12 mmol, moderate, 60 ± 5 mmol). Fatty acid oxidation was somewhat greater in marathon runners than in moderately trained runners during 60 min of exercise [marathon, 196 ± 29 mmol; moderate, 148 ± 16 mmol; not significant (NS)]. Total fatty acid oxidation was substantially greater than FFA availability over 30 min of exercise, regardless of training or exercise group ($P < 0.01$; Fig. 5). During the 60 min of exercise below the LT, FFA availability and fatty acid oxidation were not different between the marathon or moderately trained groups (Fig. 5, *inset*) and fatty acid oxidation was substantially greater than FFA availability.

Total energy expenditure over 30 min was greater in the groups running above than below the LT (2,293 ± 130 and 1,899 ± 54 kJ, respectively; $P < 0.01$), whereas total fatty acid oxidation during 30 min of exercise was not significantly different above and below the LT (698 ± 25 and 787 ± 88 kJ, respectively).

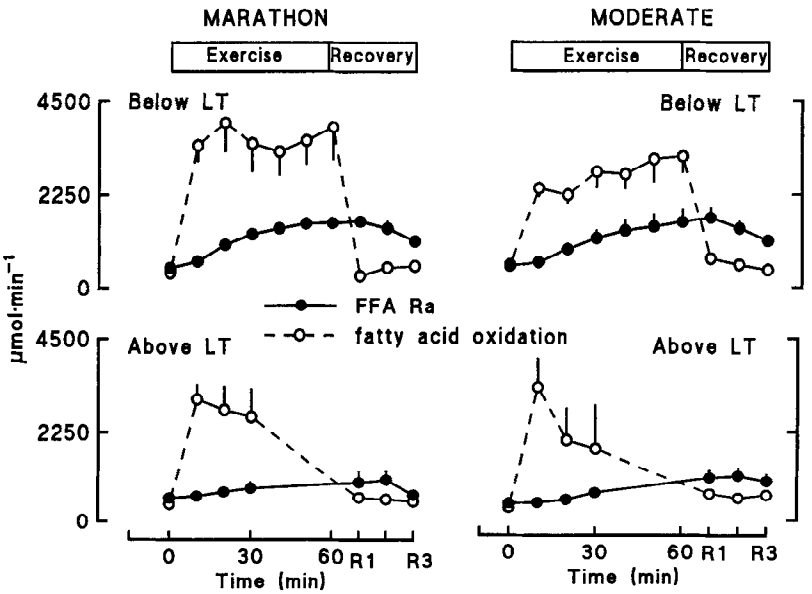

FIG. 4. Pattern of response for FFA rate of appearance (FFA Ra) and fatty acid oxidation in moderately and marathon-trained subjects both above and below LT. Values are means ± SE. Zero time point values are mean of 4 baseline measurements.

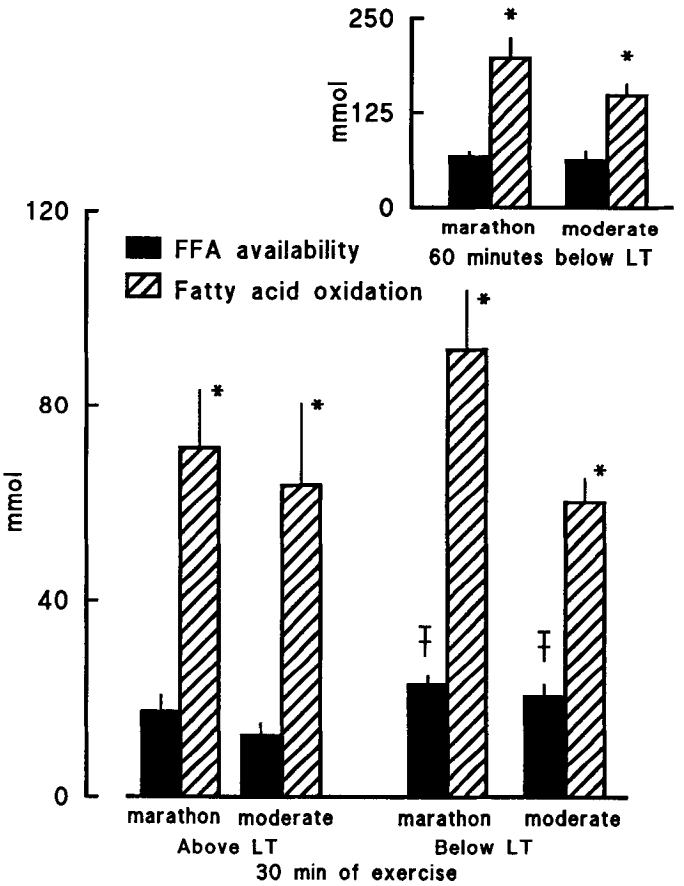

FIG. 5. FFA availability (area under curve of FFA Ra) and total fatty acid oxidation (indirect calorimetry) during 1st 30 min of exercise in moderately and marathon-trained subjects running above and below LT. *Inset*, FFA availability and fatty acid oxidation during 60 min of exercise below LT. Values are means ± SE. * Significantly greater fatty acid oxidation compared with FFA availability, $P < 0.01$. † Significantly greater FFA availability below LT compared with that above LT, $P < 0.01$.

Recovery. During recovery, $\dot{V}O_2$ returned to baseline values within the first 10 min in all groups. FFA Ra exceeded fatty acid oxidation in all groups ($P < 0.01$; Fig. 4). For the 30-min recovery period, the mean recovery FFA fluxes were 1,065 ± 120 μmol/min and 1,359 ± 116 μmol/min for the above and below LT groups, respectively. The mean fatty acid oxidation rates were 614 ± 58 μmol/min and 512 ± 47 μmol/min, respectively.

Carbohydrate Oxidation

As measured by indirect calorimetry, baseline carbohydrate oxidation was 999 ± 289 and 679 ± 195 μmol/min in the above and below LT groups, respectively (NS). The quantity of carbohydrate oxidized during 30 min of exercise was not significantly different between training groups (marathon, 415 ± 46 mmol; moderate, 360 ± 18 mmol), but when the data were collapsed across the marathon and moderate runner training groups, a greater carbohydrate oxidation was observed in the above LT group (450 ± 45 mmol) compared with that in the below LT group (325 ± 23 mmol; $P < 0.005$). Carbohydrate was the primary substrate oxidized during exercise and exceeded fat oxidation in all groups but was only significant ($P < 0.01$) in the above LT groups (Fig. 6). Above the LT, carbohydrate and fat oxidations contributed ~60 and 30% of the total energy expended, respectively, whereas below the LT the contributions were ~ 51 and 41%, respectively. The respiratory exchange ratios for the two groups and two different exercise intensities are depicted in Fig. 7.

Breath $^{14}CO_2$ Excretion

The recovery of $^{14}CO_2$ in breath relative to the [1-^{14}C]palmitate infusion rate is depicted in Fig. 8. Because of the brief interval between beginning the tracer infusion and the onset of exercise, the baseline fractional recovery of the tracer in the breath was low (~2%). With the onset of treadmill running, an immediate increase in $^{14}CO_2$ excretion occurred ($P < 0.001$), with the fractional recovery exceeding 60% in both groups at both exercise intensities. There were no statistically significant differences between groups. During recovery, the appearance of $^{14}CO_2$ in the breath decreased dramatically and was not significantly different between groups.

DISCUSSION

During exercise, the energy supply must meet the energy demands or exercise will cease. This study examined whether FFA availability differs relative to fatty acid oxidation during running above and below the LT and VT and whether this relationship is different in those who train intensively for marathons and those who choose to train less intensively. Previous studies (23, 29) have suggested that fat oxidation is greater during exercise below the LT than above the LT. We found that total fatty acid oxidation was slightly but not significantly less in those running

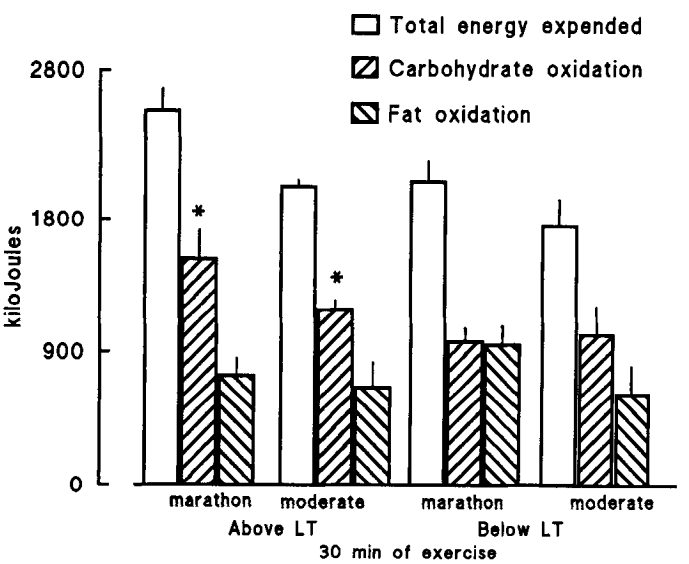

FIG. 6. Total energy expended and total body carbohydrate and fat oxidation as estimated by indirect calorimetry in moderately and marathon-trained subjects both above and below LT during 1st 30 min of exercise only. Values are means ± SE. * Significantly greater carbohydrate oxidation compared with fat oxidation, $P < 0.01$.

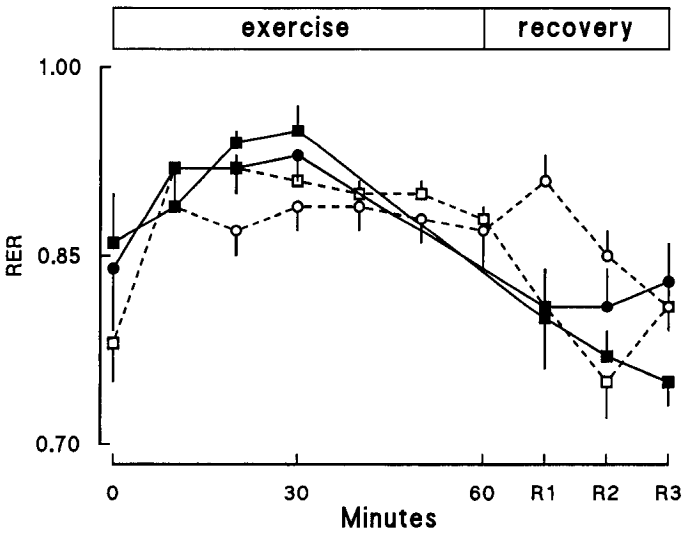

FIG. 7. Respiratory exchange ratio (RER) at rest (*time 0*) and during exercise and recovery in the 2 different exercise groups and exercise intensities. ●, Above LT, marathon runners; ■, above LT, moderately trained runners; ○, below LT, marathon runners; □, below LT, moderately trained runners. Values are means ± SE.

above the LT, whereas plasma FFA availability was significantly less during exercise above the LT. The fractional contribution of fatty acid oxidation to total energy expenditure was less during exercise above the LT. During exercise above and below the LT, fatty acid oxidation exceeded FFAs available from the circulation, suggesting that intramuscular triglyceride stores provided fatty acids for oxidation. Finally, marathon training was associated with a greater proportion of fat oxidation below the LT but not with greater FFA availability.

Previous studies have documented decreased fatty acid oxidation (29) and decreased (23) or unchanged FFA Ra (29) during bicycle exercise at work rates above the LT. In contrast, treadmill running above the LT resulted in an increase in FFA Ra above resting values, although it is not to the same degree as running below the LT. It is conceivable that slight differences are present between the metabolic response to running and bicycling that could influence effective adipose tissue lipolysis. The onset of the LT is reportedly different during bicycle and treadmill exercise (2), implying that metabolic differences may exist between these modes of exercise. It should be noted that a decrease in FFA flux was observed by Hall et al. (11) during high-intensity running. Unfortunately, FFA turnover was calculated by using antecubital venous blood FFA SA in that study. This sampling site has subsequently been shown to create ~20–25% errors in estimating arterial FFA SA (20).

Changes in the substrate milieu (3, 10, 15, 16) have been implicated as having a potential role of modulating FFA availability during exercise. Boyd et al. (3) reported that increased blood lactate concentrations due to intravenous infusion of DL-lactate in humans suppressed plasma FFA concentrations. In the present study, we observed an increase in FFA Ra above resting values during exercise above the LT, although the increase was blunted compared with exercise below the LT. There are conflicting data regarding whether lactate per se inhibits lipolysis in vivo. When hyperlactatemia (pure L-lactate) was studied under "clamped" hormonal conditions and with appropriate controls (i.e., for pH), no effect on FFA Ra was noted (30). In studies of FFA turnover in dogs, however, lactic acid was found to reduce plasma FFA (10, 15, 16). It is not possible to determine from our results whether hyperlactatemia in the above LT runners directly suppressed FFA flux relative to that seen in the below LT runners; little effect on fatty acid oxidation is apparent, however.

It is unlikely that the difference observed in FFA availability running above vs. below the LT can be accounted for by differences in plasma hormone concentrations. Growth hormone showed the expected exercise-induced increase, and plasma insulin decreased in all subjects; the exercise-induced fall in plasma insulin concentrations is essential for increases in arterial FFA concentrations (35). Unfortunately, plasma epinephrine and norepinephrine concentrations were not measured in this study. It is possible that the very high catecholamine concentrations associated with intense exercise (29) stimulate α_2-adrenergic receptors and thereby inhibit FFA release (33). The absence of plasma catecholamine concentrations in the present study limits the ability to assess whether between-group differences in FFA responses might be adrenergically mediated.

We noted that the excretion of $^{14}CO_2$ in expired air was >60% of the [^{14}C]palmitate infusion rate throughout exercise in all subjects (Fig. 8), implying that the majority of plasma FFAs are rapidly oxidized during running. Even if FFA oxidation were complete, however, circulating FFA Ra did not match fatty acid oxidation rates during exercise above or below the LT. Although FFA availability was lower while running above the LT compared with running below the LT, fatty acid

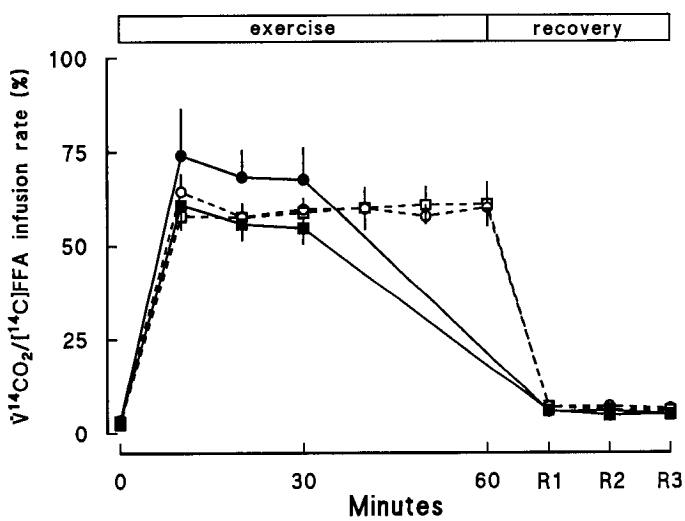

FIG. 8. Recovery of $^{14}CO_2$ in breath [$^{14}CO_2$ production ($\dot{V}^{14}CO_2$)] as a percentage of [^{14}C]FFA (palmitate) infusion rate in the 2 different exercise groups and exercise intensities. ●, Above LT, marathon runners; ■, above LT, moderately trained runners; ○, below LT, marathon runners; □, below LT, moderately trained runners. Values are means ± SE.

oxidation was not significantly lower with the high work intensity. Romijn et al. (29) also observed that with higher intensity bicycle exercise (65 and 85% maximal $\dot{V}O_2$) FFA Ra could not meet fatty acid oxidative needs. Thus, moderate- and high-intensity work (running or bicycling) necessitates the oxidation of intramuscular triglycerides, and plasma FFAs are of lesser importance as a lipid fuel source. The somewhat greater plasma glycerol concentrations we observed in subjects running above the LT (Fig. 2) may represent muscle release of glycerol as intramuscular triglycerides are hydrolyzed.

Although all subjects in the present study were endurance trained to some degree, relative fatty acid oxidation rates were somewhat greater in marathon-trained individuals running below the LT. Even though it is possible that constitutional physiological characteristics of the marathon runners, such as fiber type, could account for the differences in lipid oxidation between groups, endurance training has been shown to increase the energy able to be derived from nonplasma sources (intramuscular triglycerides) (26). We considered the inclusion of an untrained control group in this study. To obtain meaningful information, however, it was felt critical to study 1 h of running below the LT and at least 30 min of running above the LT. Finding truly untrained subjects capable of these exercise bouts seemed sufficiently unlikely that we elected to study only moderately and highly trained runners.

The measurement of fuel oxidation by indirect calorimetry during heavy exercise has been challenged (23). During heavy exercise, accelerated lactic acid production by exercising muscles increases plasma lactate concentrations and requires increased utilization of the bicarbonate buffering system to maintain a neutral pH. This results in the excretion of carbon dioxide (proportional to the decrease in body bicarbonate) over and above that generated from substrate oxidation. This excess $\dot{V}CO_2$ could result in an overestimation of the respiratory quotient. The changes we observed in the bicarbonate pool were ~50 ml/min, or ~1% of $\dot{V}CO_2$ during exercise above the LT. Although we corrected the respiratory quotient for the calculated shifts in bicarbonate, the influence of nonoxidative carbon dioxide generation on total $\dot{V}CO_2$ during heavy relatively steady-state exercise is minimal. Thus our calculations are consistent with the finding that indirect calorimetry is a satisfactory measure of net substrate oxidation during high-intensity submaximal exercise (28).

In summary, running above the LT results in reduced FFA availability compared with exercise below the LT in both moderately and well-trained endurance runners. Despite this difference, fatty acid oxidation was not different between these work intensities. As expected, the greater energy demands associated with running above the LT were met by increased carbohydrate oxidation. At both work rates, an increase in FFA Ra occurred that contrasts with what was observed during high-intensity bicycle exercise. The level of training did not influence this response nor did it influence total fatty acid oxidation (indirect calorimetry) during exercise above the LT. In conclusion, during treadmill exercise above or below the LT, adipose tissue FFA do not meet the oxidative needs and intramuscular triglyceride stores are utilized. Marathon training does not appear to provide adipose tissue lipolytic benefits over moderate-endurance training; however, this does not preclude the existence of a training effect compared with sedentary individuals.

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