Effect of high-altitude acclimation on NEFA turnover and lipid utilization during exercise in rats

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McClelland, Grant B., Peter W. Hochachka, and Jean-Michel Weber. Effect of high-altitude acclimation on NEFA turnover and lipid utilization during exercise in rats. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E1095–E1102, 1999.—Relative exercise intensity (or %maximum O_2 consumption, $\dot{V}O_{2max}$) controls fuel selection at sea level (SL) and after high-altitude acclimation (HA) in rats. In this context we used indirect calorimetry, [1-^{14}C]palmitate infusions, and muscle triacylglycerol (TAG) measurements to determine *1*) total lipid oxidation, *2*) the relationship between circulatory nonesterified fatty acid (NEFA) flux and concentration, and *3*) muscle TAG depletion after exercise in HA-acclimated rats. Aerobic capacity is decreased in trained rats after 10 wk of acclimation. Both SL and HA showed the same relative use of lipids at 60% $\dot{V}O_{2max}$ [62 ± 5% (HA) and 61 ± 3% (SL) of O_2 consumption ($\dot{V}O_2$)] and 80% [46 ± 6% (HA) and 47 ± 5% (SL) of $\dot{V}O_2$] of their respective $\dot{V}O_{2max}$. At 60% $\dot{V}O_{2max}$, plasma [NEFA] were higher in HA, but rate of appearance was essentially the same in both groups (at 30 min, 38 ± 9 vs. 49 ± 6 μmol·kg^{-1}·min^{-1} in HA and SL, respectively). At this intensity SL showed no significant decrease in muscle TAG, but in HA it decreased by 64% in soleus and by 90% in red gastrocnemius. We conclude that *1*) the relative contributions of total lipid are the same in SL and HA, contrary to differences in [NEFA], because the relationship between flux rate and [NEFA] is modified after acclimation, and *2*) muscle TAG may play a more important role at HA.

rats; maximum exercise intensity; lipid metabolism; nonesterified fatty acids; carbohydrates; oxidative fuel; muscle triacylglycerol

LIPIDS represent an abundant source of oxidative fuel for muscle contraction, and body stores of triacylglycerol can sustain low-intensity exercise for several days (17). At high altitude (HA), an increased use of lipids has been thought to be advantageous during exercise to spare valuable and limited stores of muscle glycogen (48). Increases in plasma nonesterified fatty acid concentrations [NEFA] have often been interpreted as an increase in lipid oxidation (20, 48) because of the correlation between concentration and flux during low-intensity exercise at sea level (15, 31). Extrapolations from concentration measurements can be misleading (46), and it is not known whether the relationship between [NEFA] and flux is the same at sea level and HA. For instance, some studies on training in humans have shown a decrease in [NEFA] but an increase in fat oxidation during exercise (22, 25). Increases in [NEFA] can arise from a mismatch between rate of appearance from adipose tissue (R_a) and rate of disappearance into muscle (R_d). Such a mismatch can in part be caused by a decrease in the triacylglycerol-fatty acid (TAG-FA) cycle (47) and is not necessarily linked to increased oxidation. There is also no direct information on the effect of HA acclimation on muscle TAG utilization. Carbohydrates (CHO) provide a higher yield of ATP per mole of oxygen and for this reason have been viewed as a preferred fuel source during exercise after HA acclimation (18). However, we have recently shown that the contributions of total CHO, circulatory glucose, and muscle glycogen to overall metabolism are not altered by acclimation when control and acclimated animals are compared at the same relative exercise intensity (%maximum O_2 consumption, $\dot{V}O_{2max}$) (28). Thus the O_2-saving advantage of CHO seems to be outweighed by limited availability of this critical fuel source. Consequently, the contribution of total lipid oxidation to total metabolic rate is also the same before and after acclimation. In this context, the effect of acclimation on the use of lipids and its different sources has not been studied systematically, and the potential effects of chronic exposure to hypoxia warranted closer examination.

%$\dot{V}O_{2max}$ is the major determinant of CHO and lipid utilization after HA acclimation in rats (28) as well as at sea level in other quadrupeds (36) and humans (7). In previous HA acclimation studies, comparisons have been made at the same absolute exercise intensity (i.e., same work rate; e.g., see Ref. 6). The decrease in aerobic capacity or $\dot{V}O_{2max}$ associated with HA acclimation (8, 28) makes absolute work rates a higher %$\dot{V}O_{2max}$ in HA individuals. This has important implications, because maximal rates of lipid oxidation are reached at low exercise intensities (30–60% $\dot{V}O_{2max}$, depending on species and gender), whereas the relative importance of CHO increases with exercise intensity, ultimately providing 100% of the energy at $\dot{V}O_{2max}$ (36). Therefore, the increases in CHO or decreases in lipid oxidation observed in previous acclimation studies (6, 34) may have been due to experimental design and not necessarily acclimation. Here, we used indirect calorimetry and continuous tracer infusions to quantify lipid oxidation and NEFA turnover rate in HA-acclimated and sea level control (SL) rats exercising at the same %$\dot{V}O_{2max}$. Changes in muscle TAG after exercise were also measured to estimate the contribution of intramuscular lipids. The goals of this study were to determine *1*) whether the relationship between NEFA flux and NEFA concentration is the same at SL and HA and *2*) whether exercise causes the same depletion of muscle TAG reserves at SL and HA. In addition, we were able to estimate the relative contributions of circulatory and

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intramuscular lipids during exercise in both groups of animals.

METHODS

Animals. The methods used for acclimation, training, and surgery in these experiments have been described elsewhere (28). This study has been approved by the animal ethics committees of the Universities of British Columbia and Ottawa. Thirty-two female Wistar rats were randomly assigned to one of two groups, one kept at SL conditions [fractional concentration of inspired O_2 ($F_{I_{O_2}}$) = 0.2094] and the other under hypobaric hypoxia simulating HA conditions ($F_{I_{O_2}}$ = 0.12, equivalent to 4,300 m). Starting body mass (M_b) at 5–6 wk of age was 231 ± 2 g (HA) and 225 ± 2 g (SL). Each group was given free access to food (Rodent Lab Diet, PMI Nutrition, St. Louis, MO) and water. Both groups were kept under a 12:12-h light-dark photoperiod and were housed in groups of 2 or 4 per cage at 25°C. M_b at the time of experiments was not significantly different (P = 0.21) between SL at 302 ± 6 and HA at 289 ± 14 g (28).

HA rats were acclimated to HA conditions in specially designed hypobaric chambers. The barometric pressure was decreased progressively (4) over the first 10 days of acclimation to a final value of 450 mmHg by use of an oil-less vacuum pump, as described elsewhere (28). Rats were kept at this pressure for ≥10 wk before measurements were begun. The chambers were opened for 1–2 h most days for cage cleaning and exercise training. HA and SL rats were trained four times per week under normoxia on an eight-lane motorized treadmill with air-jet stimulus (Omnitech Electronics, Columbus, OH), starting at 10 m/min, 6° incline, and 45 min/day. Both speed and incline were increased progressively to a final training regimen of 20 m/min, 10° incline, and 60 min/day. The total duration of training was 10 wk, and all exercise protocols were performed at a 10° incline.

Respirometry. Measurements of mass-specific O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were made with a flow-through respirometry system (Sable Systems TR-3, Henderson, NV). Exercise measurements were made in a Plexiglas-enclosed motorized treadmill equipped with electric stimulus (Columbus Instruments, Columbus, OH). $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER) measurements were found to be accurate to ±1% (n = 3) by burning methanol. $\dot{V}O_{2max}$ was measured for each animal (SL rats under normoxic conditions, HA rats under hypoxic conditions and after acclimation was complete). The three criteria used to determine when the animals had reached $\dot{V}O_{2max}$ were *1*) no change in $\dot{V}O_2$ as speed was increased, *2*) the animals could no longer keep their position on the treadmill, and *3*) the RER ($\dot{V}CO_2$/$\dot{V}O_2$) reached a value greater than 1 (38). $\dot{V}O_{2max}$ in acclimated (HA) rats was found to be 24% lower than in SL rats (67.6 ± 1.3 vs. 89.3 ± 1.2 ml·kg^{-1}·min^{-1}) (28).

Exercise protocols. For all protocols, HA were run at $F_{I_{O_2}}$ = 0.12 and SL were run at $F_{I_{O_2}}$ = 0.2094 after an overnight fast. Running speeds corresponding to 60 and 80% $\dot{V}O_{2max}$ were calculated for each individual. Values of $\dot{V}O_2$ and $\dot{V}CO_2$ were continuously measured for 50 or 60 min at 60% $\dot{V}O_{2max}$ and for 25 or 30 min at 80% $\dot{V}O_{2max}$. Measured intensities were 53.9 ± 0.4 and 57.1 ± 0.5% $\dot{V}O_{2max}$ and 78.7 ± 1.2 and 75.7 ± 0.6% $\dot{V}O_{2max}$ for SL and HA, respectively.

NEFA kinetics. NEFA kinetics were only quantified at the lower exercise intensity of 60% $\dot{V}O_{2max}$ because of the more invasive nature of these measurements. After completion of the respirometry measurements, the animals were transferred to the University of Ottawa and allowed to recover for a minimum of 5 days. Upon arrival, HA rats were immediately placed again in hypobaric chambers. Jugular vein and carotid artery catheters were implanted under halothane anesthesia, and the animals were allowed to recover for 3–6 days, as previously described (28). Training was suspended for both HA and SL during this period.

On the day of the experiment, extensions were added to both catheters, and the venous line was used for the continuous infusion of [1-^{14}C]palmitate (50–60 mCi/mmol, Amersham, Oakville, ON, Canada). Palmitate has been shown to be an appropriate choice of tracer for measuring NEFA kinetics during exercise in mammals (27). The isotope was dissolved in either an albumin solution (1 mM) or in 700 μl of plasma from donor rats. Infusions took place using a calibrated syringe pump (Harvard Apparatus, South Natick, MA) at a rate of 0.6 ml/h for 60 min at rest, and 1.0 ml/h for 60 min during exercise at 60% $\dot{V}O_{2max}$ and for 5 min during recovery. Resting infusion rates were 1,049,215 ± 40,579 for SL and 1,224,962 ± 29,030 dpm·kg^{-1}·min^{-1} for HA rats. To minimize fluctuations in specific activity (24), infusion rates were increased 1.7 times during exercise in both groups. The arterial catheter was used to take blood samples (100 μl for 2 of the resting samples and 300 μl for all others) at 45, 50, and 60 min after the start of the resting infusion; at 15, 30, 45, and 60 min during exercise; and at 2 and 5 min postexercise (recovery). All samples were centrifuged immediately, and the plasma was frozen at −20°C until further analysis.

Tissue sampling. Two additional groups of rats were trained for 10 wk as described for the two experimental groups. One-half of the animals in each group underwent HA acclimation for ≥10 wk. Animals were euthanized (sodium pentabarbitol at 20 mg/100 g) after an overnight fast, and resting samples were taken from the soleus, plantaris, and red and white portions of gastrocnemeus muscles from the left hindlimb and were quickly frozen using precooled aluminum clamps. Postexercise samples were obtained immediately after 60 min of exercise at 60% $\dot{V}O_{2max}$. Tissues were stored at −80°C until analysis.

Sample analysis. Plasma NEFA concentrations were measured on one resting sample and all other samples using a Hewlett-Packard 5890 series II gas chromatograph (GLC) equipped with a 30-m fused silica capillary column (Supelco SP-2330) kept at 185°C for 34 min, raised to 210°C at 5°C/min, and kept at 210°C for 11 min. The system was also equipped with an automatic injection system (HP 7673). NEFA were extracted and methylated from 75 μl of plasma by a modification of the method of Tserng et al. (43), previously used in other mammals (26, 27). The GLC was calibrated with known standards (PUFA-2, Matreya), and heptadecanoic acid (17:0) was added as an internal standard. [1-^{14}C]palmitate activity was measured with a Tri-Carb 2500 counter on 30 μl of plasma after drying under N_2, resuspension in 1 ml H_2O, and addition of ACS-II scintillant (Amersham).

Tissue TAG concentrations were determined as previously described (10). Tissue samples (100–300 mg) were powdered in a liquid N_2-cooled mortar and transferred into preweighed glass tubes. The samples were homogenized in a chloroform-methanol (Folch 1:1, vol/vol) solution (9) at 30 ml/g tissue. The homogenizer was rinsed with 1 ml of Folch 1:1, which was added to the homogenate. Lipids were extracted by shaking at room temperature for 20 min. The homogenate was centrifuged at 3,000 rpm for 10 min, the pellet was washed with another 1 ml of Folch 1:1 and centrifuged, and the supernatants were pooled. Chloroform was then added at 0.5 times the final volume to bring the Folch solution to a ratio of 2:1. A 0.2% KCl solution was added at 0.25 times the final volume, and after centrifugation the aqueous phase was discarded. Two to three milliliters of ethanol (99%) were added to the remaining organic phase before drying under N_2

Table 1. *$\dot{M}O_2$, $\dot{M}CO_2$, RER, CHO oxidation, and % total $\dot{M}O_2$ in control SL and HA rats exercising at 60 and 80% $\dot{V}O_{2max}$*

	60% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$
	5–50 min	5–25 min
$\dot{M}O_2$, µmol·kg^{-1}·min^{-1}		
SL	2,243 ± 54–2,129 ± 58	2,889 ± 87–3,275 ± 154
HA	1,810 ± 42–1,629 ± 48	2,086 ± 36–2,178 ± 39
$\dot{M}CO_2$, µmol·kg^{-1}·min^{-1}		
SL	1,840 ± 55–1,629 ± 48	2,672 ± 129–2,896 ± 178
HA	1,478 ± 39–1,285 ± 48	1,796 ± 26–1,887 ± 53
RER		
SL	0.82 ± 0.01–0.77 ± 0.01	0.92 ± 0.02–0.88 ± 0.01
HA	0.82 ± 0.01–0.75 ± 0.01	0.86 ± 0.02–0.87 ± 0.01
CHO oxidation, µmol O_2·kg^{-1}·min^{-1}		
SL	874 ± 74–431 ± 54	2,150 ± 249–1,986 ± 239
HA	682 ± 86–273 ± 36	1,100 ± 99–1,190 ± 140
% Total $\dot{M}O_2$		
SL	39 ± 3–20 ± 2	73 ± 7–59 ± 4
HA	37 ± 5–16 ± 2	53 ± 5–54 ± 5

Values are means ± SE from McClelland et al. (28). $\dot{M}O_2$, O_2 consumption; $\dot{M}CO_2$, CO_2 production; RER, respiratory exchange ratio; CHO, carbohydrate; % Total $\dot{M}CO_2$, contribution to total metabolic rate; SL, sea level rats; HA, high altitude-acclimated rats; $\dot{V}O_{2max}$, maximum O_2 consumption.

at 40°C. The lipids were redissolved in 250 µl or 500 µl of isopropanol, and TAG concentration was measured spectrophotometrically (Perkin-Elmer Lambda 2 UV/VIS) at 540 nm with a diagnostic kit (Sigma, St. Louis, MO). A subsample (15–50 mg) of each powdered tissue was transferred to a preweighed glass tissue grinder and homogenized in 19 volumes of freezable buffer (16). Protein concentrations were determined by plate reader (Molecular Devices, Thermomax) microassay at 600 nm with a kit (Bio-Rad). This was used to express TAG in the more reliable units of micromoles per gram of protein (12).

Calculations and statistics. Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using the equations of Withers (45) and converted micromolar units ($\dot{M}O_2$ and $\dot{M}CO_2$). Total lipid oxidation measured by indirect calorimetry was calculated with the equations of Frayn (11), with the assumption that the contribution of proteins to overall energy expenditure was negligible during exercise in the postabsorptive state (33). R_a palmitate rates were calculated using steady-state calculations of Steele (40) previously validated for fatty acid kinetics (30). R_a NEFA was calculated by using the percent contribution of palmitate to total NEFA concentration determined by GLC. Hypothetical relative circulatory NEFA oxidation rates were calculated on the basis of 23 moles of oxygen per mole of palmitate (29) and were converted to NEFA oxidation using the percent contribution of palmitate and then expressing the result as a percentage of total $\dot{M}O_2$. Results were analyzed using a *t*-test and a one- or two-way analysis of variance (ANOVA). When tests for normality failed, a Mann-Whitney Rank-Sum Test or a Kruskal-Wallis ANOVA was used. Pairwise multiple comparisons were made by the Student-Newman-Keuls test or by Dunnett's method. All percentages were arcsine square root transformed. Values are means ± SE.

RESULTS

Respirometry and CHO oxidation. $\dot{M}O_2$, $\dot{M}CO_2$, RER, and CHO oxidation are summarized in Table 1. Briefly, $\dot{M}O_2$ and $\dot{M}CO_2$ were greater in SL than in HA at both 60 and 80% $\dot{V}O_{2max}$. HA acclimation did not have a significant effect on RER, an indicator of fuel preference, which increased from 60 to 80% $\dot{V}O_{2max}$. CHO oxidation was higher in SL than in HA, but when corrected to total metabolic rate ($\dot{M}O_2$), there was no difference in the contribution of CHO to overall metabolism. As indicated by RER values, CHO oxidation increased as exercise intensity increased from 60 to 80% $\dot{V}O_{2max}$ (Table 1).

Total lipid oxidation. During exercise at 60% $\dot{V}O_{2max}$, lipid oxidation rates were lower in HA than in SL ($P < 0.0001$, ANOVA). Oxidation rates ranged from 1,366 ± 58 to 1,695 ± 66 µmol O_2·kg^{-1}·min^{-1} in SL and 1,126 ± 95 to 1,432 ± 49 µmol O_2·kg^{-1}·min^{-1} in HA (Fig. 1*A*). However, the percent contribution of lipid oxidation to total $\dot{M}O_2$ was not significantly different between the two groups ($P = 0.21$; Fig. 1*B*). Over 50 min of exercise, it ranged from 61 ± 3 to 80 ± 2% in SL and 62 ± 5 and 84 ± 2% in HA. At 80% $\dot{V}O_{2max}$, lipid oxidation rates were not different in the two groups ($P > 0.05$; Fig 2*A*). Lipid oxidation rates did not change significantly over time in HA, ranging from 984 ± 1,123 to 985 ± 76 µmol O_2·kg^{-1}·min^{-1} over the course of the exercise bout. SL values, on the other hand, increased over the exercise period from 737 ± 184 to 1,466 ± 102 µmol O_2·kg^{-1}·min^{-1} (Fig. 2*A*). At the beginning of exercise (10 min mark), lipid oxidation accounted for 47 ± 6% of total $\dot{M}O_2$ in HA but only 27 ± 7% in SL ($P = 0.03$). At 20 min there was no significant difference between SL and

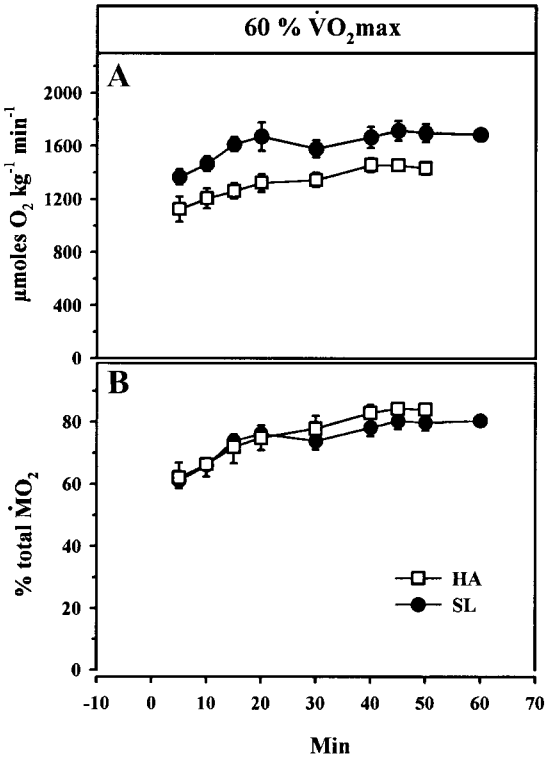

Fig. 1. Total lipid oxidation as µmol O_2·kg^{-1}·min^{-1} (*A*) and expressed relative to total O_2 consumption (in converted micromolar units) as $\dot{M}O_2$ (*B*) in rats running at 60% maximal O_2 consumption ($\dot{V}O_{2max}$). High altitude-acclimated (HA, □, running speed = 9.8 ± 0.6 m/min) and sea level (SL) control (●, running speed = 16.3 ± 0.3 m/min) rats are shown.

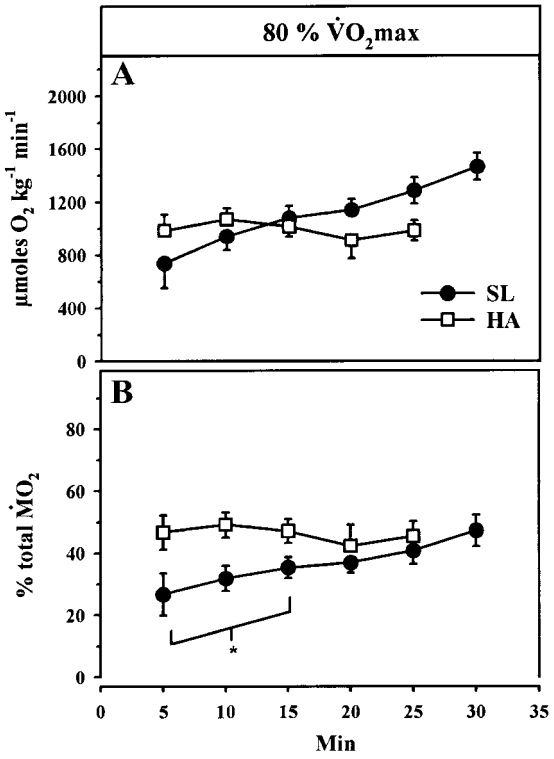

Fig. 2. Total lipid oxidation in µmol $O_2 \cdot kg^{-1} \cdot min^{-1}$ (*A*) and expressed relative to total $\dot{M}O_2$ (*B*) in rats running at 80% $\dot{V}O_{2max}$. HA (running speed = 18.0 ± 0.5 m/min, □) and SL (running speed = 28.0 ± 0.7 m/min, ●) rats are shown. *Significantly different from HA.

HA ($P = 0.57$). There was little change in lipid contribution in HA and at the end of exercise at 46 ± 6%, whereas it rose in SL to 47 ± 5% (Fig. 2*B*). When compared at the same absolute average speed of 17.2 m/min, the HA rats had a lower rate of lipid oxidation ($P < 0.0001$) and percent contribution to total $\dot{M}O_2$ (47 ± 6 to 46 ± 6%) than in SL (61 ± 3 to 80 ± 2%; Fig. 3, *A* and *B*).

Palmitate and NEFA turnover. Concentration determined by GLC, specific activity of [1-^{14}C]palmitate, and R_a of palmitate all appear in Fig. 4, *A–C*. Resting palmitate concentrations were higher in HA (356 µmol/l) than in SL (239 µmol/l) ($P < 0.0001$) and remained constant during exercise. Both groups showed a large and significant increase in palmitate concentration after exercise had stopped ($P < 0.05$). R_a, however, dropped from an average resting value of 16.2 to 11.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during exercise in HA but remained constant in SL (15.4 to 14.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; Fig. 4, *A* and *C*). Turnover of palmitate was lower in HA during exercise but not significantly different from SL. Similarly, NEFA concentrations were higher in HA than in SL (1,193 ± 85 to 1,207 ± 51 µmol/l in HA and 994 ± 108 to 795 ± 64 µmol/l in SL from rest to 60 min of exercise; $P < 0.05$). The NEFA R_a was lower in HA (but nonsignificant; $P = 0.11$) compared with SL at 30 min (38 ± 9 vs. 49 ± 6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), 45 min (32 ± 7 vs. 46 ± 9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and 60 min (29 ± 6 vs. 45 ± 12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; Fig. 5, *A* and *B*).

NEFA concentrations and composition. At rest, the plasma concentration of all individual FA contributed to the higher total [NEFA] in HA rats, and the percent contributions of stearate (18:0) and oleate (18:1) were different between the groups (Table 2; Fig. 5). Throughout exercise and recovery, the percent contribution of palmitate (16:0) to total NEFA does not deviate from ~30% despite changes in absolute concentrations (Table 2 and Fig. 5). However, 18:1 and 18:0 showed changes in both concentration and percent contribution to total NEFA between rest, exercise, and recovery as well as between HA and SL (Table 2).

Muscle TAG. TAG concentrations were determined in soleus, plantaris, and red and white portions of the gastrocnemius at rest and after 60 min at 60% $\dot{V}O_{2max}$ (Fig. 6). There was no significant difference in resting levels of TAG between the two groups. SL rats showed a decrease in TG of 42% in soleus and 64% in white gastrocnemius after exercise, but neither value was significantly different from resting level. HA did show a significant decrease ($P < 0.05$) in soleus (100 ± 19 to 42 ± 11 µmol/g protein) and red gastrocnemius (14.02 ± 0.83 to 1.4 ± 0.02 µmol/g protein) in TAG after 60 min at 60% $\dot{V}O_{2max}$ (Fig. 6).

DISCUSSION

This study shows that, in an animal model, relative exercise intensity is the major determinant of lipid utilization after HA acclimation. Contrary to previous studies on men (6, 34, 35), we found in rats that there was no change in the relative contribution of lipids after prolonged exposure to hypoxia. Trained female

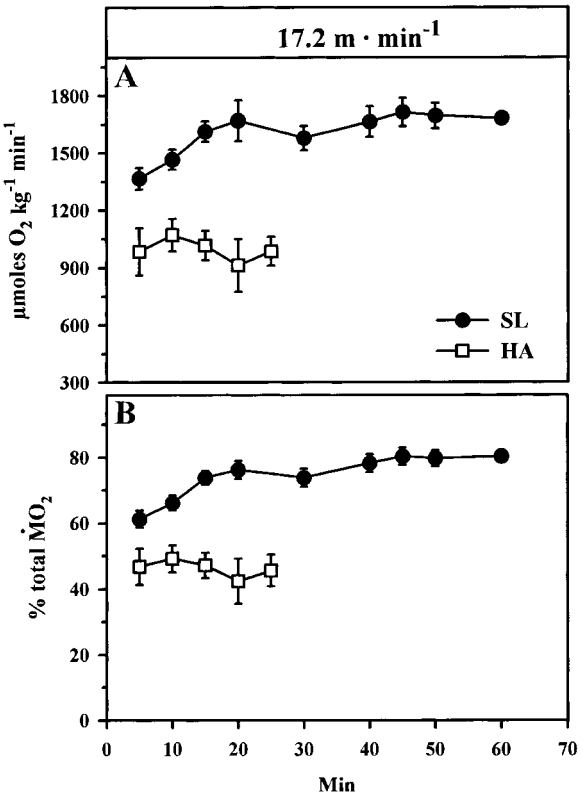

Fig. 3. Total lipid oxidation in µmol $O_2 \cdot kg^{-1} \cdot min^{-1}$ (*A*) and expressed relative to total $\dot{M}O_2$ (*B*) in rats running at the same average absolute speed of 17.2 m/min (SL at 60% $\dot{V}O_{2max}$, HA at 80% $\dot{V}O_{2max}$). Symbols are as in Fig. 1.

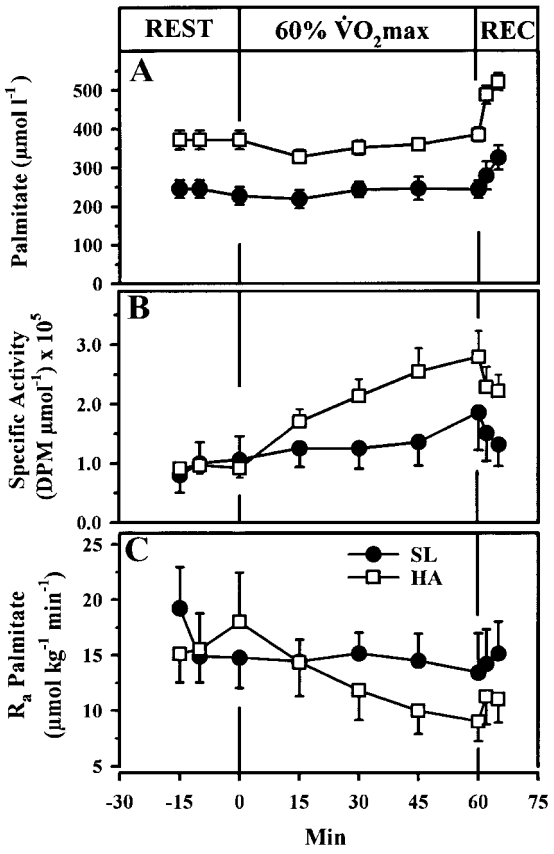

Fig. 4. Plasma concentration (μmol/l, *A*), specific activity (dpm/μmol, *B*), and rate of appearance (R_a) of palmitate (μmol·kg^{-1}·min^{-1}, *C*) before (REST), during, and after (REC, recovery) 60 min of exercise at 60% $\dot{V}O_{2max}$. Values are means ± SE; n = 8.

HA and SL rats exercising at 60 and 80% of their $\dot{V}O_{2max}$ derive the same fraction of their total energy from lipid oxidation (Figs. 1*B* and 2*B*). Higher [NEFA] seen after HA acclimation suggested an increase in lipid utilization, but flux rates were not different from those of SL (Fig. 5). Therefore, the correlation between concentration and flux seen at SL was not observed after HA acclimation. The contributions of circulatory NEFA and intramuscular TAG may be different before and after HA acclimation. The greater depletion of muscle TAG seen in HA soleus and red gastrocnemius (Fig. 6) suggests that this fuel source may be more important in this group. Along with our earlier findings on CHO oxidation (28), we have demonstrated that %$\dot{V}O_{2max}$, not acclimation, is the main determinant of whole animal oxidative fuel selection at HA. Therefore, current general models of fuel selection at SL, drawn from animals and humans (7, 36), apply to these HA acclimation experiments. Previous studies on men made comparisons at the same absolute speed that represented a higher percentage of $\dot{V}O_{2max}$ for the HA group. This resulted in HA groups using lower proportions of lipid, most likely as a consequence of experimental design, as shown here when HA and SL were compared at the same speed of 17.2 m/min (80 and 60% $\dot{V}O_{2max}$ in HA and SL, respectively; Fig. 3, *A* and *B*). Recent findings on humans suggest that this pattern does not hold true in trained vs. untrained men (3) and in men vs. women (13, 19) at the same relative exercise intensity. Therefore, in fuel selection studies, training status and gender appear to be as important as exercise intensity to considerations of experimental design.

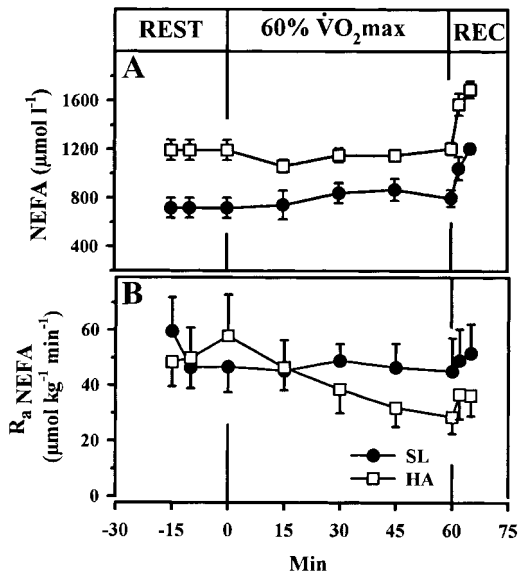

Fig. 5. Plasma concentration (μmol/l, *A*) and R_a of total nonesterified fatty acids (NEFA, μmol·kg^{-1}·min^{-1}, *B*) before (REST), during, and after (REC) 60 min of exercise at 60% $\dot{V}O_{2max}$. Values are means ± SE; n = 8.

Total lipid oxidation. Increasing lipid oxidation can have at least two obvious advantages: *1*) improving endurance to take advantage of large adipose tissue stores and/or *2*) sparing valuable muscle glycogen: the only fuel available for high-intensity exercise. Here, we found that neither of these potential benefits was directly exploited, because total lipid oxidation contributed equally to overall metabolism in HA and SL (Figs. 1 and 2). However, the fact that lipid oxidation did not decrease (as expected, if the higher ATP yield per O_2 of CHO were exploited) could be interpreted as a glycogen-sparing mechanism. The relative contribution of lipids was higher in HA than in SL at the beginning of exercise at 80% $\dot{V}O_{2max}$, so there may be, under these circumstances, active sparing of glycogen (Fig. 2*B*). Why not rely even more on lipids than observed here and be able to power locomotion for several days on adipose tissue reserves to spare glycogen even further? Part of the answer may lie in the inability to sustain high power outputs through lipid oxidation (32), which reaches maximal rates at low relative work rates. Lipid oxidation usually does not increase above 60–65% $\dot{V}O_{2max}$ (dogs, goats, and men), and the contribution of this fuel to total energy expenditure declines as exercise intensity increases (36, 37, 44). HA and SL rats showed no increase in lipid oxidation from 60 to 80% $\dot{V}O_{2max}$ (Figs. 1 and 2), but this maximum rate may have been reached at a lower intensity. Recent studies on women show that they can maintain higher lipid oxidation rates than men at the same relative exercise intensity (13, 19, 41). This may explain lower RER values seen for female rats (Table 1) compared with literature values for human males. The upper limit for lipid oxidation may be related to energetic constraints

Table 2. *Plasma NEFA composition from SL and HA rats before, during, and after exercise at 60% $\dot{V}O_{2max}$*

	16:0		16:1		18:0		18:1		18:2		Total NEFA	
	SL	HA	SL	HA	SL	HA	SL	HA	SL	HA	SL	HA
Rest	262 ± 75	372 ± 24	13 ± 3	23 ± 2	239 ± 26	288 ± 19	139 ± 21	264 ± 28	157 ± 18	249 ± 31	807 ± 69	1,193 ± 85
	(33 ± 1)	(31 ± 1)	(1.9 ± 0.2)	(2.0 ± 0.2)	(30 ± 2)	(25 ± 2)	(17 ± 2)	(22 ± 1)	(19 ± 1)	(21 ± 1)		
15 min	224 ± 21	328 ± 17	21 ± 5	38 ± 5	211 ± 20	262 ± 14	107 ± 19	235 ± 11	134 ± 20	198 ± 10	694 ± 76	1,060 ± 52
	(32 ± 1)	(31 ± 1)	(3.0 ± 0.2)	(3.5 ± 0.3)	(31 ± 3)	(25 ± 1)	(15 ± 2)	(22 ± 0)	(19 ± 1)	(19 ± 1)		
30	251 ± 21	352 ± 18	29 ± 6	40 ± 5	230 ± 21	273 ± 17	132 ± 25	268 ± 11	149 ± 21	219 ± 13	789 ± 63	1,151 ± 57
	(32 ± 1)	(31 ± 0)	(3.5 ± 0.5)	(3.4 ± 0.3)	(30 ± 3)	(24 ± 1)	(16 ± 2)	(23 ± 1)	(19 ± 1)	(19 ± 1)		
45	247 ± 23	360 ± 16	29 ± 6	39 ± 4	214 ± 20	258 ± 15	156 ± 26	272 ± 13	156 ± 22	221 ± 12	797 ± 89	1,150 ± 47
	(31 ± 1)	(31 ± 0)	(3.3 ± 0.4)	(3.3 ± 0.3)	(28 ± 2)	(22 ± 1)	(19 ± 1)	(24 ± 1)	(19 ± 1)	(19 ± 1)		
60	244 ± 16	385 ± 18	24 ± 6	39 ± 5	205 ± 22	260 ± 13	160 ± 22	285 ± 11	157 ± 14	238 ± 13	795 ± 55	1,207 ± 51
	(31 ± 1)	(32 ± 0)	(3.5 ± 0.4)	(3.1 ± 0.3)	(26 ± 2)	(22 ± 1)	(20 ± 2)	(24 ± 1)	(20 ± 1)	(20 ± 1)		
2 Rec	279 ± 28	488 ± 23	28 ± 6	52 ± 6	206 ± 20	268 ± 11	213 ± 44	408 ± 31	208 ± 34	254 ± 33	941 ± 111	1,570 ± 89
	(30 ± 1)	(31 ± 1)	(3.6 ± 0.4)	(3.3 ± 0.3)	(24 ± 3)	(17 ± 1)	(22 ± 3)	(26 ± 1)	(21 ± 1)	(22 ± 1)		
5 Rec	326 ± 24	522 ± 21	31 ± 6	54 ± 6	234 ± 21	287 ± 11	266 ± 49	449 ± 19	236 ± 24	382 ± 23	1,101 ± 95	1,693 ± 69
	(30 ± 0)	(31 ± 0)	(3.3 ± 0.2)	(3.1 ± 0.3)	(22 ± 2)	(17 ± 1)	(24 ± 1)	(27 ± 0)	(21 ± 1)	(22 ± 1)		

Values are mean concentration ± SE in μmol/l, and values in parentheses are percent total NEFA ± SE. NEFA, nonesterified fatty acid; Rest, before exercise; Rec, recovery or after exercise.

set by the lower yield of ATP per mole of O_2, or perhaps the fibers recruited at these intensities (type I and type IIa) are specialized for fat use and uptake (5), whereas the fibers recruited for more rapid work are geared for a higher glycolytic capacity (type IIb). The characteristics of the muscles recruited at these intensities [predominantly soleus, plantaris, and red gastrocnemius in SL rats (23)] appear to change little after HA acclimation. Postacclimation in rats, there are equivalent amounts of 3-hydroxy acyl-CoA dehydrogenase, a β-oxidation enzyme, in soleus and plantaris, and the conservation of fiber typology of the plantaris compared with SL (1). In any case, the reliance on lipids is neither increased nor decreased after HA acclimation, even though a decline in relative lipid use (compensated by an increase in relative CHO use) would allow animals to maintain the same rate of aerobic ATP production with a lower O_2 consumption.

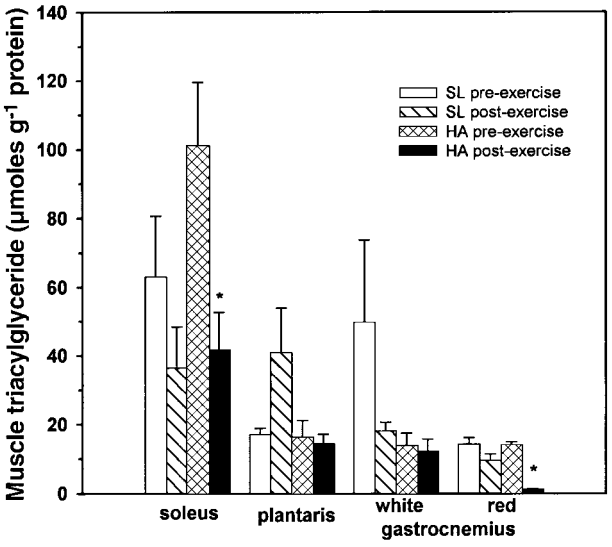

Fig. 6. Triacylglycerol concentrations (in μmol/g total muscle protein) before (preexercise) and after (postexercise) 60 min of exercise at 60% $\dot{V}O_{2max}$ in trained HA and SL rats. Values are means ± SE; n = 6 for preexercise and n = 5 and 6 for HA and SL postexercise, respectively. *Significantly different from HA preexercise.

NEFA turnover. Plasma [NEFA] was substantially higher in HA than in SL at rest, during exercise at 60% $\dot{V}O_{2max}$, and in postexercise recovery (Fig. 5*A*). Except for a sharp rise at the beginning of recovery, these values were not affected by exercise. All of the individual NEFA measured contributed to the higher NEFA in HA, but not equally, as shown by differences in percent contribution of 18:0 and 18:1 to total NEFA between the two groups. Palmitate appears to be an appropriate tracer for total NEFA in HA acclimation turnover experiments, as it deviates little from 30% of total NEFA at rest, during exercise, or in recovery (Table 2). Nonruminant mammals with the same diet should show similar plasma NEFA composition (2), but this is clearly not the case in rats after acclimation. Differences seen in stearate (18:0) and oleate (18:1) point to potential changes in stearyl desaturase activity in liver with acclimation. This enzyme has not been studied after HA acclimation. This is an area in need of further research, as it may indicate differential incorporation of NEFA into adipose tissue and muscle TAG. Fasting before each experiment also played a part in the high [NEFA] seen in both groups. Suprisingly, R_a NEFA was the same or even tended to be lower in HA than in SL during exercise and recovery (Fig. 5*B*), and these changes in flux were, therefore, independent of plasma concentration. This blunted turnover could not be the result of hyperglycemia or lactate, because plasma glucose and lactate concentrations, measured simultaneously, were the same in the two groups (28). Consistently higher [NEFA] despite similar resting and lower or similar exercise/recovery fluxes contradicts the accepted dogma that concentration and flux are positively correlated during exercise (31). Moreover, other recent experiments have shown that endurance training causes a decrease in plasma [NEFA], together with an increase in lipid utilization (25). Therefore, extrapolation from [NEFA] to turnover rate should clearly be avoided. Interestingly, this study is the first to directly quantify the effects of HA acclimation on NEFA fluxes. Other investigations investigating the impact of acclimation on fat metabolism (34, 48) have

based their conclusions on observed changes in plasma concentrations. We would probably have interpreted our results as an increase in NEFA utilization after HA acclimation, had we relied exclusively on concentration measurements.

Changes in plasma [NEFA] during exercise result from a mismatch between the rate of appearance from adipose tissue (R_a) into the circulation and uptake by working muscles (R_d). High plasma [NEFA] have previously been found at rest and during exercise under acute and chronic hypoxic conditions in humans (20, 34, 48). It has been suggested that this is the result of an increased lipolysis brought about by increased norepinephrine and glucagon (35). Our results demonstrate that high [NEFA] cannot be accounted for by an increased release from adipose tissue. High and constant [NEFA] in HA most likely indicate a chronic mismatch between R_a and R_d at rest, which is maintained in the transition to exercise. The rate of clearance (NEFA turnover/[NEFA]) is also greatly reduced in HA and probably contributes to the higher [NEFA] in these animals. It may be the result of a decrease in the (normally) large percentage of NEFA that are reesterified back into TAG at rest (47). The TAG-FA cycle, as well as other substrate cycles, has not been studied after HA acclimation.

Muscle TAG utilization. SL rats showed no significant decrease in TAG with exercise (Fig. 6). This is in contrast to studies on rats that use tetanic stimulation (39) and treadmill exercise (14) but is consistent with a recent finding on humans, in which there was no decrease in muscle TAG until exercise had finished (21). HA rats showed a decrease in TAG with exercise in soleus and red gastrocnemius. This, along with lower circulatory NEFA turnover, suggests that muscle TAG utilization is increased at HA. Based on blood flow measurements (23), soleus and red gastrocnemius muscles are recruited at this exercise intensity at SL. It is not known whether this is true for HA and suggests that comparisons of muscle recruitment patterns before and after HA acclimation warrant closer examination.

To provide a first estimate of the relative contributions of circulatory and intramuscular lipid sources, we need an estimate of circulatory NEFA oxidation rates. If we assume that 100% of the NEFA entering the circulation (R_a) are oxidized by working muscles, they would account for the same proportion of total energy expenditure in HA and SL. By the end of exercise, circulatory NEFA could account for maximally 46 ± 9 and 46 ± 13% of $\dot{M}O_2$ in HA and SL, respectively ($P = 0.17$). Therefore, turnover of NEFA in HA, at this low intensity, is decreased in proportion to the decrease in total metabolic rate ($\dot{M}O_2$). Muscle TAG would then minimally contribute ~15% of total $\dot{M}O_2$ at this intensity. This is our safest estimate, because we did not directly measure the percentage of R_a oxidized; however, at this intensity, it is likely less than 50% (44). The above calculation should be viewed cautiously because of possible contributions to whole body measurements from nonactive tissues (13). It is not known what effect nonactive tissues have, or even if there is a difference between bipedal and quadrupedal locomotion. We do know that both forms of locomotion require the same amount of energy at any given speed (42). Also, on the basis of our measurements, muscle TAG may play a more important role in HA rats. This may indicate a lower percent extraction and oxidation of NEFA by muscles in HA-acclimated rats, also suggested in human acclimation studies (34).

Conclusions and implications. This is the first study to show that total lipid utilization during exercise is unchanged after altitude acclimation. Using the rat as an animal model and eliminating the influence of exercise intensity, this study and our previous work on CHO reveal a more comprehensive picture of high-altitude fuel selection. So far, we have shown that a compromise must be struck in hypoxic environments between the O_2-saving advantage of increased CHO use and the glycogen-sparing advantage of increased lipid use. One implication is that glycogen apparently is a crucial substrate, and its use during exercise is minimized at altitude by not increasing CHO metabolism beyond SL values (28). Interestingly, muscle TAG may be a more important fuel under these conditions, as shown by a greater depletion of TAG and a blunted plasma NEFA turnover rate during exercise after acclimation. Further work will be needed to assess whether this observed pattern of fuel selection can be generalized to all mammals.

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