Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men

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Bergman, Bryan C., and George A. Brooks. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J. Appl. Physiol.* 86(2): 479–487, 1999.—We evaluated the hypotheses that endurance training increases relative lipid oxidation over a wide range of relative exercise intensities in fed and fasted states and that carbohydrate nutrition causes carbohydrate-derived fuels to predominate as energy sources during exercise. Pulmonary respiratory gas-exchange ratios [(RER) = CO_2 production/O_2 consumption ($\dot{V}O_2$)] were determined during four relative, graded exercise intensities in both fed and fasted states. Seven untrained (UT) men and seven category 2 and 3 US Cycling Federation cyclists (T) exercised in the morning in random order, with target power outputs of 20 and 40% peak $\dot{V}O_2$ ($\dot{V}O_{2\,peak}$) for 2 h, 60% $\dot{V}O_{2\,peak}$ for 1.5 h, and 80% $\dot{V}O_{2\,peak}$ for a minimum of 30 min after either a 12-h overnight fast or 3 h after a standardized breakfast. Actual metabolic responses were 22 ± 0.33, 40 ± 0.31, 59 ± 0.32, and 75 ± 0.39% $\dot{V}O_{2\,peak}$. T subjects showed significantly ($P < 0.05$) decreased RER compared with UT subjects at absolute workloads when fed and fasted. Fasting significantly decreased RER values compared with the fed state at 22, 40, and 59% $\dot{V}O_{2\,peak}$ in T and at 40 and 59% $\dot{V}O_{2\,peak}$ in UT subjects. Training decreased ($P < 0.05$) mean RER values compared with UT subjects at 22% $\dot{V}O_{2\,peak}$ when they fasted, and at 40% $\dot{V}O_{2\,peak}$ when fed or fasted, but not at higher relative exercise intensities in either nutritional state. Our results support the hypothesis that endurance training enhances lipid oxidation in men after a 12-h overnight fast at low relative exercise intensities (22 and 40% $\dot{V}O_{2\,peak}$). However, a training effect on RER was not apparent at high relative exercise intensities (59 and 75% $\dot{V}O_{2\,peak}$). Because most athletes train and compete at exercise intensities >40% maximal $\dot{V}O_2$, they will not oxidize a greater proportion of lipids compared with untrained subjects, regardless of nutritional state.

crossover concept; lipid oxidation; carbohydrate oxidation; indirect calorimetry

IT IS WELL DESCRIBED by using longitudinal experimental designs that after endurance training the pulmonary respiratory gas-exchange ratio (RER) is lower, compared with before training, in subjects exercising at an absolute submaximal power output (5, 7, 13, 15, 20, 25). A lower RER [RER = CO_2 production/O_2 consumption ($\dot{V}O_2$)] in exercising trained subjects indicates a relative increase in lipid oxidation. Increased tissue respiratory control, increased working muscle free fatty acid uptake, augmentation of beta oxidation, and down-regulation of glycogenolysis and glycolysis have been attributed to a training-induced elaboration of the mitochondrial reticulum (4, 12, 22). However, RER in trained vs. untrained subjects exercising at similar relative exercise intensities has received much less attention. Moreover, considering potential major effects of nutritional status on metabolism, we could not find a report in which effects of endurance training and nutritional status on the balance of carbohydrate (CHO) and lipid oxidation during exercise at several relative power outputs were systematically evaluated.

To evaluate the hypotheses that, in men, training decreases RER values over a wide range of exercise intensities under both fed and fasted conditions and that food intake 3–4 h before exercise increases CHO oxidation during exercise, we compared pulmonary gas-exchange ratios in trained and untrained men in fed and fasted states while they were exercising at intensities ranging from 20 to 75% peak $\dot{V}O_2$ ($\dot{V}O_{2\,peak}$).

METHODS

Subjects. Seven trained and seven untrained healthy male subjects between the ages of 19 and 32 yr were used in this study. Subjects were informed verbally by interview of the nature and purpose of the experiment, and they signed an informed consent before participation in this study, which was approved by the University of California, Berkeley, Committee for Protection of Human Subjects (no. 94–6–35). Untrained subjects performed <2 h of regular physical activity per week. Trained subjects were recruited from both the University of California Cycling Team and the Berkeley Bicycle Club and were licensed category 2 or 3 racers in the United States Cycling Federation (USCF) (Table 1). Thus cyclists could be classified as moderately to well trained but were not national or international caliber athletes.

Maximal (peak) exercise tests. To determine $\dot{V}O_{2\,peak}$ during leg cycling, subjects were tested by using a progressive maximal exercise test on two different occasions to accustom them to the testing protocol and apparatus. Untrained subjects exercised on a Monark cycle ergometer and started pedaling at an external power output of 50 W. Subjects' pedaling cadence was kept at 70 revolutions/min (rpm) with the use of a metronome. Every 2 min, the power output was increased by 50 W until the subjects could not continue despite verbal encouragement. Blood was taken from fingertips at the end of each workload stage to determine lactate thresholds. Lactate threshold was determined by the intersection of regression equation lines for both linear and exponentially increasing lactate concentration during progressive submaximal workloads (11). Trained subjects were tested on a stationary trainer with a fan resistance unit and used their own bicycle for the $\dot{V}O_{2\,peak}$ tests and exercise trials described below. Cyclists started the test with a gear ratio that would closely approximate a 50-W initial workload used for untrained subjects. Pedaling cadence was not controlled in these subjects, since trained subjects typically pedal at very high rpm, which vary from rider to rider. By allowing the subjects

Table 1. *Subject characteristics*

	Age, yr	Weight, kg	Body Fat, %	Years Racing	$\dot{V}_{O_{2\,peak}}$, l/min	$\dot{V}_{O_{2\,peak}}$, ml·kg^{-1}·min^{-1}	Lactate Threshold, %$\dot{V}_{O_{2\,peak}}$
Untrained	22.1 ± 1.3	75.6 ± 6.2	18.0 ± 2.9		2.40 ± 0.13	38.55 ± 2.39	ND
Trained							
Cat. 2 cyclists ($n=3$)	28.7 ± 2.4	77.3 ± 4.2	13.2 ± 3.1	8 ± 3.2	4.50 ± 0.14	58.40 ± 1.93	75.1 ± 9.0
Cat. 3 cyclists ($n=4$)	22.5 ± 1.9	71 ± 5.3	6.9 ± 1.0	3 ± 1.0	4.10 ± 0.35	57.80 ± 2.61	67.4 ± 1.1
Mean $\pm$ SE	25.1 ± 1.8	73.7 ± 3.5	$9.6 \pm 1.8*$	5.5 ± 1.9	$4.27 \pm 0.21*$	$58.04 \pm 1.58*$	70.7 ± 5.8

Values are means $\pm$ SE for 7 trained and 7 untrained subjects. $\dot{V}_{O_{2\,peak}}$, peak oxygen consumption, Cat., category, as defined by United States Cycling Federation; ND, not determined. *Significantly different from untrained, $P < 0.05$.

to select their own rpm, typical neuromuscular recruitment patterns and riding style were reproduced in an effort to achieve $\dot{V}_{O_{2\,peak}}$ in these subjects. $\dot{V}_{O_{2\,peak}}$ was defined as the highest rate of $\dot{V}_{O_2}$ that subjects could maintain for a full 1-min period.

Body composition was assessed in subjects by using the sum of seven skinfold measurements according to the method of Jackson and Pollock (21).

Exercise trials. After the preliminary $\dot{V}_{O_{2\,peak}}$ tests, subjects were randomly assigned to a starting exercise intensity and nutritional state, with further random assignments for remaining exercise tests, each performed during both fed and fasted states. Each subject performed all exercise trials at the same time of day to avoid diurnal variations that may alter substrate utilization. Although each subject started at a different time of the morning, all trials started before noon. Each subject was tested at exercise intensities of 20 and 40% $\dot{V}_{O_{2\,peak}}$ for 2 h, 60% $\dot{V}_{O_{2\,peak}}$ for 1.5 h, and 80% $\dot{V}_{O_{2\,peak}}$ for 45 min in trained, and for 30 min in untrained subjects. Exercise workload was continually adjusted throughout the trial to maintain the desired relative exercise intensity. Cycling cadence was kept at 70 rpm for both trained and untrained subjects throughout the trials by using a metronome. Cyclists' training programs were controlled so as to provide adequate recovery between their normal training session and experimental trials. Subjects did not exercise the day immediately preceding an exercise trial, which allowed 48 h of rest for recovery and glycogen repletion before the experimental trial.

Nutritional controls. Each subject decided on a balanced standardized meal, which they replicated and ate on nights preceding every trial. On nights before a fed trial, the subjects were also asked to eat a standard snack consisting of 500 kcal (53% CHO, 31% fat, and 16% protein) before retiring. Three hours before the start of fed exercise trials, the subjects were asked to eat a standardized breakfast consisting of 550 kcal (87% CHO, 2% fat, and 11% protein). A rest day preceding exercise trials for trained subjects was utilized to normalize muscle glycogen concentration before all trials. Pretrial meals were used to normalize replete liver glycogen stores before fed trials in all subjects.

RER determinations. Pulmonary RER values (RER = CO_2 production/$\dot{V}_{O_2}$) were calculated online via open-circuit indirect calorimetry by using a Beckman LB-2 CO_2 analyzer, Ametek S-3A1 O_2 analyzer, Fleisch no. 3 pneumotachometer, and a PC. Expired air was collected into a mixing chamber from which air samples were pumped through in-line Dririte into gas analyzers for analysis. Certified calibration gases were used to calibrate analyzers before each trial. Pulmonary minute inspiratory volume was measured to ensure constancy of temperature and water vapor content of inspired air during a given trial; minute inspiratory volume was corrected to minute expiratory volume by using the Haldane assumption. Subjects wore the mouthpiece for the first 15 min of exercise and then followed a 5-min-off, 10-min-on protocol for

the remainder of the first hour. During the second hour of exercise, subjects followed an 18-min-off, 12-min-on protocol for ventilatory gas-exchange measurements at the end of each half hour. During the last 4 min before mouthpiece removal, RER values were averaged to achieve one representative value for each time period. For each trial, total workload in kilocalories per minute as well as relative CHO and lipid oxidation rates were calculated from standard tables, assuming RER = nonprotein respiratory quotient (RQ) (1). During periods when subjects were not wearing the mouthpiece, they were allowed to drink tap water ad libitum.

Blood lactate. Blood lactate was measured to assess the stability of acid-base balance during RER measurement. Blood was sampled at rest, every 15 min for the first hour, and then every 30 min for the second hour during the 20, 40, and 60% $\dot{V}_{O_{2\,peak}}$ exercise bouts. During the 80% $\dot{V}_{O_{2\,peak}}$ exercise bout, blood was sampled every 15 min. Blood samples were taken from finger-tip punctures into microcapillary tubes, immediately transferred to 10% perchloric acid, vortexed, centrifuged, decanted, and stored at $-20°C$ until analysis. Lactate concentrations were determined enzymatically (18). To obtain a representative blood lactate value for each subject per trial (workload), data obtained during the second half of each trial were averaged. Thus representative RER and blood lactate values were determined simultaneously when each was stable.

Statistics. Two-way ANOVAs with repeated measures were used to evaluate statistical significance of mean differences in lactate concentrations during trials. Unpaired Student's t-tests were used to compare subject characteristics. Significant differences between absolute oxidation rates of CHO and fat were determined by using factorial ANOVA. Significance of differences among groups, nutritional condition, and changes over time for RER were determined by using a repeated-measures factorial ANOVA. Post hoc comparisons were made using Fisher's protected least significant difference. An α of 0.05 was used throughout for statistical significance.

RESULTS

Subject characteristics. There were no significant differences in the mean age or weight of subjects; however, percent body fat of cyclists was significantly lower ($P < 0.05$) than that of untrained subjects (Table 1). Additionally, trained cyclists had 50% greater ($P < 0.05$) $\dot{V}_{O_{2\,peak}}$ values than untrained subjects, with an average lactate threshold for trained subjects at 71% $\dot{V}_{O_{2\,peak}}$ (Table 1).

Relative exercise intensities. Target relative exercise intensities of 20, 40, 60, and 80% $\dot{V}_{O_{2\,peak}}$ were matched experimentally with average $\dot{V}_{O_2}$ values of 22 ± 0.33, 40 ± 0.31, 59 ± 0.32, and $75 \pm 0.79\%$ $\dot{V}_{O_{2\,peak}}$ for both trained and untrained subjects. The only significant

difference in relative exercise intensity between groups was during the hardest exercise trials, in which trained subjects achieved significantly lower percentages of $\dot{V}O_{2peak}$ compared with untrained subjects in the fed state (72 ± 1.51 vs. $77 \pm 1.27\%$).

Lactate concentration. In each segment of the study, stable blood lactate concentrations were achieved (Fig. 1). Furthermore, there were no significant differences in lactate concentrations between groups at exercise intensities of 22 and 40% $\dot{V}O_{2peak}$ (Fig. 1, *A* and *B*, respectively). Training, regardless of nutritional state, significantly decreased lactate concentrations during the first 30 min of exercise at 59 and 75% $\dot{V}O_{2peak}$ (Fig. 1, *C* and *D*, respectively, and Fig. 1*E*). Thus RER measurements were made with constant lactate concentrations and, presumably, stable acid-base balance during all exercise intensities (Fig. 1, *A-D*) (see above). In our studies, extended periods of measurement were used to minimize the release of "nonmetabolic" CO_2 perturbing RER measurements.

RER and nutritional and training states. When RER values were compared in two nutritional states, mean fasting RER values were significantly lower than fed values in trained subjects exercising at 22, 40, and 59% $\dot{V}O_{2peak}$ (Fig. 2, *A-C*, respectively) and in untrained subjects exercising at 40 and 59% $\dot{V}O_{2peak}$ (Fig. 2, *B* and *C*, respectively).

RER and metabolic power output. At the same absolute workload (8 kcal/min: 40% $\dot{V}O_{2peak}$ for trained, 59% $\dot{V}O_{2peak}$ for untrained; 11.9 kcal/min: 59% $\dot{V}O_{2peak}$ for trained, 75% $\dot{V}O_{2peak}$ for untrained), RER values were significantly lower in trained compared with untrained subjects in either fasted (Fig. 3*A*) or fed (Fig. 3*B*) nutritional states. Mean RER values increased as an exponential function of power output at all exercise intensities in both nutritional conditions and training states (Fig. 2*E*). Training significantly decreased RER values when subjects were fed at 40% $\dot{V}O_{2peak}$ (Fig. 2*B*). However, when fasted, trained subjects showed significantly lower RER values at 22 and 40% $\dot{V}O_{2peak}$ (Fig. 2, *A* and *B*, respectively, and Fig. 2*E*). There were no differences in RER between trained and untrained subjects in either nutritional state while they were exercising at 59 and 75% $\dot{V}O_{2peak}$ (Fig. 2, *C* and *D*, respectively).

RER and absolute substrate oxidation rate. In the fed nutritional state, relative rates of lipid oxidation were higher in trained subjects only at 40% $\dot{V}O_{2peak}$ (Fig. 4*B*). Trained subjects worked at higher absolute workloads, and, therefore, exhibited greater absolute rates of lipid oxidation at 40% $\dot{V}O_{2peak}$ and CHO oxidation at 40, 59, and 75% $\dot{V}O_{2peak}$ (Fig. 4*A*). Trained fasted subjects exhibited a greater contribution of lipid to total energy expenditure at 22 and 40% $\dot{V}O_{2peak}$ (Fig. 5*B*), with greater absolute rates of lipid oxidation at 22 and 40% $\dot{V}O_{2peak}$ as well as increased rates of absolute CHO oxidation at 59 and 75% $\dot{V}O_{2peak}$ (Fig. 5*A*).

DISCUSSION

Training decreased RER only during exercise intensities eliciting $\leq 40\%$ $\dot{V}O_{2peak}$; thus our results indicate that the hypothesis does not hold that training increases relative lipid oxidation during moderate and hard relative exercise intensities. Furthermore, food intake 3 h before exercise significantly increased RER values and CHO oxidation at exercise intensities up to 59% $\dot{V}O_{2peak}$; however, the balance of substrate utilization at 75% $\dot{V}O_{2peak}$ was not affected by training or food effects. Thus data support the hypothesis that CHO intake 3–4 h before exercise increases CHO oxidation for at least the first 1.5 h of exercise at intensities $\leq 59\%$ $\dot{V}O_{2peak}$. As evident at high relative intensities, exercise power output was more influential in determining the balance of substrate oxidation than either training status or nutritional state.

Training effects on RER. To our knowledge, a similar cross-sectional investigation concerning effects of prior nutrition, exercise intensity, duration, and training on the respiratory gas-exchange ratio has not been conducted. Christensen and Hansen (5) were the first to observe that endurance exercise training decreased RER values at a given absolute workload. However, the authors did not make comparisons at similar relative workloads. Our data agree with those of Christensen and Hansen as well as with others who report decreased RER after endurance exercise training at absolute workloads when the subjects were fasted (Refs. 7, 8, 20, 25; Fig. 3*A*) and fed (Refs. 13, 26; Fig. 3*B*).

There are only a handful of studies comparing RER values in trained and untrained subjects at the same relative exercise intensities. Whereas some investigators report no difference in RER values between young trained and untrained subjects during 1–2 h of exercise (13, 22, 24), others have found slightly lower RER values in trained subjects (8, 16, 23, 26). For example, Coggan et al. (8) found lower RER values during 30 min of exercise in trained men at 78% $\dot{V}O_{2peak}$ (0.94 ± 0.01) than in untrained men at 79% $\dot{V}O_{2peak}$ (0.97 ± 0.01). Hagberg et al. (16) also found lower average RER values in trained (0.87 ± 0.03) compared with untrained men (0.93 ± 0.03) during 60 min of exercise at 71% $\dot{V}O_{2peak}$. Similarly, Klein et al. (23) and Montain et al. (26) observed that RER values were significantly lower in trained than in untrained subjects; unfortunately, they did not report RER values for comparison with results of other studies. Hurley et al. (19) found trained subjects to have lower RER values than untrained subjects at some exercise intensities (60, 70, and 75% $\dot{V}O_{2peak}$) but not others (65 and 80% $\dot{V}O_{2peak}$). Importantly, procedures to control prestudy nutrition were not reported, and trials were only 10 min in duration. These differences make comparison with the present study difficult. Our results are similar to those of Koivisto et al. (24) through 2 h of exercise at 40% $\dot{V}O_{2peak}$, Friedlander et al. (13), and Jansson and Kaijser (22), who reported nonsignificant differences in RER values between trained and untrained subjects exercising at 65% $\dot{V}O_{2peak}$. Thus, although there are inconsistencies in the literature, overall our results agree with those of others and suggest that training influences the balance of substrate oxidation only at low relative exercise intensities.
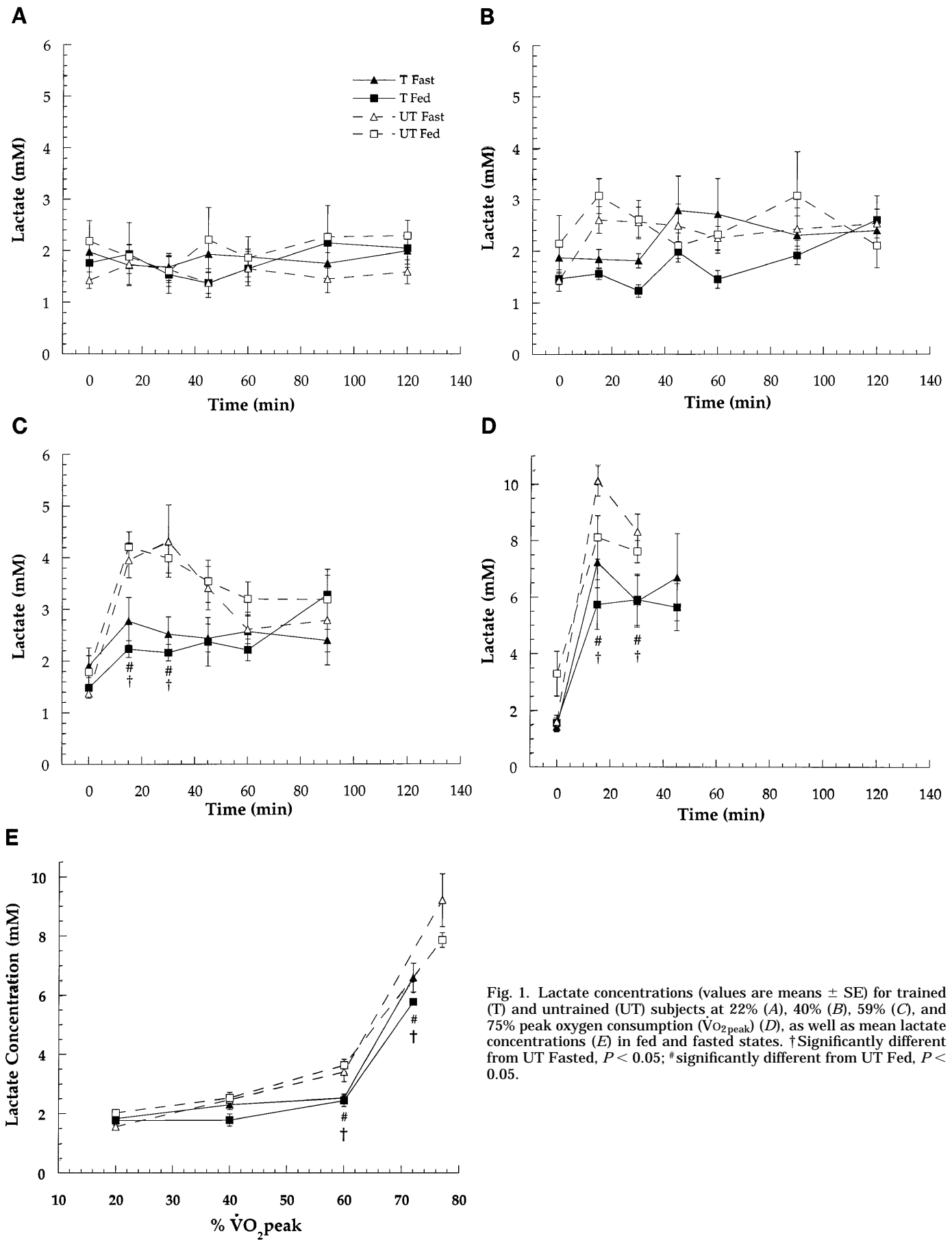
Fig. 1. Lactate concentrations (values are means ± SE) for trained (T) and untrained (UT) subjects at 22% (*A*), 40% (*B*), 59% (*C*), and 75% peak oxygen consumption ($\dot{V}O_{2peak}$) (*D*), as well as mean lactate concentrations (*E*) in fed and fasted states. †Significantly different from UT Fasted, $P < 0.05$; #significantly different from UT Fed, $P < 0.05$.
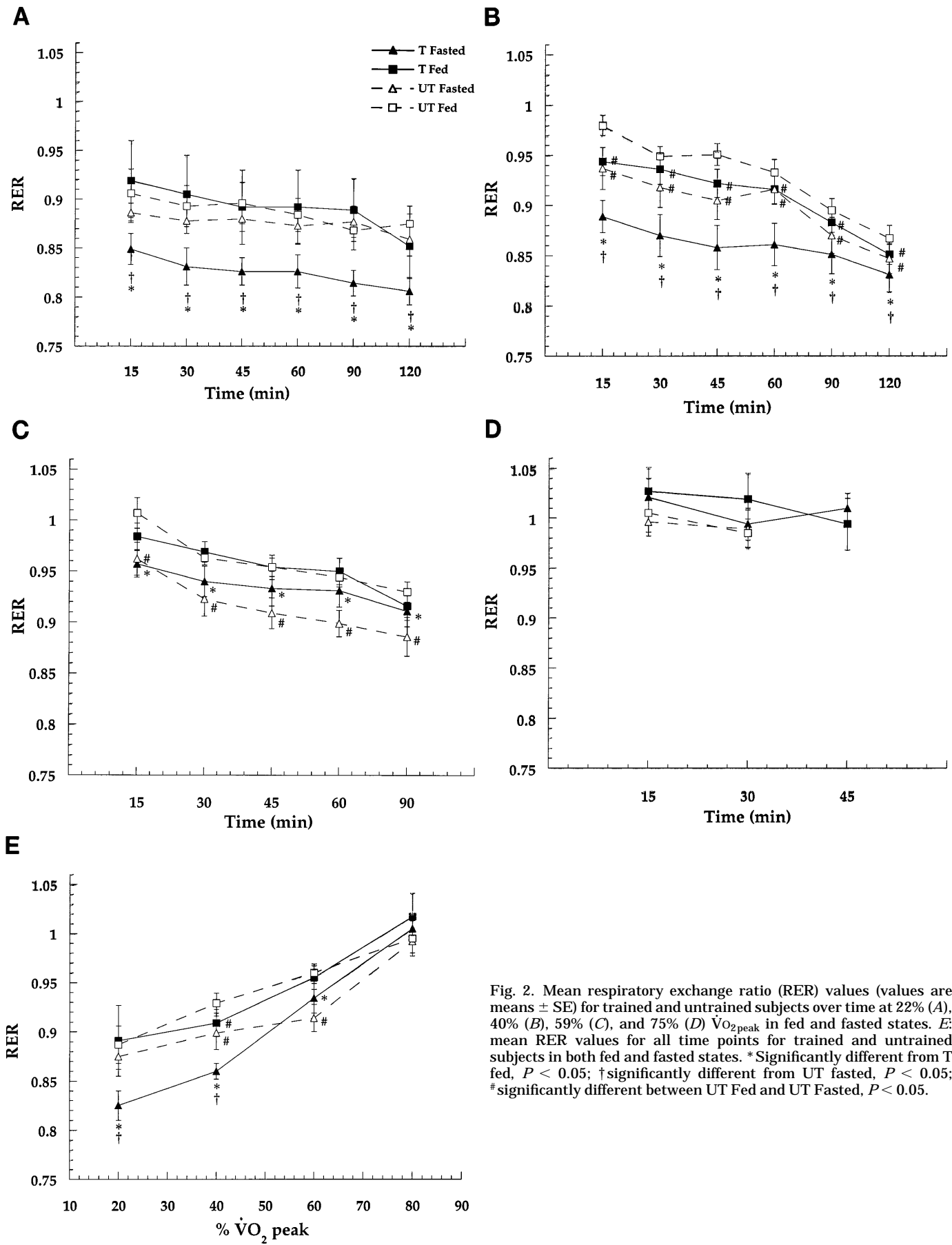
Fig. 2. Mean respiratory exchange ratio (RER) values (values are means ± SE) for trained and untrained subjects over time at 22% (A), 40% (B), 59% (C), and 75% (D) $\dot{V}O_{2peak}$ in fed and fasted states. E: mean RER values for all time points for trained and untrained subjects in both fed and fasted states. *Significantly different from T fed, $P < 0.05$; †significantly different from UT fasted, $P < 0.05$; #significantly different between UT Fed and UT Fasted, $P < 0.05$.

A

Δ = significantly different from untrained at p<0.05.

B

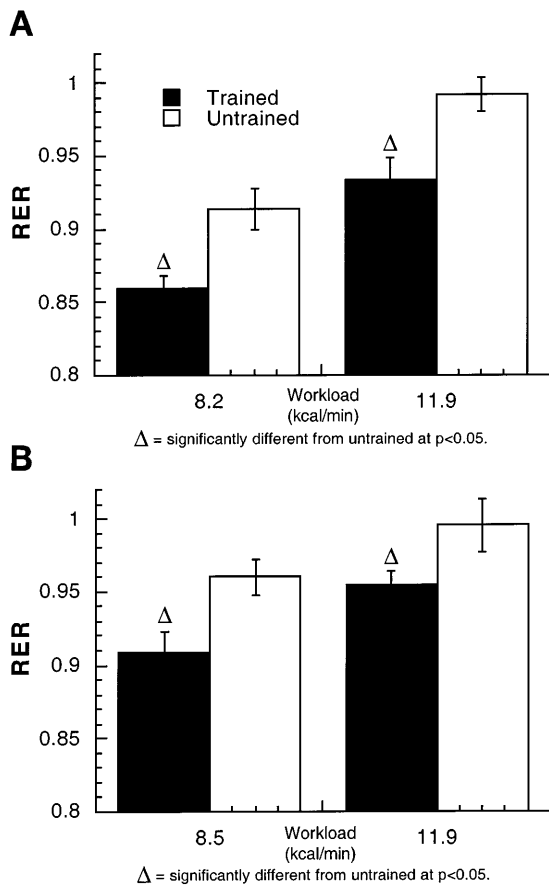Δ = significantly different from untrained at p<0.05.

Fig. 3. Mean RER values at similar absolute workloads in fasted (A) and fed (B) nutritional states. Workload was calculated from oxygen consumption and RER (see METHODS). Workloads at 8.16 and 8.47 kcal/min corresponded to exercise bouts at 40% $\dot{V}O_{2\,peak}$ in trained subjects and at 59% $\dot{V}O_{2\,peak}$ in untrained subjects. Workloads at 11.9 kcal/min corresponded to exercise bouts at 59% $\dot{V}O_{2\,peak}$ in trained subjects and at 75% $\dot{V}O_{2\,peak}$ in untrained subjects.

Using an experiment of nature, Roberts et al. (30) made comparisons of RER between dogs, which have high aerobic capacities, and similarly sized goats, which have low aerobic capacities, at graded exercise intensities. Roberts et al. found that dogs had similar RER values as goats across a broad range of relative exercise intensities. Thus, regardless of genotype or training-induced influences on phenotypic expression of oxygen transport and utilization systems, as predicted (4), humans and other mammals with widely different aerobic capacities demonstrate similar substrate utilization patterns when relative exercise intensity is considered.

We report that training increases relative lipid oxidation during mild-to-moderate exercise only when subjects exercise in the fasted state. Food intake before exercise only slightly alters the above relationship. When subjects were fed a meal 3–4 h before exercise, there were still no differences in RER between trained and untrained subjects exercising at 59 and 75% $\dot{V}O_{2\,peak}$. RER values were significantly decreased for trained subjects only at 40 and not at 22% $\dot{V}O_{2\,peak}$. It is unknown why trained subjects did not oxidize relatively more lipid while exercising at 22% $\dot{V}O_{2\,peak}$ when

fed a meal before exercise. Perhaps glucose transporter GLUT-4 translocation persisted despite the return to baseline insulin concentration 3 h after a meal (9, 26). Such an effect could have stimulated glucose uptake in muscle and mitigated adaptations, that could have led to enhanced lipid oxidation under fasting conditions (12).

Despite our inability to explain why trained fed subjects did not utilize relatively more lipid when exercising at 22% $\dot{V}O_{2\,peak}$, overall it is clear that relative power output has a major influence on substrate utilization pattern (Fig. 2E). Because most athletes compete and train at much higher relative exercise intensities than 40% $\dot{V}O_{2\,peak}$, our results suggest that they will not oxidize a greater proportion of lipid during exercise than untrained subjects, regardless of their nutritional condition.
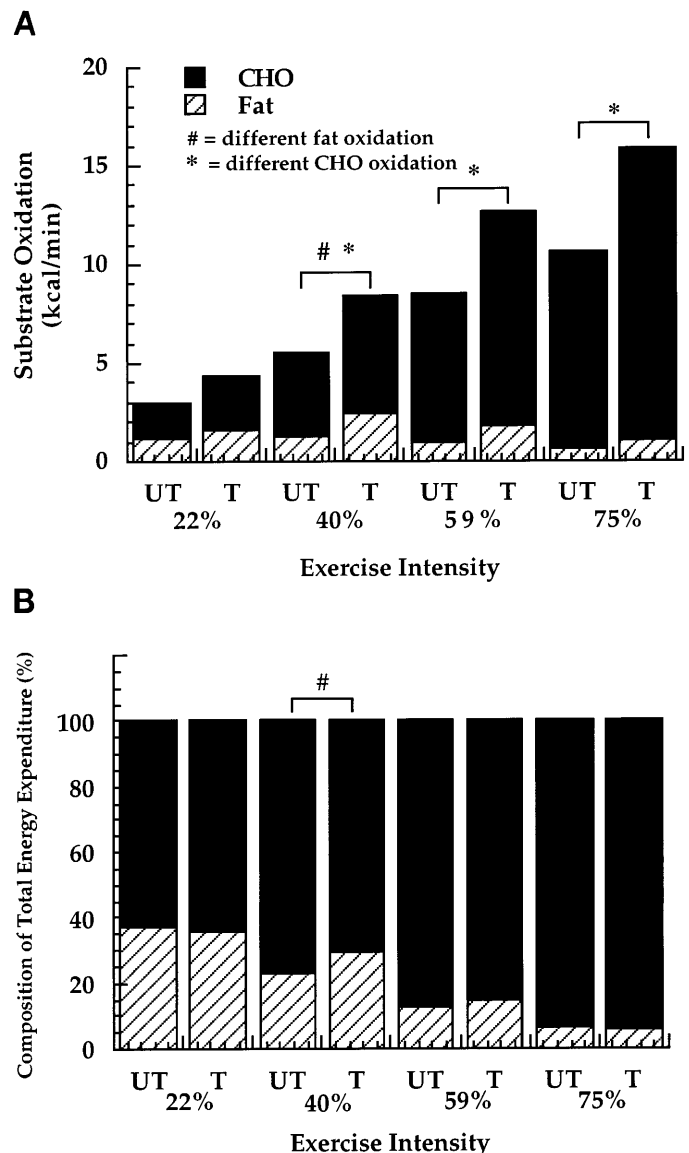
A

B

Fig. 4. Mean absolute (A) and relative (B) substrate oxidation rates in trained and untrained subjects in fed nutritional state at 22, 40, 59, and 75% $\dot{V}O_{2\,peak}$. CHO, carbohydrate. $P < 0.05$ for both * and #.
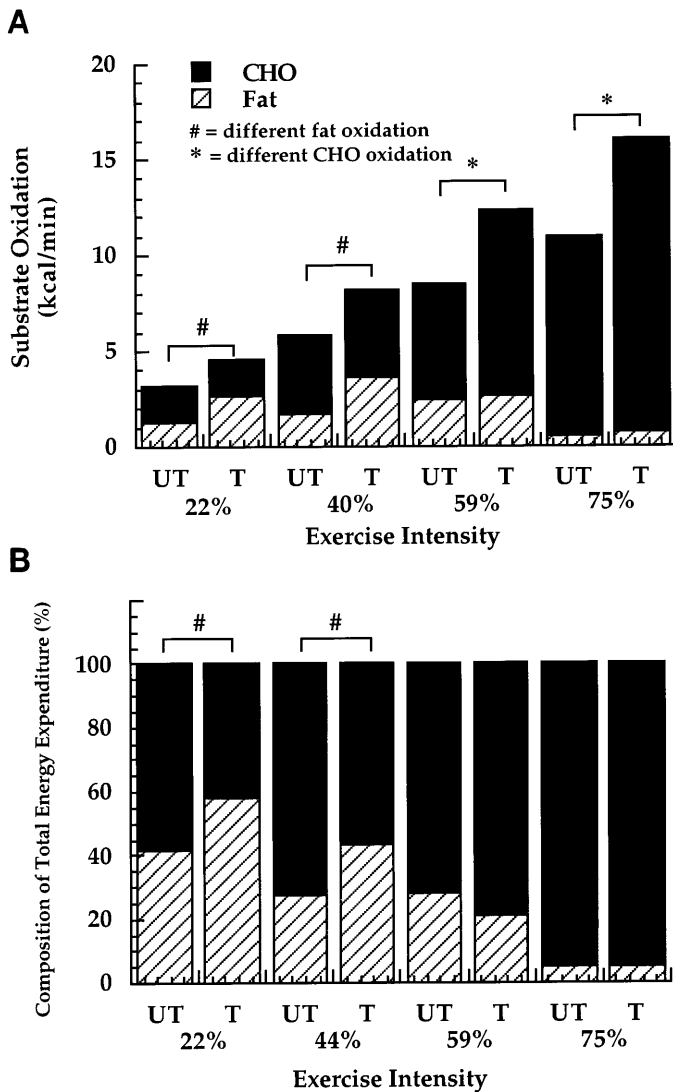
Fig. 5. Mean absolute (*A*) and relative (*B*) substrate oxidation rates in trained and untrained subjects in fasted nutritional state at 22, 40, 59, and 75% $\dot{V}O_{2\,peak}$. $P < 0.05$ for both * and #.

A concern in evaluating our results for subjects exercising at 75–80% $\dot{V}O_{2\,peak}$ was that RER approximated 1.0. We would have predicted that some lipid oxidation, either in working muscle or elsewhere, would cause RER <1.0. Thus we suspect that disturbances in acid-base balance or other factors conspired to produce our RER results at high power outputs.

Our high RER values for subjects exercising at high intensities cause concern that RER overestimated working muscle RQ. This concern is justified on theoretical bases, but the concern is not readily supported in the literature. For instance, Odland et al. (27) recently reported RER and leg RQ values in men working at 65% maximal $\dot{V}O_2$ for 1 h. In their report (their Table 1), RER varied over time, declining from 0.99 at 18 min to 0.92 after 60 min of exercise; the average RER was 0.94 ± 0.01. Leg RQ for the same period averaged 1.00 ± 0.02.

That RER and working limb RQ values are independently reproducible leads to the conclusion that fat

oxidation can be masked at high sustained power outputs. Future efforts need to be directed to establish how to evaluate working muscle lipid oxidation. For the present, we are left with the conclusion that lipid oxidation is small and minimally affected by training if relative exercise intensity is considered.

Food intake effects on RER. Our results from fed subjects are consistent with those of others (9, 26), who have found increased CHO oxidation during exercise that is preceded by a preexercise meal consumed up to 4 h earlier. Thus the fed nutritional state predisposes subjects to CHO oxidation regardless of training state or exercise intensity. The influence of nutrition on substrate utilization was reported by Helge et al. (17), who did not find decreased RER at an absolute workload after 7 wk of training when subjects were chronically fed a high-CHO diet, even though subjects were tested after a 12-h fast. After an overnight fast, our subjects oxidized greater relative percentages of energy from lipid at intensities up to 59% $\dot{V}O_{2\,peak}$ (Fig. 2*E*). Greater relative exercise intensities are likely associated with enhanced CHO oxidation, regardless of nutritional state, because of increased recruitment of type II CHO-dependent fibers and increased arterial catecholamine concentration (6), which appear to override any effect of fasting on enhancing lipid oxidation, as observed at lower relative exercise intensities. One might expect fasting to have increased relative lipid oxidation in untrained subjects at 22% $\dot{V}O_{2\,peak}$, as RER values were lower in these subjects at 40 and 59% $\dot{V}O_{2\,peak}$. It is possible that unchanged RERs could be attributed to lack of subject compliance, as three of the seven subjects showed higher RERs at 22% $\dot{V}O_{2\,peak}$ when reporting for "fasted" than for "fed" trials.

After 2 h of exercise at both 22 and 40% $\dot{V}O_{2\,peak}$, RER values in trained fed subjects were approaching trained fasted RER values. These results suggest that fasting will increase lipid oxidation in trained subjects during the first several hours of low-intensity exercise and that substrate utilization during longer duration exercise may not be influenced by either an overnight fast or a meal 3–4 h before exercise. As found previously (9), pretrial CHO feeding may have increased glycogen stores, allowing greater CHO oxidation from muscle glycogen during exercise (29). After 2 h of exercise, enhanced glycogen stores may be depleted, resulting in similar substrate utilization patterns between fed and fasted conditions. Additionally, prolonged insulin-like effects after a meal may elevate RER values, even when insulin concentration has recovered to basal values, which may attenuate after 1 h of exercise, resulting in similar substrate utilization compared with the fasting state (9).

Lack of enhanced relative lipid oxidation in fasted compared with fed subjects at 75% $\dot{V}O_{2\,peak}$ suggests that muscle power output and the intramuscular milieu dictate that CHO oxidation predominates during moderate-to-maximal intensity exercise tasks. Several studies increased glycolytic flux via a hyperinsulinemic euglycemic clamp, increased exercise intensity or CHO feeding, and found decreased long-chain fatty acid

oxidation, with unchanged medium-chain fatty acid oxidation, suggesting that increased CHO flux decreased lipid oxidation by limiting long-chain fatty acid entry into the mitochondria (10, 31, 32). Contrary to predictions of the Randle (glucose-fatty acid) cycle (28), it appears that CHO flux and oxidation control lipid oxidation, not vice versa.

Our results showing the dominance of exercise power output over dietary history in determining the fuel mix during exercise are consistent with those of Whitley et al. (33). They studied well-trained cyclists during 90 min of exercise at 70% of maximal $\dot{V}O_2$. On two occasions, subjects ingested isoenergetic high-CHO or high-fat meals 4 h before exercise; on the third occasion, subjects were studied after an overnight fast. Blood glucose, insulin, and free fatty acid levels differed before exercise in predictable ways attributable to CHO nutrition. However, during exercise, insulin levels rapidly fell and RER rose to similar values ($\simeq 0.90$) under all dietary conditions. Thus substrate selection at relatively high-intensity exercise is dominated by CHO oxidation and is remarkably resistant to alteration.

When they compete or train, most athletes do not perform after an overnight fast but typically consume a high-CHO meal several hours before exercise. Results of the present investigation suggest that athletes who eat a meal 3–4 h before exercise are more dependent on CHO oxidation for at least the first 1.5 h of exercise at intensities $<59\%$ $\dot{V}O_{2\,peak}$ compared with when subjects are fasted

Absolute substrate oxidation rates. As shown by others (7, 8, 15, 20, 25, 26), absolute lipid oxidation was greater in trained subjects when they were fed or fasted at a given absolute workload, likely because of the lower relative exercise intensity for trained subjects (Fig. 3). Discussion thus far has focused on a relative (%CHO, %lipid) comparison of substrate oxidation in trained and untrained subjects during exercise. However, trained subjects cycled at higher power outputs. Consequently, absolute substrate oxidation rates differed between groups. To compare absolute rates of lipid and CHO oxidation in our two groups of subjects, we calculated substrate oxidation rates at each relative exercise intensity in both fed and fasted states (Figs. 4 and 5). Our results support the conclusion that during exercise intensities of 59 and 75% $\dot{V}O_{2\,peak}$, regardless of nutritional state, the increased power output of trained compared with untrained subjects is supported by enhanced CHO oxidation.

Highest absolute rates of lipid oxidation in the fed state were observed at 40% $\dot{V}O_{2\,peak}$ in both trained (2.4 kcal/min) and untrained (1.3 kcal/min) subjects. Maximal lipid oxidation rates were also observed at 40% $\dot{V}O_{2\,peak}$ in trained fasted subjects (3.7 kcal/min). These maximal lipid oxidation rates agree with previously published data indicating that greatest absolute rates of lipid oxidation occur at 45% $\dot{V}O_{2\,peak}$, with an increase in exercise intensity eliciting decreased rates of lipid oxidation (3).

Limitations. A limitation to our study could be that a cross-sectional, as opposed to longitudinal, design was employed. Thus, by comparing groups of widely different aerobic capacities, exercise experiences, and, possibly, genetic differences in muscle fiber type, we may have obscured subtle differences in substrate oxidation attributable to training. However, one could also argue that a cross-sectional study, comparing subjects of widely different genetic capacities and muscle fiber types, would exaggerate any training-induced enhanced lipid oxidation at a given relative exercise intensity, as athletic selection for endurance sport may favor the ability to oxidize lipid needed for endurance events.

A major limitation of our experimental approach is that, beyond broad USCF categories, the precise identities of fuels oxidized could not be determined. For instance, we could not discriminate between oxidation of blood-borne free fatty acids and intramuscular triglycerides. Similarly, we could not discriminate between glucose and glycogen oxidation in untrained men compared with cyclists (13). Similarly, we could not know whether hexoses were oxidized directly or first converted to lactate and subsequently shuttled through the interstitium and vasculature before oxidation (2).

Our subjects were moderately-to-well-trained category 2 and 3 USCF cyclists who had been training an average of 5.5 yr; however, these subjects could not be considered elite cyclists. Wilber et al. (34) recently reported that elite national team cyclists had lactate thresholds at 80% $\dot{V}O_{2\,peak}$ (9% higher than subjects in the present study) and $\dot{V}O_{2\,peak}$ values of 5.09 l/min (0.82 l/min higher than subjects in the present study). Thus our data suggest that substrate oxidation at moderate-to-high relative exercise intensities is unaltered in moderately-to-well-trained cyclists compared with untrained subjects. We cannot exclude, however, the possibility that additional years of endurance training or a genetic predisposition for endurance exercise may influence substrate oxidation at moderate-to-high relative exercise intensities.

With regard to a training effect on sedentary controls, experimental trials were conducted on untrained subjects a week apart and in random order. Therefore, whereas it is likely that the eight trials produced a training effect, because a randomized design was employed, it is unlikely that training of sedentary subjects systematically biased results.

Finally, we caution that our results are appropriate for nonelite male athletes and sedentary counterparts. Recently, Friedlander et al. (14) contrasted metabolic responses in men ($n = 13$) and women ($n = 14$) under similar protocols and using the same equipment. RER values were consistently lower in women than in men (e.g., 0.87 ± 0.02 in women and 0.94 ± 0.02 in men) during exercise at 65% $\dot{V}O_{2\,peak}$ after training. Thus some of the variations in reported values are gender related.

Summary and conclusions. Results of our experiments do not support the hypothesis that trained subjects always oxidize relatively more lipid than do untrained subjects when exercise intensity is normalized to percentage of $\dot{V}O_{2\,peak}$. Our results do support the hypothesis that prior CHO nutrition causes CHO-

derived fuels to predominate as energy sources during exercise. Whereas endurance training increased absolute and relative lipid oxidation at exercise intensities of 22 and 40% $\dot{V}o_{2\,peak}$ after a 12- to 13-h overnight fast, increased absolute CHO oxidation rates supported the greater muscle power output by trained subjects exercising at high intensities. Thus our results suggest that trained athletes oxidize greater absolute and relative amounts of lipid only at intensities $\leq 40\%$ $\dot{V}o_{2\,peak}$. However, because most competitive athletes train and compete at intensities $>40\%$ $\dot{V}o_{2\,peak}$, they will not oxidize greater relative or absolute amounts of lipid, compared with the untrained state, regardless of nutritional condition.

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