Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men

EVA JANSSON AND LENNART KAIJSER
Department of Clinical Physiology, Karolinska Sjukhuset, S-104 01 Stockholm, Sweden

JANSSON, EVA, AND LENNART KAIJSER. *Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men.* J. Appl. Physiol. 62(3): 999–1005, 1987.—Substrate utilization during exercise at 65% of maximal O_2 uptake ($\dot{V}O_{2\,max}$) and biochemical characteristics of vastus lateralis were compared between five endurance-trained (T) and five untrained subjects (U). The oxidative enzyme activities were 100% greater in T than in U, and $\dot{V}O_{2\,max}$ was 50% higher. A greater proportion of ATP regeneration occurred through oxidative processes in T than in U (smaller leg lactate release and smaller muscle lactate accumulation). The respiratory exchange ratio together with the local leg respiratory quotient indicated a greater contribution of fat to oxidative metabolism in T than U (53 vs. 33%). No difference, however, in the ratio of plasma free fatty acid extraction to O_2 extraction by the working legs was found between T and U. Thus it could be calculated that a greater fraction of fat oxidation would have been covered by intramuscular triglycerides in T than in U (34 vs. 15%, $P < 0.05$). T in comparison to U were further characterized by a smaller glycogen breakdown and a smaller glucose uptake, which may have been one contributing factor that prevented the blood glucose level from falling in T. The greater leg muscle citrate concentration in T could have been one factor mediating a lower carbohydrate utilization as a response to an increase in the relative proportion of fat oxidation.

human skeletal muscle; endurance training; exercise; muscle glycogen; blood glucose; free fatty acids; lactate; respiratory exchange ratio; muscle citrate

A NUMBER OF STUDIES have shown that endurance training results in increased activities of enzymes in the tricarboxylic acid cycle, the respiratory chain, and the β-oxidation in trained musculature (14). It is also well known that an individual's aerobic working capacity, as measured by maximal O_2 uptake, is increased by physical training and, furthermore, that when exercise at the same absolute load is repeated after training lactate production is lowered and the respiratory exchange ratio is decreased. This suggests that a greater proportion of the energy expenditure is covered by oxidative metabolism and that oxidative metabolism, to a greater extent, is covered by fat oxidation (21). Less clear-cut results are available regarding the effect of training on the relationship of aerobic to anaerobic metabolism or the relationship of fat to carbohydrate oxidation when exercise at the same relative load (defined as the same fraction of the maximal O_2 uptake) is repeated after training. Hermansen et al. (11) also showed a decreased respiratory exchange ratio at the same relative load, but Henriksson (10) did not find a corresponding increase in the net uptake (as calculated from leg blood flow and arteriovenous difference) of plasma free fatty acids in a trained exercising leg, although the respiratory exchange ratio was decreased. It is known, however, that there is a simultaneous uptake and release of fatty acids in an exercising leg (26, 27) and, consequently, that the arteriovenous difference greatly underestimates the true plasma free fatty acid extraction.

Therefore, the aim of this study was to compare untrained and extremely endurance-trained subjects with regard to the leg extraction of plasma free fatty acids. This was done by use of a radioisotope-labeled fatty acid during prolonged exercise at the same relative load (same fraction of maximal O_2 uptake). In addition, the net leg uptake of blood glucose and the muscle glycogen utilization were measured. The leg substrate utilization was related to muscle fiber composition and the activity of mitochondrial and glycolytic enzymes.

METHODS

Subjects. Two groups of subjects with extremely different training backgrounds were studied: five endurance-trained competitive cyclists and five untrained volunteers. All subjects were fully and fairly informed of the purpose of the experiment and the procedures involved and of possible risks before giving their consent. Also, the subjects were free to withdraw their participation at any time. The study was approved by the Ethics Committee of the Karolinska Hospital. Means ± SD of age, weight, and height were for the trained 21 ± 3 yr, 66 ± 3 kg, and 178 ± 3 cm for the trained subjects and 27 ± 3 yr, 67 ± 10 kg, and 182 ± 12 cm for the untrained subjects.

Procedures. Maximal O_2 uptake ($\dot{V}O_{2\,max}$) was determined in a preparatory test on an electrodynamically braked cycle ergometer according to the criteria of Åstrand and Rodahl (1) and was 4.81 ± 0.47 l/min for the trained group and 3.29 ± 0.39 l/min for the untrained subjects ($P < 0.01$). Maximal heart rate (HR_{max}) was 195 ± 7 and 199 ± 14 beats/min for the trained and untrained subjects, respectively. The studies were done the morning after an overnight fast. Catheters were introduced percutaneously into the left brachial artery (a) and the femoral vein (fv) for blood sampling and into an antecubital vein for the infusion of [^{3}H]palmitate. At rest O_2 uptake ($\dot{V}O_2$), a-fv differences of blood O_2, glucose, and lactate, and arterial plasma free fatty acid (FFA) concen-

tration were measured, and a muscle biopsy was taken percutaneously from vastus lateralis of quadriceps femoris muscle (2). The subjects exercised at loads chosen to correspond to 65% of their $\dot{V}O_{2\,max}$ for 60 min on an electrodynamically braked cycle ergometer. Measurements of $\dot{V}O_2$ and a-fv differences were repeated after 15 and 60 min of exercise. In addition, during the last measurement period (after 60 min of exercise) leg FFA extraction was estimated from the extraction of [^{3}H]-palmitate infused intravenously at constant rate (1.3 μCi/min) for 10 min before the start of blood sampling after an initial bolus (10 μCi; Ref. 23). Muscle biopsies were taken after 15 and 60 min of exercise.

Analyses. $\dot{V}O_2$ and CO_2 elimination were measured by the Douglas bag technique, and gas analyses were performed according to Scholander (30). The respiratory exchange ratio (R) was calculated. Mechanical efficiency in percentage was calculated from the formula

$$R = \frac{\text{mechanical work performed (W)} \times 0.293}{\text{total body } \dot{V}O_2 \text{ (l/min)}}$$

Blood O_2 saturation was measured spectrophotometrically, and O_2 content was calculated from the saturation and the hemoglobin concentration (3). For the calculation of local leg respiratory quotient (RQ), total CO_2 and O_2 in blood samples were measured in duplicate by the Van Slyke method on arterial and venous blood samples taken after 60 min of exercise (28). To strengthen the methods statistically, mean values of R and leg RQ were calculated for each individual. An earlier study showed that R and leg RQ did not differ systematically during submaximal exercise, thus justifying a mean value calculation (17).

Blood glucose and lactate concentrations were analyzed on a neutralized perchloric acid extract by fluorometric methods (13, 25), and plasma FFA concentration was determined by the ^{63}Ni method (12). ^{3}H activity in the FFA fraction was measured in a Packard liquid scintillation spectrometer (model 3375) after separation of FFA by thin-layer chromatography. The FFA fraction was added to a scintillation solution containing 2,5-diphenyloxazole and *p*-bis[2-(4-methyl-5-phenyloxazolyl)]benzene.

Calculations. FFA turnover, extraction, and release and ^{3}H specific activity of FFA were calculated as described previously (23, 27).

fractional extraction of [^{3}H]palmitate

= a-fv difference of [^{3}H]palmitate/arterial concentration of [^{3}H]palmitate

gross extraction of plasma FFA

= fractional extraction of [^{3}H]palmitate × arterial concentration of plasma FFA

net extraction of plasma FFA

= a-fv difference of plasma FFA

release of plasma FFA

= gross extraction − net extraction

turnover rate of plasma FFA

= infusate radioactivity (cpm/ml) × infusion rate (ml/min)/arterial plasma FFA specific activity (cpm/mmol)

The expression "gross extraction of plasma FFA" is in the abstract and DISCUSSION shortened to "extraction of FFA."

The resting biopsy was quickly divided into three parts, one of which was frozen in isopentane, precooled in liquid N_2 and used for histochemical analysis of fiber types (4). The second part was frozen in liquid N_2 and, after homogenization in a 0.1 M phosphate buffer (pH 7.7), was analyzed for succinate dehydrogenase (SDH) according to the principles of Lowry and Passonneau (25) as modified by Häggmark et al. (9), 3-hydroxyacyl-CoA dehydrogenase (HAD), and 1-phosphofructokinase (PFK; Ref. 24). After being frozen in liquid N_2 the third part was freeze-dried, cleaned of visible fat, blood, and connective tissue under a dissection microscope at constant temperature (21°C) and humidity (30%), and analyzed for glycogen, ATP, lactate, glucose 6-phosphate (G-6-*P*), and citrate (25). The biopsies obtained after exercise were immediately plunged into liquid N_2 and analyzed for glycogen, ATP, lactate, G-6-*P*, and citrate as described above.

The results are expressed as means ± SD for the groups. The statistical significance of the mean differences were tested by the Student's *t* test. The level of statistical significance was set to 95% ($P < 0.05$). A nonsignificant difference is indicated by NS.

RESULTS

O_2 uptake and heart rate. The work load was 229 ± 13 W in the trained subjects and 146 ± 21 W in the untrained subjects. $\dot{V}O_2$ after 15 min of exercise was 3.00 ± 0.14 and 2.21 ± 0.33 l/min ($P < 0.01$) in trained and untrained subjects, respectively, and after 60 min of exercise 3.06 ± 0.19 and 2.25 ± 0.32 l/min ($P < 0.01$), respectively. After 15 min of exercise $\dot{V}O_2$ corresponded to 62 ± 6% of $\dot{V}O_{2\,max}$ in the trained subjects and 67 ± 5% in the untrained subjects and after 60 min to 64 ± 5 and 68 ± 4%, respectively (NS).

Heart rate did not differ significantly between the groups, either in absolute value or expressed as percent of HR_{max}. The trained subjects increased from 155 ± 10 beats/min at 5 min to 173 ± 8 beats/min after 60 min of exercise (79 to 89% of HR_{max}) and the corresponding increase for the untrained was 163 ± 7 to 173 ± 15 beats/min (82 to 89% of HR_{max}).

After 15 min of exercise R was 0.84 ± 0.05 in trained subjects and 0.91 ± 0.10 in untrained subjects (NS), and after 60 min of exercise it was 0.84 ± 0.04 and 0.90 ± 0.07, respectively (NS). After 60 min of exercise the leg RQ was 0.84 ± 0.09 in trained subjects and 0.90 ± 0.07 in untrained subjects (NS). The calculated mean for R and leg RQ after 60 min of exercise was 0.84 ± 0.04 in trained subjects and 0.90 ± 0.03 in untrained subjects ($P < 0.05$).

Blood: O_2, substrates, and metabolites. At rest the a-fv

difference for O_2 was 49.3 ± 11.6 and 38.5 ± 16.3 ml/l in the trained and untrained subjects, respectively. During exercise the a-fv difference for O_2 was greater in the trained than untrained subjects. At 15 min of exercise it was 173.4 ± 9.5 and 148.4 ± 15.6 ml/l ($P < 0.01$) and at 60 min of exercise 181.1 ± 6.9 and 159.5 ± 16.6 ml/l ($P < 0.05$), respectively.

Arterial blood glucose concentration, both at rest and during exercise, was significantly higher in trained than in untrained subjects. It increased significantly during exercise in the trained ($P < 0.05$), whereas it decreased in the untrained subjects ($P < 0.05$; Fig. 1). The a-fv difference of glucose after 15 min of exercise was of similar magnitude in trained and untrained subjects, but after 60 min of exercise it was 0.08 ± 0.16 in the trained and 0.27 ± 0.80 mmol/l in the untrained subjects ($P < 0.05$; Fig. 1).

Arterial blood lactate concentration at rest was significantly higher in the trained than in the untrained subjects. During exercise, however, it increased insignificantly in the trained subjects, whereas a pronounced increase above resting level occurred in the untrained subjects, ending at a level significantly above that of the trained subjects (Fig. 1). The a-fv difference of lactate after 15 min of exercise was −0.14 ± 0.24 in the trained and −0.47 ± 0.14 mmol/l in untrained subjects ($P < 0.05$). After 60 min of exercise it was −0.04 ± 0.06 and −0.31 ± 0.28 mmol/l, respectively (NS).

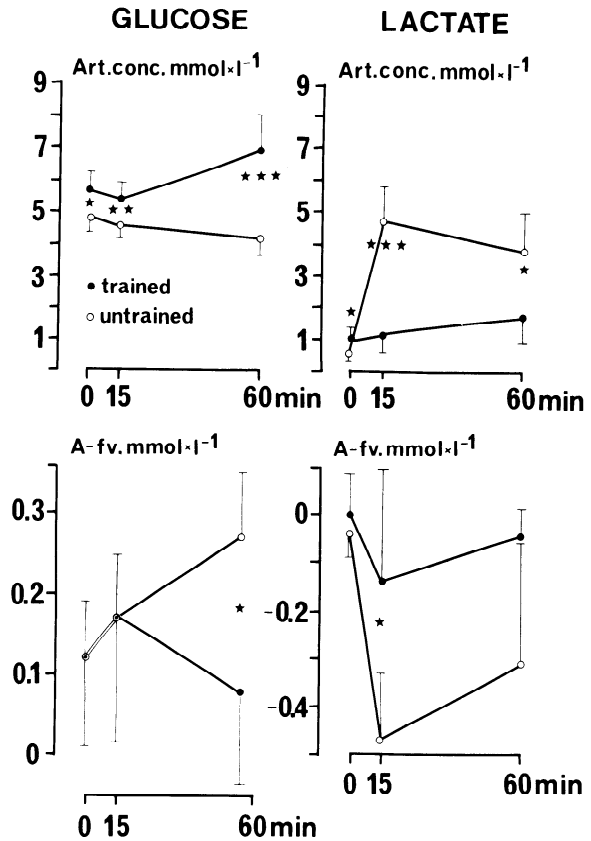

FIG. 1. Arterial blood glucose and blood lactate concentration and arterial-femoral venous differences (a-fv) of glucose and lactate at rest and during exercise in untrained (*open circles*) and trained (*filled circles*) subjects. All statistical comparisons are between untrained and trained subjects: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Arterial plasma FFA concentration at rest was 0.651 ± 0.181 in the trained and 0.582 ± 0.081 mmol/l in untrained subjects. After 60 min of exercise it was 0.696 ± 0.266 mmol/l in the trained, which did not differ significantly from their level at rest, but had increased in the untrained to 0.769 ± 0.023 mmol/l ($P < 0.05$). The total turnover rate, fractional extraction, gross extraction, net extraction, and release of plasma FFA at 60 min of exercise did not differ significantly between the two groups (Table 1).

Muscle: fiber composition and biochemical data at rest. The trained subjects had a significantly higher fraction of type I fibers (70 ± 11 vs. 40 ± 9%) and lower fraction of type IIa (26 ± 8 vs. 41 ± 9%) and type IIb (4 ± 4 vs. 19 ± 3%) fibers than the untrained subjects (Fig. 2). SDH and HAD activities were about twice as high in the trained as in untrained subjects, but PFK activity did not differ significantly between the groups (Fig. 2).

Glycogen and citrate concentrations were significantly higher, and lactate and G-6-*P* concentrations were significantly lower, in the trained than in the untrained subjects. ATP concentration did not differ between the two groups (Table 2).

Muscle: substrates and metabolites during exercise. Decrease in glycogen concentration during the 60 min of exercise was significantly smaller in trained than in the untrained subjects (Fig. 3; $P < 0.05$). During the first 15 min the decrease in glycogen concentration was 4.0 ± 3.9 and 8.7 ± 2.4 mmol·kg dry $wt^{-1} \cdot min^{-1}$ in trained and untrained subjects, respectively, and from 15 to 60 min the corresponding values were 2.1 ± 2.2 and 3.4 ± 0.5

TABLE 1. *Plasma FFA concentration, extraction, release, and turnover rate at 60 min of exercise*

Variable	Trained	Untrained	*P*
Arterial plasma FFA, mmol/l	0.696±0.266	0.769±0.123	NS
Fractional extraction of [^{3}H]palmitate	0.160±0.040	0.121±0.062	NS
Gross extraction of plasma FFA, mmol/l	0.109±0.032	0.091±0.037	NS
Net extraction of plasma FFA, mmol/l	0.036±0.035	0.019±0.046	NS
Release of plasma FFA, mmol/l	0.073±0.013	0.072±0.044	NS
Turnover rate of plasma FFA, mmol/min	1.10±0.25	1.14±0.36	NS

Values are means ± SD. FFA, free fatty acids; *P*, significance of difference between values for trained and untrained subjects (NS, not significant).

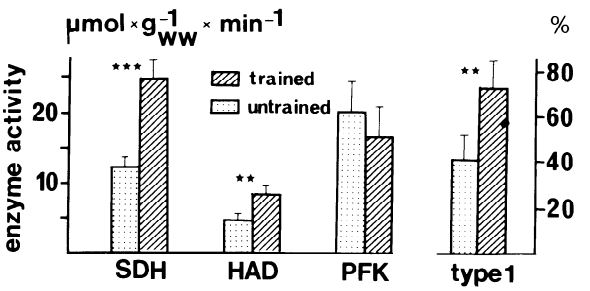

FIG. 2. Succinate dehydrogenase (SDH), 3-hydroxyacyl-CoA dehydrogenase (HAD), and phosphofructokinase (PFK) activities (μmol·g wet $wt^{-1} \cdot min^{-1}$) and percent type I fibers in vastus lateralis of untrained and trained subjects. All statistical comparisons are between untrained and trained subjects: ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. *Muscle metabolites at rest and during exercise*

Variable	Trained	Untrained	*P*
Citrate			
Rest	1.17±0.32	0.51±0.07	<0.01
Exercise, 15 min	1.66±0.47	0.78±0.14	<0.01
Exercise, 60 min	1.93±0.82	1.01±0.20	<0.05
Lactate			
Rest	6.0±1.8	7.9±1.0	<0.05
Exercise, 15 min	6.6±2.3	37.2±17.4	<0.01
Exercise, 60 min	10.2±3.6	39.3±15.9	<0.01
ATP			
Rest	25.5±2.0	27.0±2.3	NS
Exercise, 15 min	25.1±2.6	24.0±3.6	NS
Exercise, 60 min	24.3±4.6	20.8±2.5	NS
Glucose 6-phosphate			
Rest	1.00±0.28	1.58±0.40	<0.05
Exercise, 15 min	1.99±1.15	6.03±1.25	<0.001
Exercise, 60 min	2.03±0.92	3.03±1.71	NS

Values are means ± SD expressed as mmol/kg dry muscle. *P*, significance of difference between values for trained and untrained subjects (NS, not significant).

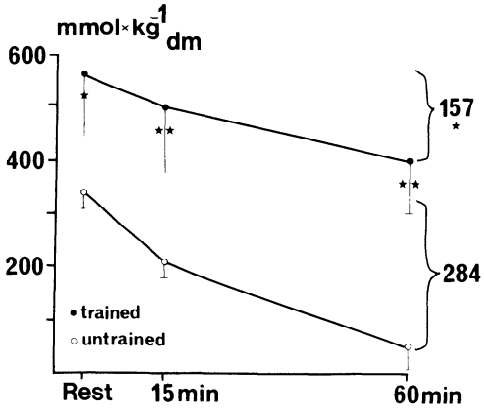

FIG. 3. Muscle glycogen concentration in vastus lateralis at rest and during exercise in untrained (*open circles*) and trained (*filled circles*) subjects. All statistical comparisons are between untrained and trained subjects: * $P < 0.05$; ** $P < 0.01$.

mmol·kg dry wt^{-1}·min^{-1}. The lactate concentration was insignificantly increased in the trained but fourfold increased above initial value in the untrained subjects by the end of the exercise period (Table 2). The citrate concentration at rest, as well as after 15 and 60 min of exercise, was significantly higher in the trained than in the untrained subjects, with a similar relative increase during exercise in the two groups (Table 2). ATP concentration was significantly decreased at the end of exercise in the untrained subjects but not in the trained subjects (Table 2). After 15 min of exercise G-6-*P* concentration was significantly lower in trained than in untrained subjects. This difference was almost eliminated by the end of exercise (Table 2).

Relationship between blood lactate and muscle enzyme levels. There was an inverse correlation between arterial blood lactate concentration during exercise and the ratio SDH/PFK or the percent type I fibers in biopsies from the resting leg muscle (Fig. 4).

DISCUSSION

In the present study, loads were selected to correspond to 65% of individual $\dot{V}O_{2\,max}$, as determined from a normal relationship between $\dot{V}O_2$ and load (1). The actual relative $\dot{V}O_2$ during the experiment was found to be 64% of $\dot{V}O_{2\,max}$ in the trained and 68% in the untrained (mean of measurements at 15 and 60 min). The insignificantly lower relative $\dot{V}O_2$ in the cyclists corresponded to a higher mechanical efficiency (22 vs. 19%), not surprising considering their bicycle training background. The difference in relative $\dot{V}O_2$ was not significant, however, and consequently it was possible to compare the groups at the same fraction of individual $\dot{V}O_{2\,max}$. Likewise, the loads selected also produced the same relative heart rate (% of HR_{max}) in the later phase of exercise in the two groups, although the relative heart rate had tended to be slightly higher in the untrained subjects in the early phase. The work intensity per kilogram active muscle (for the calculation of active muscle mass, see below) was calculated to be 21 W for both the trained and untrained subjects. Thus the subjects were compared at a corresponding activity level in this respect as well.

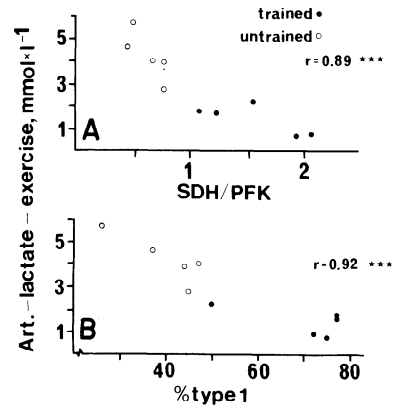

FIG. 4. Relationship between arterial blood lactate concentration during exercise and ratio of succinate dehydrogenase to phosphofructokinase (SDH/PFK) or percent type I fibers in vastus lateralis of untrained and trained subjects. Significant coefficient of correlation (*r*): *** $P < 0.001$.

Calculation of active leg muscle mass and leg blood flow. The contribution of muscle glycogen to the oxidative metabolism, as calculated from R and the glucose extraction, gave the values 48% for the untrained and 38% for the trained subjects (based on the mean of 15- and 60-min exercise values). The rate of total glycogen consumption in the leg was calculated from R and leg substrate exchange as follows: the fraction of oxidative metabolism covered by carbohydrate was estimated from R, and the fraction thereof covered by glucose calculated from the arteriovenous difference of glucose multiplied by the O_2 equivalent of glucose. Additional carbohydrate oxidized was considered to be glycogen. To this was added the additional utilization detected as a venoarterial difference of lactate. The total one-leg utilization was then calculated taking into account the leg blood flow, 4.3 for the untrained and 5.5 l/min for the trained subjects, as estimated from pulmonary $\dot{V}O_2$ and leg arteriovenous O_2 difference according to Jorfeldt and Wahren (19). This gives an absolute glycogen consumption rate of 2.8 (1 leg) for the trained and 3.0 mmol glucose units/min for the untrained subjects.

From the biopsies the measured mean glycogen consumption rate per kilogram muscle (between 15 and 60

min) was 0.52 for the trained and 0.82 mmol·kg wet $wt^{-1} \cdot min^{-1}$ for the untrained subjects. Recalculation into kilograms of wet weight from measurements in dry weight was performed, assuming 77% water in the muscle tissue. By dividing the value for one leg glycogen consumption as estimated above with the measured glycogen consumption per kilogram muscle, it is possible to make an approximate estimation of the active muscle mass if it is assumed that glycogen utilization in vastus lateralis is representative of the whole active leg musculature. The mass would then be 5.4 for the trained and 3.5 kg/leg for the untrained subjects.

Aerobic vs. anaerobic metabolism. Both smaller muscle lactate accumulation in and lactate release from the exercising legs in the trained subjects suggest that a greater proportion of the ATP regeneration occurred through oxidative processes in accordance with previous findings (10).

Plasma FFA vs. intramuscular triglyceride utilization. As in previous studies (10, 22), R as well as the leg RQ tended to be lower in the trained than in the untrained subjects. When R and RQ were combined the difference was significant. This suggests a greater contribution of fat to oxidative metabolism. A difference in fat oxidation could not be explained by a difference in plasma FFA utilization, since the FFA extraction did not differ between the groups (for explanation see Fig. 5), and, in addition, turnover rates of FFA were the same in the trained and the untrained subjects. The greater contribution of fat oxidation in the cyclists must then have been met by a greater utilization of intramuscular triglycerides (TG). As calculated from RQ (combined R and leg RQ) and the leg FFA extraction, the oxidation of TG would have made a net contribution of 34% in the trained and 15% in the untrained subjects of the total oxidative metabolism (based on 60-min exercise values; for explanation see Fig. 5). These values include a possible contribution from plasma. However, previous studies strongly indicate that such a contribution is very small (26). The rate of muscle consumption was calculated using the values given above for the relative contribution of muscle TG to the oxidative metabolism and the calculated leg blood flow values, as will be explained below. The one-leg muscle TG consumption was calculated to be 0.21 and 0.06 mmol TG/min for the trained and the untrained, respectively. From the estimated values for the active muscle mass, the TG consumption per kilogram active muscle over 60 min of exercise would have been 1.8 and 1.3 mmol TG/kg wet wt for the trained and the untrained subjects, respectively. Recently, Hurley et al. (16) showed a greater utilization of muscle TG during exercise of the same absolute intensity in the trained compared with the untrained state. The above-calculated magnitude of TG consumption, i.e., 1.3–1.8 mmol TG/kg wet wt, during 60 min of exercise is very close to that measured in biopsies by others during similar type and duration of exercise (5, 16, 31).

Similar total turnover rates in trained (1.10 mmol/min) as in untrained (1.14 mmol/min), but a 36% higher $\dot{V}O_2$ in the cyclists, would suggest either a smaller relative contribution of FFA to the oxidative metabolism in the trained group or that fatty acids had been mobilized for a purpose other than oxidation in the untrained group. The leg uptake of FFA was of a magnitude that would have covered 18% in the untrained and 19% of the oxidative metabolism in the trained subjects. If it is assumed that the FFA extraction in the legs is representative for the total working muscle mass, a total of 1.06 mmol/min FFA (96% of the FFA turnover) would have been taken up in the musculature of the trained and 0.74 mmol/min FFA (65% of the FFA turnover) in the untrained subjects. Thus, in addition to the difference in muscle substrate utilization between untrained and trained, these two groups also differ in so far as in the trained subjects all the fatty acid mobilized is taken up in the working musculature, whereas in the untrained only 65% of it is taken up. This value of 65% agrees well with what has been found in a previous study of untrained young men exercising slightly longer, 2 h at a slightly lower load, 40% of $\dot{V}O_{2\,max}$ (20).

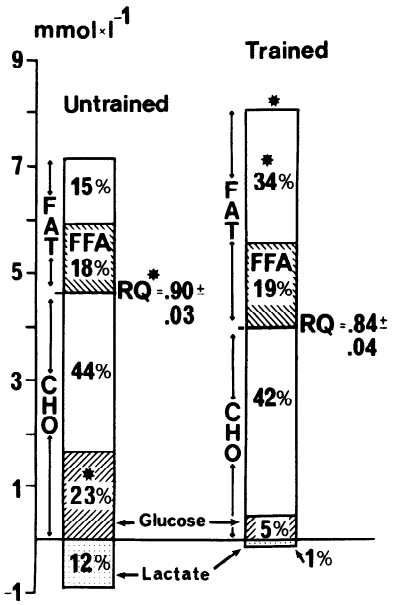

FIG. 5. Estimated contribution of fat and carbohydrate (CHO) to oxidative metabolism in untrained and trained subjects at 60 min of exercise. RQ indicates mean of respiratory exchange ratio and leg respiratory quotient. *Height of bars* indicates leg O_2 extraction [brachial arterial-femoral venous (a-fv) difference, 60-min exercise values]. Blood glucose and plasma free fatty acid (FFA, 60-min values) contributions were calculated from measured a-fv differences and O_2 equivalent of substrates (*hatched areas of bars*). *Upper and lower white areas of bars* represent that relative proportion of total O_2 consumption that would have been covered by other substrates, i.e., mainly triglycerides and glycogen oxidation, respectively. *Dotted areas* indicate lactate release (a-fv difference at 60 min of exercise). All statistical comparisons are between untrained and trained subjects: * $P < 0.05$. *Star above bar* for trained subjects denotes a significant difference in leg O_2 extraction between trained and untrained subjects.

Blood glucose vs. muscle glycogen utilization. The leg extraction of blood glucose and the muscle glycogen concentration was determined after 15 and 60 min of exercise. The rate of muscle glycogen utilization expressed per kilogram muscle as measured from biopsies was lower for the trained subjects during the whole exercise period, although the difference between trained and untrained was larger during the first 15 min than the rest of the exercise period. It is of note that the

preexercise muscle glycogen concentration was ~50% higher in the trained subjects and that their 60-min value was of similar magnitude as the preexercise value for the untrained (see Fig. 3). For the untrained, on the other hand, the glycogen stores were almost depleted after 60 min of exercise. Thus the trained subjects not only have the advantage of a lower utilization rate but also of a higher preexercise concentration of glycogen. One reason why the difference between the groups in rate of glycogen utilization decreased with time may well have been that the untrained subjects approached total depletion. In regard to the blood glucose extraction a different time course was seen for trained and untrained. After 15 min of exercise the a-fv differences were similar for the two groups, whereas after 60 min of exercise they had decreased for the trained and increased for the untrained. If the leg extraction of blood glucose at 60 min was expressed as the fraction of the total oxidative metabolism that it could have covered, it was 5% for the trained and 23% for the untrained subjects. It is possible that blood glucose extraction was increased in the untrained subjects because of their low muscle glycogen concentration. Similar results demonstrating an inverse relationship between blood glucose extraction and muscle glycogen concentration have been shown earlier (5, 7). The low dependence on blood glucose for muscle substrate provision in the trained subjects in the later phase of work may have been the reason why they, unlike the untrained subjects, were able to maintain, or even increase, their arterial glucose concentration throughout the duration of exercise.

Oxidative enzymes and substrate utilization. There are several reasons to believe that the relatively greater proportion of oxidative ATP regeneration in the trained subjects could be due to the increased activities of oxidative enzymes in their leg musculature (8, 15). In the present study the activity of SDH was 100% larger in the trained than in the untrained subjects at the same time that the $\dot{V}O_{2\,max}$ was only 50% higher. The subjects were compared at the same fraction of $\dot{V}O_{2\,max}$. If instead, the actual work load is related to the level of oxidative muscle enzymes, the relative load would be lower in the trained than in the untrained subjects.

It is less clear, however, why the muscle cell would prefer acetyl-CoA derived from fat rather than from carbohydrates. In earlier studies the citrate concentration in the cytosol has been suggested as a link between fat and carbohydrate metabolism by being an inhibitor of PFK (6, 29). In the present study both the basal concentration of muscle citrate and the absolute increase during exercise was higher in the trained subjects (see Table 2). Although it was not possible to differentiate between intra- and extramitochondrial citrate in the present study, part of the measured citrate may have been cytoplasmic producing an inhibition of PFK. Earlier studies have shown that there is a continuous release of citrate from muscle to blood both at rest and during submaximal exercise which indicates a citrate exchange between mitochondria and cytoplasm (5, 18). An accumulation of citrate in the cytoplasm, as indicated in the present study, would have inhibited PFK, thereby contributing to further retard carbohydrate relative to fat utilization.

In conclusion, during exercise at the same fraction of $\dot{V}O_{2\,max}$ a relatively greater proportion of ATP regeneration occurred oxidatively in the trained than in the untrained subjects. Furthermore, the trained subjects oxidized more fat in relation to carbohydrates, yet no difference was found in the leg extractions of plasma FFA. This suggests a greater muscle TG utilization in the trained than in the untrained subjects. The resulting carbohydrate-sparing effect applied not only to muscle glycogen but also to blood-borne glucose, thereby making it possible for the well-trained subjects to maintain their arterial glucose concentration throughout the exercise period.

This study was supported by grants from the Research Council of the Swedish Sport Federation and Swedish Medical Research Council Grants 204 and 4494.

Received 21 January 1986; accepted in final form 29 September 1986.

REFERENCES

1. ÅSTRAND, P.-O., AND K. RODAHL. *Textbook of Work Physiology.* New York: McGraw-Hill, 1977, p. 334, 341.
2. BERGSTRÖM, J. Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest. Suppl.* 68: 1–110, 1962.
3. DRABKIN, D. L., AND C. F. SMIDT. Spectrophotometric studies. *J. Biol. Chem.* 157: 69–83, 1945.
4. ENGEL, W. K. The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology* 12: 778–784, 1962.
5. ESSÉN, B., L. HAGENFELDT, AND L. KAIJSER. Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. *J. Physiol. Lond.* 265: 489–506, 1977.
6. GARLAND, P. B., P. J. RANDLE, AND E. A. NEWSHOLME. Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes and starvation. *Nature Lond.* 200: 169–170, 1963.
7. GOLLNICK, P. D., B. PERNOW, B. ESSÉN, E. JANSSON, AND B. SALTIN. Availability of glycogen and plasma FFA for substrate utilization in leg muscle of man during exercise. *Clin. Physiol. Oxf.* 1: 27–42, 1981.
8. GOLLNICK, P. D., AND B. SALTIN. Significance of skeletal muscle oxidative enzymes with endurance training. *Clin. Physiol. Oxf.* 2: 1–12, 1982.
9. HÄGGMARK, T., E. JANSSON, AND E. ERIKSSON. Fibre type area and metabolic potential of the thigh muscle in man after surgery and immobilization. *Int. J. Sport Med.* 2: 12–17, 1981.
10. HENRIKSSON, J. Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J. Physiol. Lond.* 270: 661–675, 1977.
11. HERMANSEN, L., E. HULTMAN, AND B. SALTIN. Muscle glycogen during prolonged exercise. *Acta Physiol. Scand.* 71: 129–139, 1967.
12. HO, R. J. Radiochemical assay of long chain fatty acids using ^{63}Ni as tracer. *Anal. Biochem.* 36: 105–113, 1970.
13. HOHORST, H. J. L(+) Lactatbestimmung mit Lactat Dehydrogenas und DPN. In: *Metoden der Enzymatischen Analyse.* Weinheim, FRG: Verlag Chemie, 1962, p. 226–270.
14. HOLLOSZY, J. O. Adaptation of skeletal muscle to endurance exercise. *Med. Sci. Sports* 7: 155–164, 1975.
15. HOLLOSZY, J. O., AND E. F. COYLE. Adaptations of skeletal muscle to endurance training and their metabolic consequences. *J. Appl. Physiol.* 56: 831–838, 1984.
16. HURLEY, B. F., P. M. NEMETH, W. H. MARTIN III, J. M. HAGBERG, G. P. DALSKY, AND J. O. HOLLOSZY. Muscle triglyceride utilization during exercise: effect of training. *J. Appl. Physiol.* 60: 562–567, 1986.
17. JANSSON, E. On the significance of the respiratory exchange ratio after different diets during exercise in man. *Acta Physiol. Scand.* 114: 103–110, 1982.

18. JANSSON, E., AND L. KAIJSER. Leg citrate metabolism at rest and during exercise in relation to diet and substrate utilization in man. *Acta Physiol. Scand.* 122: 145–153, 1984.
19. JORFELDT, L., AND J. WAHREN. Leg blood flow during exercise in man. *Clin. Sci. Oxf.* 41: 459–473, 1971.
20. KAIJSER, L., B. W. LASSERS, M. L. WAHLQVIST, AND L. A. CARLSON. Myocardial lipid and carbohydrate metabolism in fasting men during prolonged exercise. *J. Appl. Physiol.* 32: 847–858, 1972.
21. KARLSSON, J., L.-O. NORDESJÖ, AND B. SALTIN. Muscle glycogen utilization during exercise after physical training. *Acta Physiol. Scand.* 90: 210–217, 1974.
22. KOIVISTO, V., R. HENDLER, E. NADEL, AND P. FELIG. Influence of physical training on the fuel-hormone response to prolonged low intensity exercise. *Metab. Clin. Exp.* 31: 192–197, 1982.
23. LASSERS, B. W., L. KAIJSER, AND L. A. CARLSSON. Myocardial lipid and carbohydrate metabolism in healthy men at rest: studies during continuous infusion of ^{3}H-palmitate. *Eur. J. Clin. Invest.* 2: 348–358, 1972.
24. LOWRY, C., J. KIMMEY, S. FELDER, M. CHI, K. KAISER, P. PASSONNEAU, K. KIRK, AND O. LOWRY. Enzyme patterns in single human muscle fibers. *J. Biol. Chem.* 253: 8269–8277, 1978.
25. LOWRY, O. H., AND J. V. PASSONNEAU. *A Flexible System of Enzymatic Analysis.* New York: Academic, 1972.
26. OLSSON, A. G., B. EKLUND, L. KAIJSER, AND L. A. CARLSON. Extraction of endogenous plasma triglycerides by the working human forearm muscle in the fasting state. *Scand. J. Clin. Lab. Invest.* 35: 231–236, 1975.
27. OLSSON, A. G., B. EKLUND, G. RICCARDI, G. WALLDIUS, AND L. KAIJSER. Free fatty acid and carbohydrate metabolism of the working forearm muscle in type IV hyperlipoproteinaemia. *Eur. J. Clin. Invest.* 7: 549–556, 1977.
28. PETERS, P. P., AND D. V. VAN SLYKE. *Quantitative Clinical Chemistry.* London: Ballière, Tindal, & Cox, 1932, vol. II.
29. RENNIE, M. J., AND J. O. HOLLOSZY. Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. *Biochem. J.* 168: 161–170, 1977.
30. SCHOLANDER, P. F. Analyzes for accurate estimation of respiratory gases in one-half cubic centimeters samples. *J. Biol. Chem.* 167: 235–250, 1947.
31. SHERMAN, W. M., AND D. L. COSTILL. The marathon: dietary manipulations to optimize performance. *Am. J. Sports Med.* 12: 44–51, 1984.