

SKELETAL MUSCLE SUBSTRATE UTILIZATION DURING SUBMAXIMAL EXERCISE IN MAN: EFFECT OF ENDURANCE TRAINING

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SUMMARY

1. The influence of training-induced adaptations in skeletal muscle tissue on the choice between carbohydrates (CHO) and lipids as well as the extra- *vs.* intracellular substrate utilization was investigated in seven healthy male subjects performing one-legged knee-extension exercise. In each subject one of the knee extensors was endurance trained for eight weeks, whereafter the trained (T) and non-trained (NT) thighs were investigated a week apart.

2. The activity of β -hydroxy-acyl-coenzyme A dehydrogenase (HAD) and capillary density in the knee extensors were significantly larger in T than in NT.

3. During dynamic knee-extension exercise, performed at the same absolute intensity for 2 h, femoral venous blood flow was lower in T than in NT ($P < 0.05$), but oxygen uptake was similar.

4. Respiratory quotient (RQ) values over the exercising thigh, averaging 0.81 (T) *vs.* 0.91 (NT; $P < 0.05$) indicated that a shift towards a larger fat combustion occurred with endurance training.

5. Both free fatty acids (FFA) and serum triacylglycerol contributed to the utilization of fat in NT and T muscles with no significant contribution from muscle fibre triacylglycerol.

6. At high plasma FFA concentrations net uptake of FFA plateaued in NT but not in T muscles.

7. The findings suggest that FFA uptake in exercising muscle is a saturable process and that the transport capacity is enhanced by training. The lower CHO utilization in the T leg was mainly a function of the glycogenolysis of the muscle being reduced. Hormones such as insulin, noradrenaline and adrenaline are unlikely to play a role in this shift as differences in plasma levels during T and NT leg exercise were small and insignificant, implying that local structural and functional adaptations of the training muscle are crucial for the observed shifts in the metabolic response to exercise.

INTRODUCTION

The relative utilization of lipid and carbohydrate in exercising muscle is determined by a complex system of regulatory mechanisms, and so is the preference of the muscle in using extra- *vs.* intramuscular energy sources. Following physical training the metabolic response to exercise is changed; for instance lower respiratory quotient (RQ) values during submaximal exercise are found in trained individuals compared to untrained indicating a metabolic shift towards a larger lipid oxidation (Christensen & Hansen, 1939). Such a contention has been supported by the findings of a higher net uptake of free fatty acids and a lower release of lactate by the trained compared with the non-trained leg during exercise (Henriksson, 1977). It has been suggested that the shift towards a higher lipid metabolism with training is a function of an increased muscle oxidative capacity (Henriksson, 1977; Gollnick & Saltin, 1982). One of the limitations in experiments in man is that the metabolic response observed during exercise represents the composite picture of the whole body i.e. not only the muscle tissue, but also all other tissues and organs of the body contribute to the whole-body response. An investigation of a single muscle group allows a more direct characterization of muscle metabolism and the local effect of exercise training. Thus, in the present study, exercise was confined to the knee extensors of one leg. Furthermore, one of the knee extensors was endurance trained for 8 weeks.

As the physiological effects of a single exercise session may last several days, responses in a trained muscle may, to some extent, be due to the last work bout. One way to deal with the problem of acute exercise effects when comparing trained and non-trained muscle groups is to subject both the non-trained control and the trained muscle to a single exercise session of the same intensity and duration and then study each muscle after a given period of recovery time. Such an approach was taken in the present study in which the non-trained and the trained muscle groups were studied 22 h after an exercise session.

METHODS

Experimental model

A modified Krogh bicycle ergometer for knee-extension exercises was used (Andersen, Adams, Sjøgaard, Thorboe & Saltin, 1985). The subjects were seated in an exercise chair and secured with support straps to maintain a uniform and relaxed body position throughout the exercise. The dynamic work was performed with sixty contractions per minute, with the contraction causing the lower part of the leg to move from about 90 to 170 deg. During exercise all four heads of the muscle group are activated. In contrast to the observed EMG activity of the knee extensors very little or no EMG activity was measured in other leg muscles, such as the gluteal muscles, or in the abdominal and back muscles. This is in accordance with the findings of Andersen *et al.* (1985).

Subjects

Seven healthy men participated in the study. Their age was 21–26 years, weight 69–82 kg, and maximal pulmonary oxygen uptake (\dot{V}_{O_2}) during two-legged cycle ergometer exercise was between 3.35 and 3.84 l min⁻¹.

Each subject was informed about the procedures and the risks involved in the experiment before giving oral consent to participate. The study was approved by the Copenhagen Ethics Committee.

Preliminary experiments

Initially, the subjects were familiarized with the exercise model to learn the cadence and to relax the non-involved muscles. In order to define the maximal work load and peak pulmonary oxygen uptake for the knee extensors, a maximal test was performed with each leg, separately. The test

started with exercise at a low work load and increased by 10 W increments. Pulmonary oxygen uptake increased linearly with increasing work load until various muscle groups were recruited to stabilize the body and the hip during exercise at which point pulmonary oxygen uptake increases sharply (Andersen *et al.* 1985). This point is defined as peak oxygen uptake for the knee extensors, and the corresponding work load as peak work load.

Training

Training consisted of dynamic knee extensor exercise. During the first 3 weeks of training the subjects exercised 3 days per week with the duration of each session increasing from $\frac{1}{2}$ to $1\frac{1}{2}$ h. From week 4 the subjects exercised 4 days per week, 2 h each session, and this was maintained during the final 5 weeks. The work load was progressively increased in relation to the changes in maximal performance assessed every 2 weeks, thus keeping the work load at 65% of peak \dot{V}_{O_2} for the knee extensors.

Each training session was supervised and heart rate, pulmonary oxygen uptake and blood pressure were measured systematically. The subjects did not participate in any other physical training activities during the study.

Post-training experiments

The first day after the last training session (D1) the subjects consumed a controlled diet which consisted of $37.0 \pm 2.5\%$ energy from fat, $47.0 \pm 1.8\%$ energy from carbohydrates, and $16.0 \pm 1.3\%$ energy from proteins (means \pm s.e.m.). The mean ratio between polyunsaturated and saturated fatty acids was 0.54 ± 0.08 and total energy intake was 11.7 ± 1.1 MJ. No alcohol was allowed. The controlled diet was chosen to be similar to their normal eating habits. Physical training was not performed during D1.

On the next day (D2) the subjects arrived in the morning after an overnight fast of 10–12 h. After 30 min of rest, resting metabolic rate (RMR) was determined from pulmonary oxygen uptake. A biopsy was taken from m. vastus lateralis for histochemical and enzyme analysis. Then the subjects performed knee-extension exercise for 2 h at a work level demanding 65% of maximal work capacity for the non-trained muscle group. The same dietary regime as on D1 was followed during the rest of the day. No more exercise was performed during the rest of D2.

On day 3 – the experimental day (D3) – the subjects arrived in the morning after an overnight fast of 10–12 h. After 30 min of rest, RMR was determined from pulmonary oxygen uptake. The femoral artery and vein were catheterized 2 and 4 cm distal to the inguinal ligamentum, respectively, and the catheters were advanced proximally. A thermistor catheter (Edslab TD-probe) was inserted 8–10 cm proximally through the venous catheter for the measurement of blood flow (Andersen & Saltin, 1985). After insertion of the catheters the subjects rested for 45 min in the supine position. After another 15 min of rest in the sitting position resting blood samples were drawn from the femoral artery and vein simultaneously. A muscle biopsy was taken from m. vastus lateralis for histochemical and biochemical analyses. The subjects were then seated in the knee-extension chair to perform 2 h of work at the same work load as on D2. Arterial and venous blood samples were drawn simultaneously, and pulmonary oxygen uptake was measured at 10, 60, and 120 min of exercise. Heart rate and blood pressure were continuously recorded. A cuff was placed just below the knee of the working leg. It was inflated (280 mmHg) during each period of measurement of blood flow and blood sampling. At termination of work, another biopsy was taken from m. vastus lateralis.

D1, D2, and D3 were repeated 4 days later with the knee extensor muscle of the other leg, exercising at the same absolute work load, following the same protocol. The trained leg was tested first in four and the non-trained leg first in three subjects. When the non-trained muscle was tested first, this was done during the last week of the training period.

Blood analyses

Plasma free fatty acids (FFA) were determined by an enzymatic micromethod in accordance with Shimizu, Inoue, Tani & Yamada (1979) with some modifications. The principle of the method is based on the activation of free fatty acids by acyl coenzyme A (CoA) synthetase. The enzyme catalyses activation of long-chain fatty acids in the presence of ATP and CoA with the production of AMP. The reagent solution contained (given in final concentrations): 50 mM Tris buffer, pH 8.0, 1% Triton X-100, 2 mM $MgCl_2$, 200 μM EDTA, 50 μM ATP, 10 mM phosphoenolpyruvate, 300 μM NADH, 5 mM 2-mercaptoethanol, 5 U ml^{-1} lactate dehydrogenase, 4 U ml^{-1} pyruvate kinase, 4 U ml^{-1} adenylate kinase (myokinase), and 0.06 U ml^{-1} acyl CoA synthetase.

The sampled blood was added to 25 μl of 200 mM EGTA, pH 7.0, centrifuged and the plasma stored in ice-cold water until analysed. Fresh plasma was used in the assay. The EGTA was added to ensure no FFA precipitation with Ca^{2+} . Plasma (90 μl) was added to 700 μl reagent solution. The reaction was initiated by an addition of 15 μl of 12 mM CoA synthetase (final concentration 220 μM). After incubation at 25 °C for 2 h the samples were read in a fluorometer against a blank of 10% bovine serum albumin (BSA; fatty acid free). Recovery experiments were made using a palmitic acid standard and plasma. Standards added to plasma showed a 96% recovery. Carboxylic acids containing up to eight carbon atoms are not measured by this method.

Very low density lipoprotein (VLDL) in serum was isolated in an ultracentrifuge at density 1.006, then analysed for triacylglycerol using an enzymatic method (Boehringer/Mannheim, West Germany) in a Multistat III (Instrumentation Laboratories, Lexington, MA, USA) and for free glycerol.

Glucose and lactate were determined by standard enzymatic fluorometric methods as described by Lowry & Passonneau (1972). β -Hydroxybutyrate and glycerol were analysed in accordance with Bergmeyer, Dermot & Williamson (1974) and Wieland (1974) adjusted to fluorometric assays.

Oxygen and carbon dioxide content of the blood was determined by the technique of Van Slyke & Neill (1924). Blood oxygen saturations and haemoglobin were measured on an OSM-2 hemoximeter (Radiometer, Copenhagen). The accuracy of the method for saturation determinations was frequently verified by determinations of oxygen content on a Van Slyke apparatus. Moreover, the OSM-2 was calibrated with fully oxygenated blood samples analysed spectrophotometrically by the cyan-methaemoglobin method (Drabkin & Austin, 1935). Triple micro-capillary haematocrit tubes were centrifugated. Insulin in serum was assayed using a modified radio-immunosorbent technique (Phadebas, Pharmacia Diagnostics AB, Uppsala, Sweden).

Adrenaline and noradrenaline were measured by a single isotope derivative assay (Christensen, Vestergaard, Sørensen & Rafaelsen, 1980).

Muscle biopsy analysis. A piece of each muscle biopsy was immediately freed from blood and visible connective tissue, rapidly frozen in liquid nitrogen, and stored at -80 °C for subsequent biochemical analysis. The remaining muscle was mounted in embedding medium, frozen in isopentane, cooled to its freezing point in liquid nitrogen, and then stored at -80 °C for subsequent histochemical analysis. Serial transverse sections (10 μm) were cut with a microtome at -20 °C and stained for myofibrillar ATPase to identify fibre types (Brooke & Kaiser, 1970) and with the amylose-periodic acid-Schiff reagent (PAS) method to visualize capillaries (Andersen, 1975). Fibre area and capillary density were calculated (Andersen & Henriksson, 1977). Serial sections were stained for glycogen using the PAS reaction (Pearse, 1968). The relative glycogen content of individual muscle fibres was estimated from PAS staining intensity and glycogen depletion pattern was evaluated (Gollnick, Piehl & Saltin, 1974). For muscle enzyme and substrate assays the muscle samples were weighed before and after freeze-drying to determine the total water content. The freeze-dried muscle tissue was placed under a dissection microscope in a room where the temperature was 20 °C and relative humidity below 30%, and thoroughly dissected free of all visible connective tissue, fat and blood. The remaining muscle fibres were pooled and used for assays. Citrate synthase (CS), β -hydroxyacyl-CoA dehydrogenase (HAD), and α -glyceraldehyde-phosphate dehydrogenase (GAPdH) were determined fluorometrically (Lowry & Passonneau, 1972). The triacylglycerol (TG) content of pooled muscle fibres was determined by extraction of neutral fats with a Folch extract (Folch, Lees & Sloane Stanley, 1957). The chloroform phase was retained and after evaporation the glycerol content was measured (Chernick, 1969). Glycogen was determined as glucose residues, after hydrolysis of the muscle specimen in 1 M HCl at 100 °C for 2 h (Lowry & Passonneau, 1972).

Blood flow. This was measured by a thermodilution technique. At rest, a bolus injection of ice-cold saline was used (Gaffney, Sjøgaard & Saltin, 1990), whereas constant infusion of ice-cold saline was used during the exercise (Andersen & Saltin, 1985).

Muscle mass. Thigh volume (V) was calculated based on measurements of the thigh: three circumferences and three skin-fold measurements of the thigh (Jones & Pearson, 1969). The quadriceps femoris muscle mass (M) was then calculated as:

$$M = 0.307 \times V + 0.353$$

($n = 12$, $r = 0.93$, $P < 0.001$; autopsy study, O. Halskov, personal communication). This

anthropometric approach gave values similar to those from estimations based on multiple CAT scans (Saltin, 1985).

Pulmonary oxygen uptake. This was measured during rest and exercise by collecting expired air in Douglas bags. A Tissot spirometer was used for volume analysis and O₂ and CO₂ content of the exercise bags were determined with a paramagnetic (Servomex) and infrared (Beckman LB-2) system, respectively. O₂ and CO₂ content of the expired air in the bags collected at rest were analysed by the Scholander micro-technique.

Blood pressure. This was measured through the arterial catheter connected to a Gould pressure transducer.

Heart rate. Recordings were made of the ECG on a Siemens Elema Mingograph.

Calculations. Uptake and release of substrates were calculated from femoral arterial and venous differences multiplied by plasma, or blood flow. Substrate oxidation was estimated in accordance with Frayn (1983) and unbound FFA concentrations were calculated in accordance with Abumrad, Perkins, Park & Park (1981) utilizing the association constants given by Spector (1975).

Statistics. Results in the text, tables and figures are given as means \pm S.E.M. or means and range. Data were analysed by non-parametric statistics using the paired Wilcoxon signed rank test and Friedmans two-way analysis of variance.

RESULTS

Muscle adaptations

Capillary density

Capillary density averaged 343 capillaries mm⁻² muscle (276–403) in NT and 417 (350–497) in T ($P < 0.05$). An increased capillarization was observed for all three fibre types and the number of capillaries per fibre was 1.79 in NT and 2.25 in T ($P < 0.05$). Relative occurrence of fibre types and fibre areas were similar in the two legs.

Enzyme activities

Lower activities of β -hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase were found in NT compared with T ($P < 0.05$). In contrast, measurements of α -glyceraldehydephosphate dehydrogenase (GAPdH) revealed similar values in both (see Table 1).

Peak muscle oxygen uptake

After 8 weeks of one-legged knee-extension exercise peak oxygen uptake of the knee extensors estimated from peak pulmonary \dot{V}_{O_2} minus RMR (≈ 0.24 l min⁻¹)

TABLE 1. Enzyme activities of β -hydroxyacyl-CoA dehydrogenase (HAD), citrate synthase (CS) and glyceraldehydephosphate dehydrogenase (GAPdH) in m. vastus lateralis in non-trained and trained thigh

	Non-trained	Trained
HAD ($\mu\text{mol g}^{-1} \text{min}^{-1}$)	48 (35–61)	66* (57–75)
CS ($\mu\text{mol g}^{-1} \text{min}^{-1}$)	51 (41–69)	64* (54–78)
GAPdH ($\mu\text{mol g}^{-1} \text{min}^{-1}$)	634 (590–699)	588 (493–667)

Values (in dry wt) are means and range.

* Indicates $P < 0.05$ between non-trained and trained thigh.

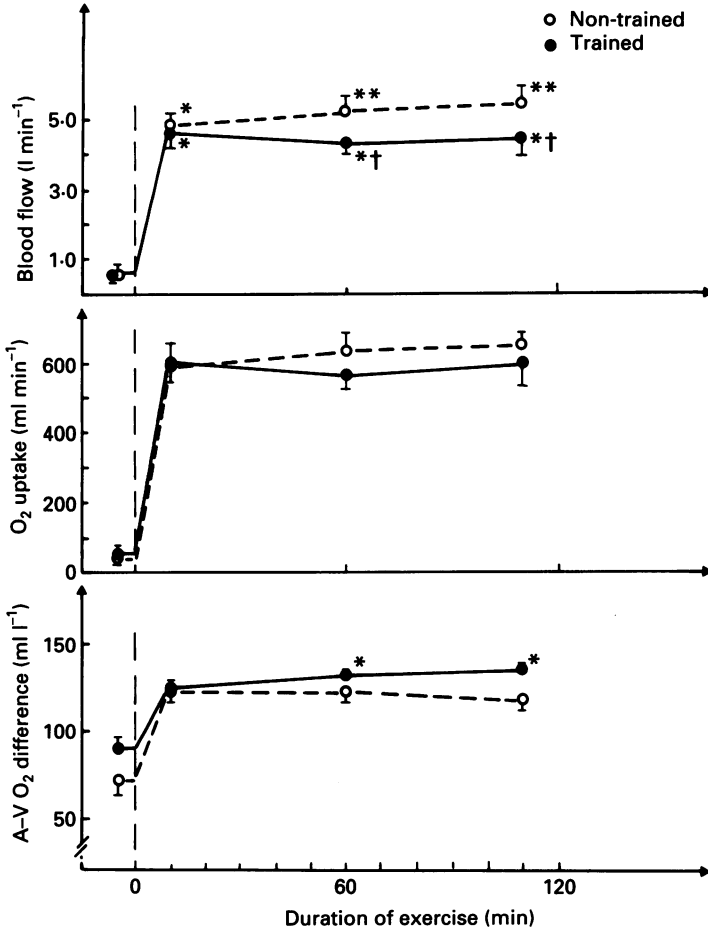


Fig. 1. Femoral venous blood flow, thigh oxygen uptake and arterial-femoral venous oxygen differences during exercise with the trained and non-trained leg (means \pm s.e.m.). * $P < 0.05$ compared to resting values; ** $P < 0.05$ compared to 10 min values; † $P < 0.05$ between trained and non-trained leg.

averaged 0.87 l min^{-1} ($0.84\text{--}0.95$) in NT and 1.06 l min^{-1} ($0.92\text{--}1.14$) in T, a difference of 24% ($P < 0.05$). Before training peak work load for the knee extensors was similar in NT and in T (45 ± 4 and 46 ± 4 W, respectively). After training peak work load in NT remained unchanged (46 ± 3 W) whereas peak work load had increased to 60 ± 5 W ($P < 0.05$) in T. Thus, peak work load was significantly higher in T than in NT. The muscle mass averaged 2.3 kg ($1.7\text{--}2.6$) and 2.4 kg ($1.7\text{--}2.8$) for NT and T, respectively, thus mean muscle \dot{V}_{O_2} peak expressed per kilogram of muscle mass was significantly higher in T ($452 \text{ ml min}^{-1} \text{ kg}^{-1}$) than in NT ($394 \text{ ml min}^{-1} \text{ kg}^{-1}$).

Experimental day (D3)

Exercise for NT and T knee extensors was at the same absolute work load (32 W; 20–40). Thus, the work load averaged 68% (60–84) for NT and 52% (47–60) for T of peak muscle \dot{V}_{O_2} for NT and T muscle, respectively.

During submaximal prolonged exercise with NT mean pulmonary oxygen uptake was 827 ml min^{-1} (623–957) after 10 min and 982 ml min^{-1} (698–1462) after 120 min ($P > 0.05$). In T pulmonary oxygen uptake was 807 ml min^{-1} (583–961) after 10 min and remained constant during the remaining exercise period. Heart rate in NT was $110 \text{ beats min}^{-1}$ at 10 min followed by a continuous increase and averaged $128 \text{ beats min}^{-1}$ at 120 min of exercise ($P < 0.05$). In T heart rate was similar to NT after 10 min ($110 \text{ beats min}^{-1}$) and remained on this level at 60 min and at 120 min.

Femoral venous blood flow in NT averaged 4.89 l min^{-1} (3.35–5.99) after 10 min and 60 min and increased to 5.49 l min^{-1} (4.14–7.56) after 120 min ($P < 0.05$) (Fig. 1). In T femoral venous blood flow averaged 4.61 l min^{-1} (2.91–6.59) after 10 min of exercise and remained unchanged throughout. Blood flow was lower in T than in NT at 60 and 120 min of exercise ($P < 0.05$) (Fig. 1). At 60 and 120 min of exercise the lower venous blood flow in T compared with NT was compensated for by a higher muscular oxygen extraction resulting in a similar oxygen uptake of the thigh in T and NT (Fig. 1). RQ determinations for the exercising thigh at 60 min were 0.89 (0.85–0.96) in NT *vs.* 0.80 (0.75–0.89) in T ($P < 0.05$), and at 120 min RQ was 0.92 (0.87–0.97) in NT and 0.82 (0.76–0.84) in T ($P < 0.05$) (Fig. 2). Similar differences were found in the respiratory exchange ratio (RER) (Fig. 2).

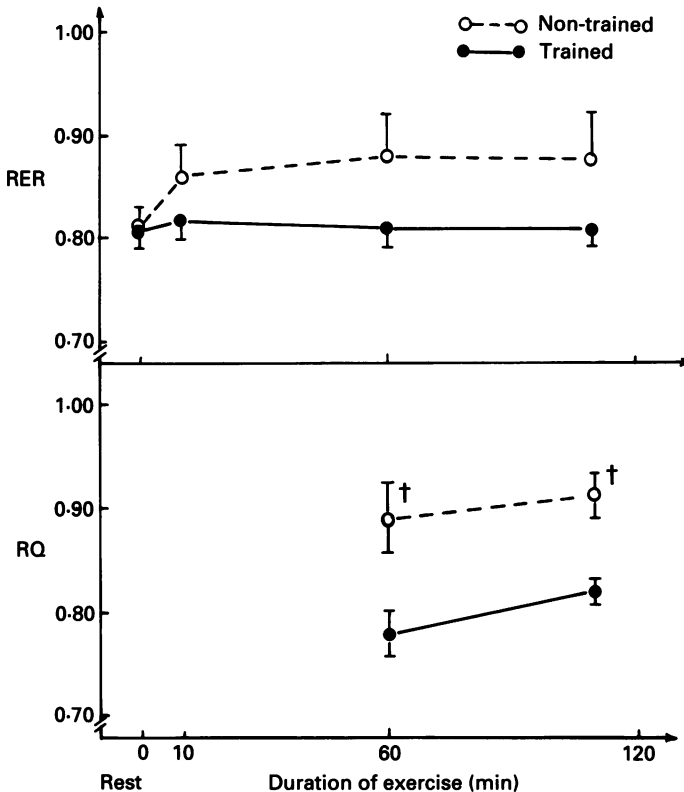


Fig. 2. Respiratory exchange ratio (RER) and respiratory quotient (RQ) across the thigh during exercise with the trained and non-trained leg (means \pm S.E.M.). For explanation of symbols see legend to Fig. 1.

Blood substrates

At rest similar values in arterial blood and plasma for substrates and metabolites were noted in both NT and T (Table 2).

TABLE 2. Arterial concentrations of substrates in non-trained and trained thigh at rest and after 10, 60 and 120 min of submaximal knee-extension exercise

	Rest	10 min	60 min	120 min
Glucose (mmol l ⁻¹)				
Non-trained	4.35 (3.73-4.61)	4.19 (3.87-4.56)	4.02 (3.45-4.48)*	3.93 (3.45-4.16)**†
Trained	4.40 (3.83-4.91)	4.23 (3.37-4.83)	4.18 (3.55-4.58)	4.11 (3.51-4.51)
Lactate (mmol l ⁻¹)				
Non-trained	0.48 (0.29-0.76)	1.35 (0.71-3.07)*†	0.64 (0.33-1.51)**	0.62 (0.43-0.83)**
Trained	0.40 (0.04-0.68)	0.66 (0.20-1.05)*	0.49 (0.03-0.68)**	0.47 (0.03-0.70)**
FFA (μmol l ⁻¹)				
Non-trained	422 (179-598)	363 (154-635)	599 (211-1313)*	795 (580-1052)**
Trained	400 (163-851)	413 (166-634)	633 (254-921)*	880 (531-1240)**
β-Hydroxybutyric acid (μmol l ⁻¹)				
Non-trained	37 (14-62)‡	46 (16-111)	72 (25-149)**†	150 (71-314)†
Trained	45 (28-232)	55 (22-259)	94 (29-310)**†	231 (112-436)†
Glycerol (μmol l ⁻¹)				
Non-trained	58 (12-205)	56 (30-340)‡	105 (56-390)**	142 (113-700)†
Trained	73 (20-205)	78 (43-390)	118 (57-540)**	145 (106-920)†
VLDL-TG (μmol l ⁻¹)				
Non-trained	361 (236-584)		415 (220-1168)	495 (264-1086)**
Trained	360 (210-560)		392 (180-655)	439 (210-761)**

Values are means and range. FFA, free fatty acids; VLDL-TG, very low density lipoprotein triacylglycerol.

* $P < 0.05$ compared to resting values; ** $P < 0.05$ compared to 10 min values; † $P < 0.05$ compared to 60 min values; ‡ $P < 0.05$ between non-trained and trained thigh.

Arterial glucose concentration at rest averaged 4.38 mmol l⁻¹ for both NT and T. During exercise a significant decrease in arterial glucose concentration only appeared in NT (Table 2). Arterial-venous (A-V) glucose differences at rest were similar in NT and T (Table 3). After 10 min of exercise the A-V glucose difference increased significantly in NT but decreased significantly in T (Table 3). Thus, glucose uptake averaged 1.41 mmol min⁻¹ at 10 min of exercise in NT, which was markedly higher than in T (0.60 mmol min⁻¹) ($P < 0.05$). A further increase in glucose uptake

was seen in T at 60 min of exercise to $1.20 \text{ mmol min}^{-1}$ ($P < 0.05$), but not in NT ($1.15 \text{ mmol min}^{-1}$). During the second hour of exercise glucose uptake was similar in both legs (Fig. 3).

Arterial lactate concentrations increased to 1.35 mmol l^{-1} in NT ($P < 0.05$) and 0.66 mmol l^{-1} in T at 10 min of exercise ($P < 0.05$, Table 2). Subsequently, the arterial lactate concentration decreased in both NT and T (Table 2). Lactate release at 10 and 60 min was significantly higher in NT than T, but similar at 120 min of exercise (Fig. 3).

TABLE 3. Arterial (A)–femoral venous (V) differences of substrates in non-trained and trained thigh at rest and 10, 60, 120 min of submaximal knee-extension exercise

	Rest	10 min	60 min	120 min
A–V				
Glucose (mmol l^{-1})				
Non-trained	0.17 (0.09–0.36)	0.27 (0.02–0.42)	0.22 (0.01–0.41)	0.28 (0.11–0.43)†
Trained	0.21 (0.13–0.48)	0.10 (0.05–0.22)*‡	0.21 (0.01–0.47)**	0.30 (0.09–0.66)
FFA ($\mu\text{mol l}^{-1}$)				
Non-trained	–74 (–3 to –66)	22 (2–40)*	34 (5–72)	28 (0–88)
Trained	–46 (–90–7)	28 (–7–66)*	44 (34–69)**	77 (50–90)†‡
Glycerol ($\mu\text{mol l}^{-1}$)				
Non-trained	–34 (–52 to –12)	–22 (–75 to –4)*	–11 (–18 to –3)	–14 (–35 to –2)
Trained	–62 (–185 to –18)‡	–36 (–140 to –3)*	–31 (–70 to –10)‡	–23 (–45 to –11)‡

Values are means and range. * $P < 0.05$ compared to resting values; ** $P < 0.05$ compared to 10 min values; † $P < 0.05$ compared to 60 min values; ‡ $P < 0.05$ between non-trained and trained thigh.

Arterial FFA concentration increased during exercise to close to $800 \mu\text{mol l}^{-1}$ at the end of exercise in both NT and T (Table 2). The A–V FFA differences rose to $21 \mu\text{mol l}^{-1}$ (NT) after 10 min of exercise ($P < 0.05$), to $34 \mu\text{mol l}^{-1}$ at 60 min and remained at this level. In T A–V FFA differences rose to $28 \mu\text{mol l}^{-1}$ after 10 min of exercise ($P < 0.05$), to $44 \mu\text{mol l}^{-1}$ at 60 min and $77 \mu\text{mol l}^{-1}$ at 120 min ($P < 0.05$, Table 3).

A similar net FFA uptake was noticed at 10 min in NT ($54 \mu\text{mol min}^{-1}$) and T ($76 \mu\text{mol min}^{-1}$) with a further rise to $71 \mu\text{mol min}^{-1}$ (NT) and to $112 \mu\text{mol min}^{-1}$ (T; $P < 0.05$) at 60 min. At 110 min of exercise no further increase was noticed in NT ($72 \mu\text{mol min}^{-1}$) whereas in T the uptake rose significantly to $199 \mu\text{mol min}^{-1}$ (Fig. 4) resulting in a larger net FFA uptake in T compared to NT ($P < 0.05$).

Arterial β -hydroxybutyric acid concentration rose gradually during exercise to $150 \mu\text{mol l}^{-1}$ (NT) and $231 \mu\text{mol l}^{-1}$ (T) at the end of exercise ($P < 0.05$, Table 2). No A–V differences were observed in either of the legs during the first hour of exercise,

whereafter A-V differences of $18 \mu\text{mol l}^{-1}$ were found after 120 min in NT and $27 \mu\text{mol l}^{-1}$ in T ($P < 0.05$). During exercise no uptake of β -hydroxybutyric acid was found until at the end of exercise, where a higher uptake was observed in T ($103 \mu\text{mol min}^{-1}$ (23-209)) than in NT ($76 \mu\text{mol min}^{-1}$ (0-92)) ($P < 0.05$).

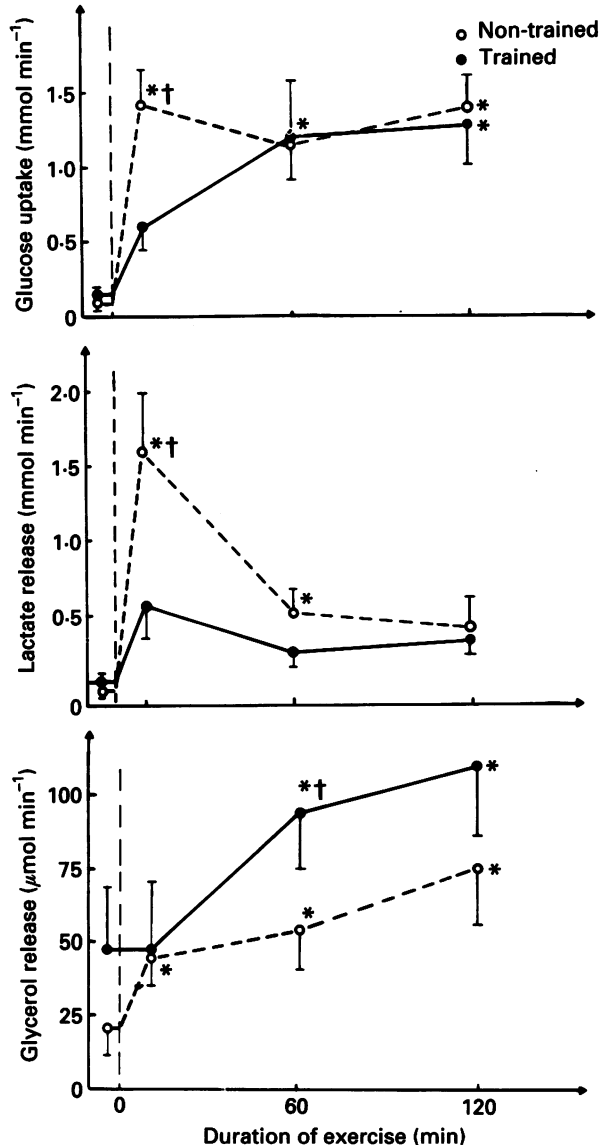


Fig. 3. Glucose uptake, lactate release and glycerol release, calculated from A-V differences multiplied by plasma or blood flow, during exercise with the trained and non-trained leg (means \pm s.e.m.). For explanation of symbols see legend to Fig. 1.

Arterial triacylglycerol content of very low density lipoproteins (VLDL-TG) increased significantly to $495 \mu\text{mol l}^{-1}$ (NT) and $439 \mu\text{mol l}^{-1}$ (T) at 120 min of exercise. A-V VLDL-TG differences at rest averaged $30 \mu\text{mol l}^{-1}$ in NT and

$55 \mu\text{mol l}^{-1}$ in T ($P < 0.05$). During exercise VLDL-TG A-V differences were not consistently detectable. However, in an effort to obtain an estimate of total degradation of VLDL-TG during exercise, the areas under the arterial and venous concentration curves were integrated from 0 to 120 min of exercise. The difference

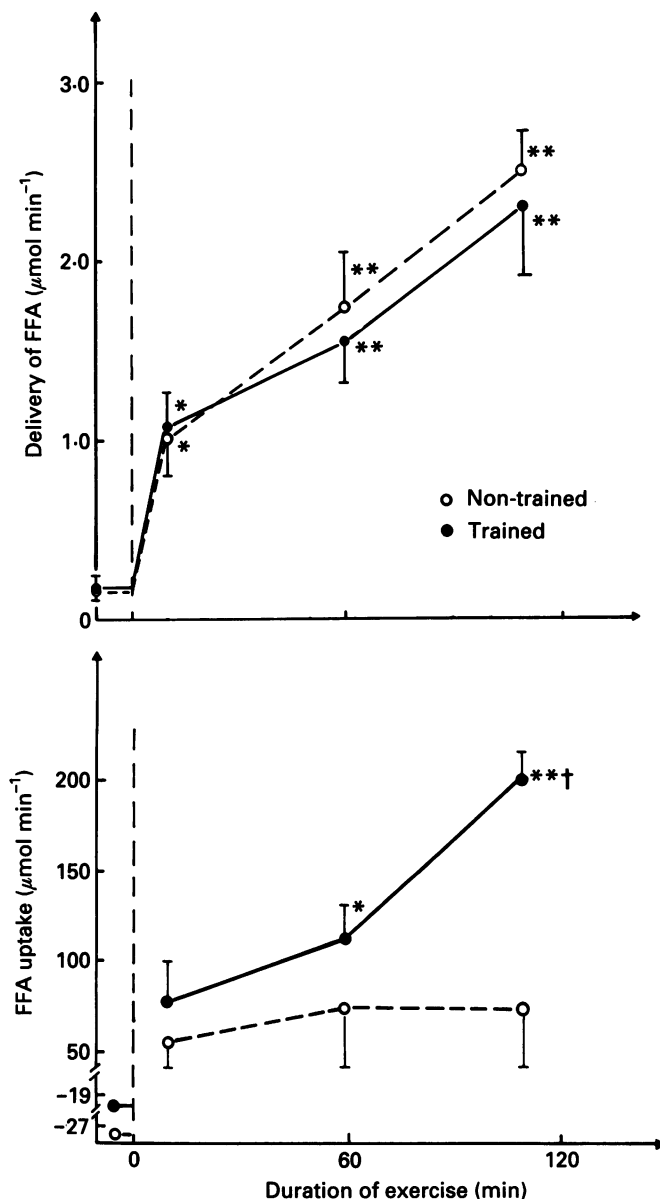


Fig. 4. FFA delivery and FFA uptake during exercise with the trained and the non-trained leg (means \pm s.e.m.). For explanation of symbols see legend to Fig. 1.

between these areas multiplied by plasma flow thus represents an estimate of total leg degradation of VLDL-TG, which averaged 9.3 mmol in trained and 8 mmol in the non-trained state ($P > 0.05$).

Changes in arterial glycerol concentrations from resting values were not seen after 10 min of exercise. Thereafter a similar and significant increase was found in NT and T towards the end of exercise (Table 2). The release of glycerol was higher in T than NT at 60 min ($P < 0.05$) and such a tendency was also observed at 120 min (Fig. 3).

Hormones

Arterial concentration of serum insulin decreased continuously during exercise to 3.7 mU l^{-1} (NT) and 4.4 mU l^{-1} (T) at 120 min of exercise ($P < 0.05$). Arterial concentration of adrenaline was 0.12 ng ml^{-1} in NT and T at rest and no changes were observed with exercise. Resting arterial concentration of noradrenaline was 0.38 and 0.33 ng ml^{-1} in NT and T, respectively, and remained unchanged during exercise. During exercise no significant differences were seen between NT and T, either in insulin, adrenaline or noradrenaline concentrations.

Muscle energy substrates

The initial resting muscle glycogen level was markedly higher in T than in NT ($P < 0.05$, Table 4). Muscle glycogen concentration after 120 min of exercise had decreased to 160 mmol kg^{-1} dry wt in NT and to 436 mmol in T ($P < 0.05$, Table 4). Utilization of muscle glycogen averaged 233 mmol kg^{-1} dry wt in NT and significantly larger than in T (176 mmol kg^{-1} dry wt).

TABLE 4. Muscle (m) substrates obtained from m. vastus lateralis before and after submaximal knee-extension exercise with the non-trained and trained thigh

	Non-trained	Trained
m-Glycogen (mmol kg^{-1})		
Before exercise	393 (297–505)	612 (459–840)†
After exercise	160 (25–263)*	436 (265–589)†
m-Lactate (mmol kg^{-1})		
Before exercise	6.4 (3.6–9.1)	6.1 (4.4–8.7)
After exercise	11.0 (5.3–21.0)	6.4 (2.7–11.3)
m-Triacylglycerol (mmol kg^{-1})		
Before exercise	21.5 (15.2–29.0)	32.9 (20.3–51.0)†
After exercise	24.0 (8.0–30.0)	30.6 (13.1–69.0)

Values (in dry wt) are means and range. * $P < 0.05$ between before and after exercise values; † $P < 0.05$ between non-trained and trained thigh.

The PAS staining revealed a small difference between NT and T before exercise in glycogen content (Fig. 5). In NT 39% of slow twitch, 55% of fast twitch a, and 59% of fast twitch b fibres were dark stained – the rest of the fibres were moderately to lightly stained. In T 69% of slow twitch fibres were dark and the remaining moderately stained. All fast twitch fibres were PAS dark stained.

After 2 h of exercise 77% of slow twitch fibres and 10% of fast twitch a and b fibres were PAS negative (NT) (Fig. 5). In T only 11% of slow twitch fibres, but none of the fast twitch a or b fibres were glycogen depleted.

At rest the concentration of lactate in the muscle averaged 6 mmol kg^{-1} dry wt in NT and T (Table 4). At termination of exercise muscle lactate was at the same level in NT and T and not different from resting values.

At rest a significant difference in pooled muscle fibre triacylglycerol (muscle TG) concentration was noticed (22 mmol kg^{-1} dry wt in NT and 33 mmol kg^{-1} dry wt in T) but in neither leg was a significant reduction detected after exercise (Table 4).

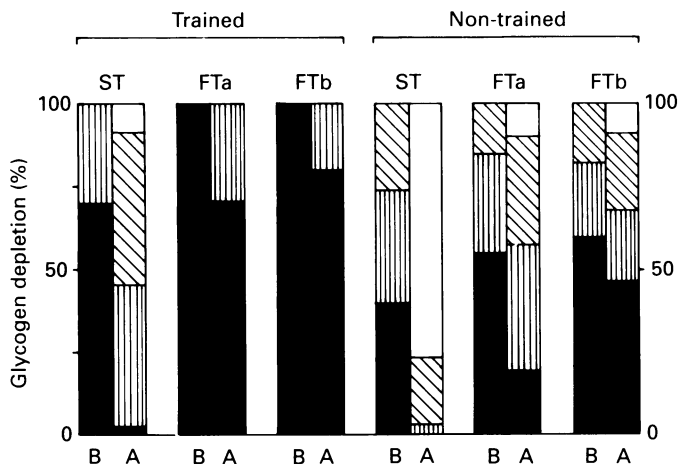


Fig. 5. Glycogen depletion pattern of slow twitch (ST) and fast twitch (FT) a and b fibres before (B) and after (A) exercise in the trained and non-trained muscle. ■, dark stained, glycogen filled; ▨, moderately stained; ▩, lightly stained; □, negatively stained, glycogen depleted. For explanation see text.

DISCUSSION

The old problem of the pronounced shift to enhanced fat combustion after physical conditioning was studied in an isolated muscle group in man. It could be demonstrated that the reduction in carbohydrate utilization was almost completely due to reduced glycogenolysis and that the elevation in lipid oxidation was a function of an enhanced FFA uptake from plasma by the trained muscles, with no significant contribution of TG stored within the muscle fibres.

During exercise the muscle tissue is offered ample amounts of free fatty acids, but only a small percentage of FFA delivered is taken up by the muscle. A linear relationship between plasma FFA concentration and the rate of plasma FFA uptake and oxidation is usually described (Paul, 1970; Hagenfeldt & Wahren, 1971) and taken as implying that the magnitude of FFA uptake is not regulated primarily by the muscle but largely determined by factors such as the rate of lipolysis in adipose tissue (Hagenfeldt & Wahren, 1971). However, in the present study, in the non-trained limb, the net uptake of FFA only increased initially during exercise as delivery of FFA increased. As exercise proceeded, a levelling off in net FFA uptake occurred in spite of increasing delivery. In contrast, in the trained muscle being offered the same amount of FFA as the non-trained muscle during exercise (Fig. 4), net uptake of FFA increased linearly with delivery during the whole exercise period (Fig. 4). Thus the net uptake of FFA was considerably higher in the trained compared to the non-trained muscle at high levels of FFA delivery.

With regard to the mechanism of FFA uptake several steps for regulation are obvious: limitations in FFA utilization could be either in transport of FFA out of the capillaries, transport in the interstitial space, transport into the muscle cell and mitochondria or oxidation by the mitochondria. FFA flux through the plasma membrane has long been considered a passive, non-specific process that occurred either by diffusion or by partitioning into the lipid bilayer (Heimberg, Goh, Lausner, Soler-Argilaga, Weinstein & Wilcox, 1978; DeGrella & Light, 1980). Recent studies, however, on suspensions of isolated hepatocytes (Stremmel, Strohmeyer & Berk, 1986), cultured adipocytes (Abumrad *et al.* 1981; Schweiterman *et al.* 1988), cardiac myocytes (Sorrentino *et al.* 1988) and intact liver (Goresky, Daly, Mishkin & Arias, 1978), strongly indicate that transport of FFA across the plasma membrane is a saturable process and conforms to the basic criteria of a carrier-mediated transport system. Also, in studies of resting perfused rat skeletal muscle, uptake of palmitate followed saturation kinetics (Turcotte, Kiens & Richter, 1991). In the present non-trained state, FFA uptake did not increase as a function of concentration which might be a reflection of transport saturation. FFA uptake, once thought to be regulated at the metabolic and intracellular level, thus may be subject to regulation at the membrane level as well. The important fraction of FFA is the unbound FFA concentration. Studies in Ehrlich ascites tumour cells (Spector, 1975) and in intact liver (Goresky *et al.* 1978) indicate that FFA uptake is a function of unbound rather than the total FFA concentration and much of the data implying saturation kinetics are derived by plots of FFA uptake *vs.* the unbound FFA concentration (Goresky *et al.* 1978; Stremmel *et al.* 1986; Sorrentino *et al.* 1988; Tucotte *et al.* 1991). The difference in the kinetics for net FFA uptake within the trained and the non-trained muscle in the present study was apparent whether FFA uptake was plotted against the total or the unbound FFA delivery (Fig. 6).

An explanation for the observed difference between the kinetics of net FFA uptake in trained and non-trained muscle could also be the training-induced adaptations within the muscle tissue that might prevent saturation of net FFA uptake within the range of concentrations studied. Such adaptations include increases in capillary density which was 15% higher in the trained muscle. An obvious effect of the increased number of capillaries would be to reduce the diffusion distance and increase the surface area for exchange within the muscle. Moreover, the larger capillary density, in addition to the lower femoral blood flow in the exercising trained muscle, would imply that the mean capillary transit time was longer in the trained than in the non-trained muscle. This may be of importance for the FFA extraction within the muscle.

The increased metabolic capacity induced by training could be another, and complimentary, mechanism, as a rate-limiting factor for FFA uptake could be the capacity of the tissue to assimilate the fatty acids. Thus the marked elevation (40%) of β -hydroxyacyl-CoA dehydrogenase would increase the sensitivity for metabolic control even at low substrate levels and also increase reaction velocity (Gollnick & Saltin, 1982). These training-induced adaptations in muscle tissue may thus be of significance for the FFA uptake. However, with the strong experimental evidence for a carrier-mediated transport of FFA across the plasma membrane, a change in structure and/or function of the membrane transport system, induced by training,

should be considered as an additive explanation for the differences observed in FFA uptake between trained and non-trained muscle.

The uptake of FFA by the muscle is, however, not sufficient to cover the whole fat oxidation, neither in the non-trained (net FFA uptake covering 20 and 23% at 60

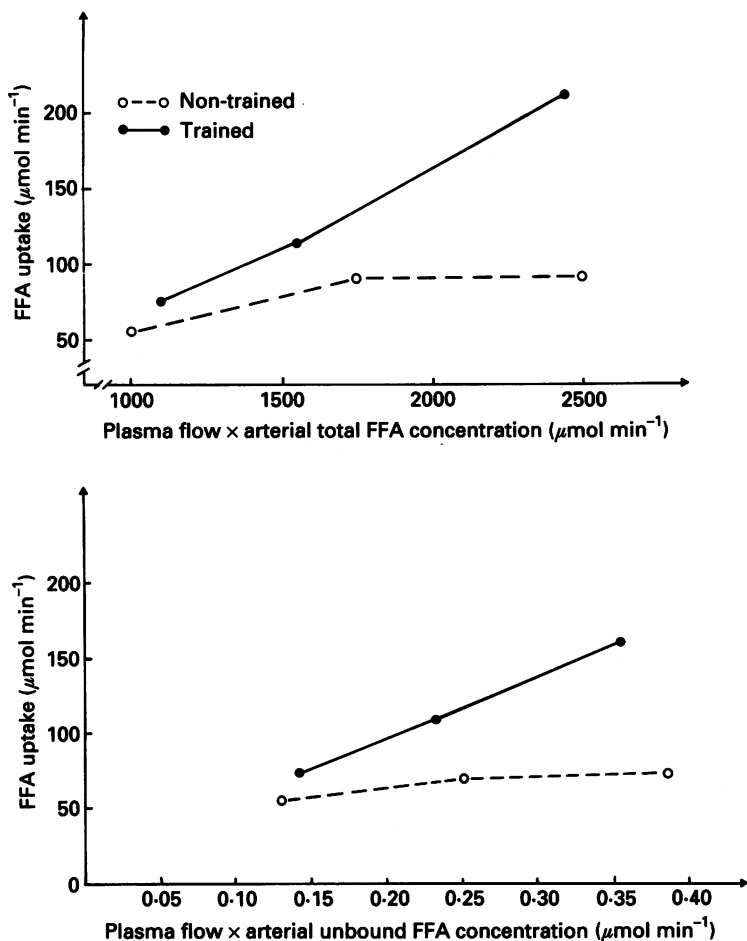


Fig. 6. Mean net FFA uptake related to delivery of total FFA (upper panel) or related to delivery of unbound FFA (lower panel) in trained and non-trained legs.

and 120 min, respectively) nor in the trained muscle (net FFA uptake covering 16 and 30% at 60 and 120 min, respectively) during exercise. Triacylglycerol represents another energy source. It is questionable whether muscle intracellular triacylglycerol is of significance as a fuel for the aerobic metabolism in human skeletal muscle. In the present study, where freeze-dried muscle fibres were dissected free from all visible fat, leaving the pure muscle fibre for analysis, no detectable decrease was found in muscle intracellular triacylglycerol level (Table 4). This is in contrast to the results by Hurley, Nemeth, Martin III, Hagberg, Dalsky & Holloszy (1986) who observed almost twice as much utilization of quadriceps muscle triacylglycerol during exercise

at the same absolute intensity after a 12 week training programme. They concluded that the increased utilization of fat in the trained state was due to an increased lipolysis of intramuscular triacylglycerol and not to an increased uptake of circulating FFA. Support for their notions is available from studies using infusion of [³H]palmitate (Jansson & Kaijser, 1987). No differences in plasma FFA utilization

TABLE 5. Calculated O₂ utilized for total measured fuel combustion during 2 h exercise with non-trained and trained knee extensors, and calculated utilization of triacylglycerols (TG)

Fuel	Non-trained			Trained		
	Uptake/release (mmol)	Fuel (g)	O ₂ utilized (l)	Uptake/release (mmol)	Fuel (g)	O ₂ utilized (l)
FFA (MW 269)	8.7	2.3	4.8	12.0	3.2	6.6
Glucose (MW 180)						
Muscle glycogen	134.0	—	—	106.0	—	—
Blood glucose	140.0	—	—	93.0	—	—
Total	274.0	—	—	199.0	—	—
Blood lactate release	35.0	—	—	20.0	—	—
Muscle lactate accumulation	1.0	—	—	0.1	—	—
Total	36.0	—	—	20.1	—	—
Total glycosyl units	238.0	42.9	32.0	179.0	32.2	24.0
Total O ₂ utilized	—	—	36.8	—	—	30.6
Total leg \dot{V}_{O_2} measured	—	—	60.0	—	—	59.0
Total \dot{V}_{O_2} rest	—	—	23.2	—	—	28.4

Calculated TG utilization:

$$23.2 \text{ l O}_2 : 2.03 \text{ g l}^{-1} :$$

$$861 \text{ g mol}^{-1} \times 10^3 = 13 \text{ mmol TG}$$

$$28.4 \text{ l O}_2 : 2.03 \text{ g l}^{-1} :$$

$$8.61 \text{ g mol}^{-1} \times 10^3 = 16 \text{ mmol TG}$$

Volumes of O₂ utilized in oxidation of fat was 2.03 l (g fuel)⁻¹ and for glucose 0.746 l (g fuel)⁻¹. Taking a weighted mean of the fatty acid composition of normal human adipose tissue gives the average fatty acid as being C₁₇H₃₃O₂ (MW 269); the triacylglycerol formed from three such molecules would have the approximate formula C₅₅H₁₀₄O₆. Palmitoyl-stearoyl-oleoyl-glycerol has this formula and so has been taken as representative molecule (MW 861). Values are from the work of Frayn, 1983. From former knee-extension studies (Graham, Kiens, Hargreaves & Richter, 1991; T. E. Graham, L. P. Turcotte, B. Kiens & E. A. Richter, unpublished observations) estimated contribution of protein amounts to approximately 2% of total fuel utilization and therefore disregarded in the above calculations.

between trained and untrained subjects were present in that study, where exercise was performed at the same relative intensity for 60 min. Thus, Jansson & Kaijser (1987) concluded that the greater contribution of fat oxidation in the trained state was met by a greater utilization of intramuscular triacylglycerol. When calculating the total amount of triacylglycerol oxidized during exercise in the present study from uptake of circulating substrates, lactate release, muscle glycogen breakdown and muscle oxygen uptake, a total triacylglycerol utilization was estimated to average 13 mmol in the non-trained and 16 mmol in the trained state (Table 5). However, it is known from studies using labelled free fatty acids that the net extraction of FFA in working muscle represents an underestimation of unidirectional FFA uptake since FFAs are both taken up and released from a working limb (Hagenfeldt & Wahren,

1966). During knee-extension exercise, we have recently observed that net FFA uptake underestimates, by 35–40%, the unidirectional FFA uptake determined with isotopes (Turcotte, Richter & Kiens, 1992). This would decrease the estimated triacylglycerol utilization to some extent. Nevertheless, these numbers are in close agreement with total glycerol release over the 2 h of exercise estimated to be 10.1 and 15.3 mmol in the non-trained and trained leg, respectively. However, it should be noted, that glycerol in femoral venous blood may stem from lipolysis of circulating triacylglycerols and of triacylglycerols stored in subcutis and in muscle within and between muscle cells. Being aware of these limitations in interpretation of glycerol release it probably provides a good estimate of total triacylglycerol breakdown in the leg. The question is, then, which sources provided the triacylglycerol that was hydrolysed. Previous studies during two-legged bicycling exercise indicate that a contribution from plasma triacylglycerol is very small (Havel, Pernow & Jones, 1967), but in the present study an estimate of total uptake of serum VLDL-triacylglycerol from arteriovenous differences times plasma flow revealed a total uptake for the whole exercise period of serum VLDL-triacylglycerol in both the exercising non-trained (8.0 mmol) and trained muscle (9.3 mmol). If oxidized this plasma triacylglycerol could thus cover a major part of the triacylglycerol oxidation during exercise. Still, some triacylglycerol breakdown (\approx 5 mmol in non-trained and 6–7 mmol in trained) is to be accounted for. It is not unreasonable to believe that triacylglycerol stored either within and/or between muscle cells provided this triacylglycerol. The present study, however, showed no difference in breakdown of triacylglycerol stored within the muscle cells of the non-trained or trained muscle. Taking all the assumptions in these calculations into consideration along with the variability in the muscle triacylglycerol assay (interassay coefficient of variation of 15%) the reasonable conclusion seems to be that there was no major difference in intracellular triacylglycerol breakdown in non-trained and trained and that the possibility exists that some of the intercellular fat may be mobilized during exercise.

The above-mentioned studies in humans showing utilization of intramuscular triacylglycerol as a major lipid energy source during exercise with a minor contribution from plasma lipids (Fröberg, 1971; Essén, 1978; Hurley *et al.* 1986; Jansson & Kaijser, 1987) are not necessarily opposed to the present findings, as the former studies involved whole-body exercise in contrast to our experimental model where exercise and training were confined to only one muscle group. Our model allows studying the choice of energy substrate by the working muscle *per se*, as the hormonal response during exercise was very small. This is in contrast to the findings in whole-body exercise, where large increments in adrenaline and noradrenaline are obtained (Holmquist, Secher, Sander-Jensen, Knigge, Warberg & Schwartz, 1986; Gaffney *et al.* 1990). This lack of increase in adrenaline with exercise in the present study might be decisive for the degree of muscle triacylglycerol utilization during exercise because it was demonstrated recently in man that stimulation of β_2 -adrenoceptors is essential for muscle lipolysis (Cleroux, Nguyen, Taylor & Leenen, 1989). This is supported by our recent findings in which infusion of a β_2 -agonist enhanced glycerol release during one-legged knee extension (Rolett, Strange, Sjøgaard, Kiens & Saltin, 1990). It might be speculated that the absence of intramuscular triacylglycerol net breakdown could be responsible for the large

utilization of blood-borne fat substrates seen in the present study. Conversely, during conditions with high rates of intramuscular triacylglycerol breakdown an inhibition may occur of an uptake of extracellular fatty acids.

The present study reveals, that when exercising at the same absolute intensity, the FFA offered to the trained and the non-trained muscle was similar but the net uptake of FFA within the muscle was significantly larger in the trained compared to the non-trained state. The findings give support to the suggestions that FFA uptake *in vivo* is a saturable process that is influenced by training. In contrast, muscle intracellular triacylglycerol does not play a role as a substrate in a small muscle group during exercise either in the trained or in the non-trained state. The sparing of muscle glycogen was mainly a function of reduced glycogenolysis because the uptake by the muscle of glucose from the bloodstream was unaffected by training. Both structural and functional muscle adaptations appear to cause the observed training-induced metabolic shifts.

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