

Evaluation of exercise and training on muscle lipid metabolism

B. C. BERGMAN,¹ G. E. BUTTERFIELD,² E. E. WOLFEL,³
G. A. CASAZZA,¹ G. D. LOPASCHUK,⁴ AND G. A. BROOKS¹

¹Department of Integrative Biology, University of California, Berkeley 94720; ²Geriatric Research, Education, and Clinical Center, Palo Alto Veterans Affairs Health Care System, Palo Alto, California 95304; ³University of Colorado Health Sciences Center, Division of Cardiology, Denver, Colorado 80262; and ⁴Departments of Pediatrics and Pharmacology, Cardiovascular Disease Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Bergman, B. C., G. E. Butterfield, E. E. Wolfel, G. A. Casazza, G. D. Lopaschuk, and G. A. Brooks. Evaluation of exercise and training on muscle lipid metabolism. *Am. J. Physiol.* 276 (Endocrinol. Metab. 39): E106–E117, 1999.—To evaluate the hypothesis that endurance training increases intramuscular triglyceride (IMTG) oxidation, we studied leg net free fatty acid (FFA) and glycerol exchange during 1 h of cycle ergometry at two intensities before training [45 and 65% of peak rate of oxygen consumption ($\dot{V}O_{2\text{peak}}$)] and after training [65% pretraining $\dot{V}O_{2\text{peak}}$, same absolute workload (ABT), and 65% posttraining $\dot{V}O_{2\text{peak}}$, same relative intensity (RLT)]. Nine male subjects (178.1 ± 2.5 cm, 81.8 ± 3.3 kg, 27.4 ± 2.0 yr) were tested before and after 9 wk of cycle ergometer training, five times per week at 75% $\dot{V}O_{2\text{peak}}$. The power output that elicited $66.1 \pm 1.1\%$ of $\dot{V}O_{2\text{peak}}$ before training elicited $54.0 \pm 1.7\%$ after training due to a $14.6 \pm 3.1\%$ increase in $\dot{V}O_{2\text{peak}}$. Training significantly ($P < 0.05$) decreased pulmonary respiratory exchange ratio (RER) values at ABT (0.96 ± 0.01 at 65% pre- vs. 0.93 ± 0.01 posttraining) but not RLT (0.95 ± 0.01). After training, leg respiratory quotient (RQ) was not significantly different at either ABT (0.98 ± 0.02 pre- vs. 0.98 ± 0.03 posttraining) or RLT (1.01 ± 0.02). Net FFA uptake was increased at RLT but not ABT after training. FFA fractional extraction was not significantly different after training or at any exercise intensity. Net glycerol release, and therefore IMTG lipolysis calculated from three times net glycerol release, did not change from rest to exercise or at ABT but decreased at the same RLT after training. Muscle biopsies revealed minor muscle triglyceride changes during exercise. Simultaneous measurements of leg RQ, net FFA uptake, and glycerol release by working legs indicated no change in leg FFA oxidation, FFA uptake, or IMTG lipolysis during leg cycling exercise that elicits 65% pre- and 54% posttraining $\dot{V}O_{2\text{peak}}$. Training increases working muscle FFA uptake at 65% $\dot{V}O_{2\text{peak}}$, but high RER and RQ values at all work intensities indicate that FFA and IMTG are of secondary importance as fuels in moderate and greater-intensity exercise.

exertion; free fatty acid; glucose; lactate; crossover concept; substrate shuttling

EFFECTS OF EXERCISE intensity and training state on muscle lipid metabolism in humans are unclear, as the relatively few studies have utilized different experimental approaches. Some investigations have employed

isotopic tracers and indirect calorimetry (25, 29, 33), whereas others have utilized arteriovenous (a-v) balance methods (17, 22, 35) and muscle biopsies (18, 22). In addition, longitudinal and cross-sectional designs have been employed that have tested trained and untrained subjects at either the same absolute or relative exercise intensity, but rarely both. Additionally, almost all studies have tested subjects after an overnight fast, in a glycogen-depleted state, or both (1, 2, 14, 16, 17, 20, 22, 30), making difficult comparisons to real life exercise, which is usually preceded by resting and eating.

Using a-v measurements, some studies have shown significant free fatty acid (FFA) uptake by resting limbs (14, 15, 35). In contrast, other investigations report net limb FFA release at rest (2, 22, 30, 32). In parallel, net glycerol release from resting limbs has been a consistent observation (2, 14, 15, 22, 30, 32, 35). Although Hagenfeldt and Wahren (14) did not observe increased net limb FFA uptake in the transition from rest to exercise, most studies report increased net FFA uptake during exercise compared with rest (2, 22, 30, 32, 35). In addition, some investigators report higher net glycerol release during exercise compared with rest (2, 22, 30, 32, 35). This result is, however, not without contradiction (14, 17).

To date, only three studies have examined training effects on working limb FFA and glycerol uptake and release. Investigators using single leg kicking protocols have found increased net FFA uptake at 2 (22) and 3 (35) h of exercise at the same absolute and relative exercise intensity, respectively. Although one study has found increased net glycerol release after 2 h of exercise (22), others have not (17, 35).

Recently, several groups of investigators used FFA isotope tracers and indirect calorimetry to estimate the contribution of intramuscular lipid stores to total lipid oxidation (25, 29, 33). Because the difference between whole body FFA oxidation measured by indirect calorimetry and tracer-measured FFA flux was greater after training, the data were interpreted to mean that training increased reliance on intramuscular triglycerides (IMTG; see Refs. 25 and 29).

The effect of training on IMTG use during exercise has also been addressed by means of the muscle biopsy technique. Hurley et al. (18) measured decreases in intramuscular lipid via muscle biopsies and concluded

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

that training produced greater reliance on intramuscular lipid sources for oxidation. However, Kiens et al. (22) did not confirm this result. Most recently, Kiens and Richter (23) reported unchanged IMTG concentration after 90 min of exercise.

Because of controversies over the effects of exercise intensity and training status on the roles played by IMTG and plasma FFA as fuels during exercise, the present study was undertaken. Using a longitudinal design and careful nutritional control, we evaluated the hypothesis that endurance training increases intramuscular FFA oxidation at given absolute and relative exercise intensities. Results of limb metabolite a-v and respiratory quotient (RQ) measurements do not support the hypothesis; results indicate that training does not appreciably change reliance on IMTG or plasma FFA, which are of minor importance as fuels for moderate and greater-intensity exercises.

METHODS

Subjects

Nine healthy untrained male subjects age 19–33 yr were recruited from the University of California, Berkeley campus, by posted notices. Subjects gave informed consent and were considered untrained if they engaged in no more than 2 h of physical activity/wk for 1 yr and had a peak rate of oxygen consumption ($\dot{V}O_{2\text{peak}}$) of $<45 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Subjects were included in the study if they had $<25\%$ body fat, were nonsmokers and diet and weight stable, had a 1-s forced expiratory volume (FEV₁) of 70% or more, and were injury and disease free as determined by physical examination. This study was approved by the Committee for the Protection of Human Subjects at Stanford University and the University of California, Berkeley (CPHS 97–6–34).

Experimental Design

After preliminary screening and interviews, subjects performed two graded exercise tests to determine the $\dot{V}O_{2\text{peak}}$ during leg cycle ergometry. Subjects were then tested in a random order at 45 and 65% $\dot{V}O_{2\text{peak}}$, with 1 wk between trials. Two days after the second trial, subjects began training on cycle ergometers 5 days/wk for 9 wk at 75% $\dot{V}O_{2\text{peak}}$. Midtraining stress tests and subsequent workload adjustments were performed to maintain relative training intensity at 75% $\dot{V}O_{2\text{peak}}$. Posttraining experimental trials were also performed in a random order at 65% of pretraining $\dot{V}O_{2\text{peak}}$ (ABT) and 65% of posttraining $\dot{V}O_{2\text{peak}}$ (RLT), corresponding to the same absolute and relative exercise intensities before and after training, respectively. Subjects continued training during the 1 wk separating the two posttraining trials.

Preliminary Testing

All exercise tests were performed on an electronically braked cycle ergometer (Monark Ergometric 829E). For determination of $\dot{V}O_{2\text{peak}}$, exercise started at a power output of 50 W, which was increased by 25 or 50 W every 3 min until exhaustion. Respiratory gases were analyzed via an indirect open circuit system (Ametek S-3A1 O₂ and Ametek CD-3A CO₂ analyzers) and were recorded by an on-line, real time PC-based system. During the second maximal exercise test performed at the metabolic ward, a catheter was placed in an antecubital vein for withdrawal of blood for lactate threshold determination as well as for routine blood analysis. Body

composition was determined via both skin-fold measurements (19) and underwater weighing. Three-day diet records were kept to obtain baseline dietary habits and to monitor macronutrient composition and caloric intake over the course of study. Dietary analyses were performed using the Nutritionist III software (N-Squared Computing, Salem, OR). FEV₁ was determined via a 9L spirometer.

Testing Protocol

The night preceding each experimental trial, subjects were admitted to the metabolic ward where they remained until testing was completed the following day. Subjects were fed a standardized dinner [1,174 kcal: 66% carbohydrate (CHO), 21% fat, 13% protein] that was replicated the night before each experimental trial. Later that evening, subjects ate a standardized snack (500 kcal: 53% CHO, 31% fat, and 16% protein) before retiring. Two subjects were tested per day with morning and afternoon testing randomly assigned to each subject and replicated for every trial. Morning procedures started at 7:00 AM, whereas preliminary afternoon procedures began at 1:00 PM. Each subject was tested at the same time of day throughout the study. Morning subjects ate a standardized pretrial meal (448 kcal: 72% CHO, 10% fat, 18% protein) at 6:00 AM, 1 h before procedures started, and 4.5–5 h before exercise. Afternoon subjects ate a standardized breakfast in the morning (729 kcal: 57% CHO, 33% fat, 10% protein) and the standardized pretrial meal at noon, again 1 h before procedures began.

Catheterizations

After local lidocaine anesthesia, the femoral artery and vein of the same leg were cannulated using standard percutaneous techniques as previously described (37). A 5.1-Fr, 50-cm, Cordis arterial catheter (model 91100900; North American Medical Instrument) was inserted 25 cm and was positioned in the distal abdominal aorta via the femoral artery. A 6-Fr thermomodulation venous catheter (model 93–135–6F; American Edwards Laboratories) was placed with the tip in the distal iliac vein through a venous sheath in the femoral vein 20 cm from the skin. Both catheters were sutured to the skin and further secured by an Ace bandage wrap. The external portions of each catheter were directed toward the hip for easy access during exercise. Alternate legs were used for both pretraining and posttraining testing. One subject experienced blood leaking from catheter placements during the beginning minutes of exercise at 65% pretraining and did not perform further exercise. Two different subjects did not receive a venous catheter for one of their trials. As a result, a sample size of 6–9 was used for calculations and comparisons.

Blood Sampling

Blood temperature was obtained from a thermistor at the end of the venous thermomodulation catheter immediately before blood sampling. Arterial and venous blood samples were drawn simultaneously and anaerobically over 5 s after 75 and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise. PO₂, PCO₂, and pH were measured within 30 min (ABL 300; Radiometer, Copenhagen, Denmark). Blood for FFA concentration ([FFA]) and glycerol concentration ([glycerol]) determination was immediately transferred to tubes containing EDTA, shaken, and placed on ice. After the final blood sample at the end of exercise, samples were centrifuged at 3,000 rpm for 10 min, and plasma was transferred to storage tubes and frozen at -80°C until analysis. Hematocrit measurements were performed on both arterial and venous blood using the

microhematocrit method. Whole blood hemoglobin concentration was determined on each arterial and venous sample using the cyanomethemoglobin method.

Muscle Sampling and Analysis

During preparation for blood sampling, one vastus lateralis was prepared for percutaneous needle biopsy. For each experimental trial, biopsies were taken from two locations separated by 1.5 cm: the distal site for preexercise sampling and the proximal site for immediate postexercise sampling. Right and left vastus muscles were alternated between trials. Biopsies taken at rest, and within 10 s of exercise cessation, were immediately plunged in liquid nitrogen and subsequently stored under liquid nitrogen or at -80°C until analysis. Samples were analyzed for metabolite contents as described previously (24). Briefly, tissue lipids were extracted and separated, and a known weight of frozen, powdered tissue was extracted in ice-cold chloroform and methanol (2:1, vol/vol) using a Polytron homogenizer. Methanol was added, and the mixture was stirred and then allowed to sit for 1 h at 4°C . After centrifugation, the supernatant was decanted and dried under N_2 at 50°C . The residue was dissolved in chloroform and transferred to a silicic acid column (Bio-Sil HA, 325 mesh). The neutral lipid plus FFA fraction was eluted with chloroform and then dried under N_2 at 50°C . Neutral lipids were then saponified by heating at 75°C for 45 min in 4% KOH in 95% ethanol. Triacylglycerol content was then determined by measuring FFAs released (9).

Hemodynamics

Heart rate and electrocardiogram (ECG) were continuously recorded and displayed using a three-lead ECG connected to a MacLab analog-to-digital converter (ADInstruments, Castle Hill, Australia) and were tracked on a Macintosh 7200/200 Power Mac computer (Apple Computer, Cupertino, CA). Arterial blood pressure was also continuously recorded and displayed using a Transpac pressure transducer (Baxter) positioned at the level of the heart connected to the MacLab system and was calibrated before every trial. Iliac venous blood flow was determined by the thermodilution technique using a cardiac output computer (model 9520; American Edwards Laboratories) with a 10-ml bolus injection of sterile saline cooled to 0°C via an ice slurry (American Edwards Laboratories-Set II, 93-520). Measurements were made in triplicate or quadruplicate during rest and exercise immediately after blood sampling. The validity and precautions associated with this technique have been described previously (3).

Metabolite Analysis

FFAs were measured in duplicate using the Wako NEFA-C kit (Wako Chemicals, Richmond, VA). Glycerol was also measured in duplicate using a triglyceride kit (GPO-Trinder), and glucose was measured in duplicate using a hexokinase enzymatic kit, both from Sigma Chemical (St. Louis, MO). Lactate was measured in duplicate using the method of Gutmann and Wahlefeld (13) using lactate dehydrogenase.

Training Protocol

All training was performed on stationary cycle ergometers 5 days/wk with workloads adjusted to the required intensity based on maximal exercise test results, and heart rates were recorded daily by personal trainers who had taken the exercise physiology course at the University of California, Berkeley. Subjects were asked to exercise 1 day/wk on their

own in addition to cycle ergometry training so that total training was 6 days/wk. All subjects were exercising at 75% of their $\dot{V}\text{O}_{2\text{peak}}$ for 1 h by the end of the second week of training. After 4 wk of training, subjects performed another maximal exercise test to exhaustion to quantify increases in $\dot{V}\text{O}_{2\text{peak}}$, and training workloads were increased accordingly to maintain relative training intensity at 75% $\dot{V}\text{O}_{2\text{peak}}$. Two weeks before posttraining testing, subjects began interval training during the last 10 min of each 1-h workout. Subjects continued training throughout the 1 wk between posttraining experimental trials. One day of rest was imposed before each trial. Subjects were weighed daily and were asked to increase food intake to maintain weight during the training program without changing normal macronutrient composition. Three-day diet records were collected after 4 wk of training and at the end of training to ensure maintenance of baseline diet composition. Dietary analysis of these records was performed using the Nutritionist III program (N-Squared Computing, Salem, OR).

Calculations

Leg RQ. Leg RQ was calculated from the ratio of venous-arterial CO_2 content difference ($v\text{-aCO}_2$) and arteriovenous O_2 content difference ($a\text{-vO}_2$)

$$\text{RQ} = \frac{v\text{-aCO}_2}{a\text{-vO}_2}$$

Blood CO_2 content. Blood PCO_2 , PO_2 , pH, and hemoglobin (Hb) were measured on both arterial and venous samples and were used in the calculations by Douglas et al. (8) for determination of blood CO_2 content (C_{CO_2})

$$\text{C}_{\text{CO}_2} \text{ blood} = \text{C}_{\text{CO}_2} \text{ plasma}$$

$$\times 1 - 0.0289 \times \text{Hb}/3.352 - [0.456 \times \text{SO}_2 \times (8.142 - \text{pH})]$$

where plasma C_{CO_2} is determined as follows

$$\text{C}_{\text{CO}_2} \text{ plasma} = 2.226 \times s \times \text{PCO}_2 \text{ plasma} \times (1 + 10^{\text{pH}-\text{pK}'})$$

and where the plasma solubility coefficient s is calculated as

$$s = 0.0307 + [0.00057 \times (37 - T)] + [0.00002 \times (37 - T)^2]$$

where T is temperature ($^{\circ}\text{C}$). The apparent dissociation constant (pK') is calculated as

$$\text{pK}' = 6.086 + [0.042 \times (7.4 - \text{pH})]$$

$$+ [(38 - T) \times \{0.00472 + [0.00139 \times (7.4 - \text{pH})]\}]$$

from the equations of Kelman (21).

Blood O_2 content. Blood O_2 content was calculated using hemoglobin concentration and oxygen saturation (SO_2) determined from the equation of Nunn (27)

$$\text{SO}_2 = 100 \times (\text{PO}_2^3 + 2.667 \times \text{PO}_2)/\text{PO}_2^3 + 2.667 \times \text{PO}_2 + 55.47$$

$\dot{V}\text{O}_2$ of the legs. Leg $\dot{V}\text{O}_2$ was calculated using the Fick equation as follows

$$\text{Leg } \dot{V}\text{O}_2 = 2 \times \text{one leg } Q \times a\text{-vO}_2$$

where Q is flow.

Net metabolite exchange. Net metabolite exchange differences were calculated by the product of leg blood flow and $a\text{-v}$ differences where arterial and venous hematocrit values were

used to correct for changes in plasma volume

Net glycerol release (mmol/min)

$$= 2 (\text{one leg Q})[(\text{Hct}_a/\text{Hct}_v)[\text{glycerol}]_v - [\text{glycerol}]_a]$$

Net FFA uptake (mmol/min)

$$= 2 (\text{one leg Q})[[\text{FFA}]_a - [(\text{Hct}_a/\text{Hct}_v)[\text{FFA}]_v]]$$

where Hct is hematocrit, and the subscripts a and v represent arterial and venous, respectively.

Fractional FFA uptake. Fractional FFA uptake was calculated from the ratio of [a-v]FFA and arterial [FFA] with correction of venous metabolite concentrations for blood volume shifts as determined from hematocrit measurement

Fractional FFA uptake (%)

$$= [\text{FFA}]_a - [(\text{Hct}_a/\text{Hct}_v)[\text{FFA}]_v]/[\text{FFA}]_a(100)$$

Whole body and leg FFA oxidation. Total triglyceride oxidation for both whole body [from respiratory exchange ratio (RER)] and exercising leg (from leg RQ) were determined from stoichiometric equations (10), assuming a whole body nitrogen excretion rate of $135 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (5). Triglyceride oxidation was converted to fatty acid oxidation using the molar equivalent assuming an average triglyceride mass of 860 g/mol (10) and multiplying molar triglyceride oxidation by three because each mole of triglyceride contains 3 mol of fatty acids.

IMTG lipolysis. Assuming glycerol kinase is not present in myocytes or adipocytes to reesterify glycerol and that glycerol is not oxidized in skeletal muscle, glycerol release across the limb represents intramuscular lipolysis. IMTG lipolysis can then be calculated from the product of three times net glycerol release. (Note that this method overestimates IMTG lipolysis due to the contributions of other lipid stores; see *IMTG Concentration*.)

$$\text{IMTG lipolysis (mmol/min)} = 3 \times \text{net glycerol release}$$

Putative IMTG oxidation. IMTG oxidation was calculated using a modification of the formula of Martin et al. (25), which takes the difference between whole body FFA oxidation and FFA disposal to estimate IMTG oxidation

IMTG oxidation (mmol/min)

$$= \text{whole body FFA oxidation (mmol/min)} \\ - \text{net FFA uptake (mmol/min)}$$

Statistical Analyses

Average arterial glucose and lactate concentration from the last 30 min of exercise were analyzed using a one-factor ANOVA with repeated measures. Differences between groups and changes over time in concentration, a-v difference, net uptake/release of FFA and glycerol, and FFA fractional extraction were determined using a repeated-measures factorial ANOVA. Post hoc comparisons were made using Fisher's protected least significant difference. One-hour averages of leg and body FFA oxidation, IMTG lipolysis, and IMTG oxidation were analyzed using unpaired Student's *t*-tests. Muscle biopsy concentration differences were determined with paired Student's *t*-tests. Statistical significance was set at $\alpha = 0.05$. All data are presented as means \pm SE.

Other Measurements

In addition to parameters included here, systemic blood pressure, cardiac output, glucoregulatory hormones, muscle

metabolites, glucose and lactate fluxes, and tissue exchange rates were measured. These results will be reported separately.

RESULTS

Subject Characteristics

Anthropometric data for subjects pre- and posttraining are shown in Table 1. Subjects were weight stable throughout the study period, although absolute changes in percent body fat decreased significantly as determined by both skin-fold measurements (-2.2%) and underwater weighing (-1.7%). $\dot{V}O_{2\text{peak}}$ significantly increased by 14.6% as a result of the training regimen. Consequently, posttraining trials at $66.1 \pm 1.1\%$ of pretraining $\dot{V}O_{2\text{peak}}$ (the same absolute workload as pretraining) were performed at $54.0 \pm 1.7\%$ of posttraining $\dot{V}O_{2\text{peak}}$. Workloads for the four trials are presented in Table 2.

Muscle Triglyceride Concentrations

Resting quadriceps triglyceride concentrations decreased after training and tended to increase during exercise (Table 3). However, the increases in IMTG concentration were quantitatively small, and the significant increase during exercise at $45\% \dot{V}O_{2\text{max}}$ before training represented only 5% of leg net FFA uptake. The change in muscle triglyceride concentration was not different between exercise intensities.

Indirect Calorimetry

Mean steady-state $\dot{V}O_2$ and $\dot{V}CO_2$ for the four trials are presented in Table 2. RER from the last 30 min of exercise showed significant increases at 65% pretraining compared with 45% pretraining (13% increase in CHO oxidation, 43% decrease in fat oxidation) as expected (Table 2). When subjects were retested after training at ABT, there was a significant decrease in RER (13% decrease in CHO oxidation, 43% increase in fat oxidation). There were no differences in RER values when subjects were tested at the same relative intensity after training.

Table 1. Subject characteristics before and after 9 wk of leg cycle endurance

Variable	Pretraining	Posttraining	Difference, %
Age, yr	27.4 ± 2.0		
Height, in.	70.1 ± 1.0		
Weight, kg	81.8 ± 3.3	81.3 ± 3.2	-0.6
Body fat, %			
Skin folds	19.7 ± 1.5	$17.5 \pm 1.6^*$	-11.0
Underwater weighing	19.5 ± 1.5	$17.2 \pm 1.4^*$	-11.6
$\dot{V}O_{2\text{peak}}$			
l/min	$3.5 \pm .10$	$4.02 \pm .15^*$	14.6
ml · kg ⁻¹ · min ⁻¹	43.5 ± 1.3	$50.1 \pm 1.6^*$	15.5
Lactate threshold, % $\dot{V}O_{2\text{peak}}$	60.9 ± 2.7	65.4 ± 2.6	7.4

Values are means \pm SE; $n = 9$ subjects. $\dot{V}O_{2\text{peak}}$, peak O_2 consumption. *Significantly different from pretraining values at $P < 0.05$.

Table 2. Mean indirect calorimetry and blood flow data for last 30 min of exercise

Variable	45% Pretraining	65% Pretraining	65% Old (ABT)	65% New (RLT)
Workload, W	85.8 ± 3.6	152.0 ± 7.6	149.4 ± 7.1	173.5 ± 6.7
Whole body $\dot{V}O_2$, l/min	1.45 ± 0.03	2.23 ± 0.08	2.01 ± 0.09	2.50 ± 0.08
Whole body $\dot{V}O_2$, l/min	1.57 ± 0.04	2.32 ± 0.09	2.17 ± 0.09	2.62 ± 0.09
RER, %	0.93 ± 0.01	0.96 ± 0.01*	0.93 ± 0.01†	0.95 ± 0.01
CHO	77	87	77	84
Fat	23	13	23	16
One leg $\dot{V}O_2$, l/min	0.57 ± 0.02	0.83 ± 0.09	0.90 ± 0.06	1.13 ± 0.09
One leg blood flow, l/min	3.9 ± 0.1	5.2 ± 0.3*	5.8 ± 0.2†	7.0 ± 0.3†‡
Leg RQ, %	0.89 ± 0.05	0.98 ± 0.02*	0.98 ± 0.03*	1.01 ± 0.02*
CHO	63.7	94.3	94.3	100
Fat	36.3	5.7	5.7	0

Values are means ± SE; $n = 9$ subjects for $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER); $n = 6$ subjects for leg respiratory quotient (RQ), blood flow. CHO, carbohydrate; ABT, absolute workload (65% of pretraining $\dot{V}O_{2peak}$); RLT, relative workload (65% of posttraining $\dot{V}O_{2peak}$). * Significantly different from 45% pretraining at $P < 0.05$. † Significantly different from 65% pretraining at $P < 0.05$. ‡ Significantly different from posttraining (65% Old) at $P < 0.05$.

Leg Blood Flow

Leg blood flow increased from rest to exercise in all groups. Exercise intensity significantly increased leg blood flow throughout exercise before and after training, with higher intensities eliciting significantly greater blood flows (Table 2). Furthermore, training status affected blood flow, as the same absolute workload after training resulted in significantly higher leg blood flows (10.3%) across all time points compared with before training. Likewise, the same relative workload elicited 25.7% higher leg blood flows after training.

Arterial Glucose and Lactate Concentrations

Arterial glucose concentrations did not change at rest after training, nor were average glucose concentrations different for the last 30 min of exercise before or after training regardless of exercise intensity (Fig. 1A). Resting lactate concentrations were also not significantly different before and after training (Fig. 1B). Lactate concentrations increased significantly from rest to exercise in all but the 45% pretraining intensities. Increasing exercise intensity significantly raised lactate concentrations compared with rest by 44.5% (45% pretraining) and 366% (65% pretraining) before training and by 159% (ABT) and 332% (RLT) after training. Training resulted in significantly lower lactate concentrations at the same absolute (55% decrease) and relative workloads (26% decrease).

Arterial [FFA], [Glycerol], Net Uptake, and Release Across the Legs

FFA. Although there were no differences among exercise intensities, after an initial decline at exercise

onset, arterial [FFA] increased over time (Fig. 2A). Negative limb [a-v] differences at rest indicated net FFA release, with a switch to positive [a-v] differences indicating net limb FFA uptake during exercise. FFA a-v differences were similar among exercise intensities, with mean values increasing over time (Fig. 2B). Fractional limb FFA extraction was not significantly different with training or exercise intensity, at 5.84% pretraining and 6.25% posttraining (Fig. 2C). Thus neither exercise intensity nor training status affected fractional FFA uptake. Mean net FFA uptake tended to be higher at 65% pretraining than 45% pretraining, although the difference was not significant ($P = 0.06$; Fig. 2D). After training at both ABT and RLT, mean net FFA uptake was significantly greater than 45% pretraining ($P < 0.05$). At ABT, mean net FFA uptake was not significantly different from 65% pretraining. However, at RLT, mean net FFA uptake was significantly greater than 65% pretraining, even though values were similar at 45 and 60 min, indicating that net FFA uptake increased more quickly at the beginning of exercise after training (Fig. 2D). There was a trend for increased mean net FFA uptake after training at RLT vs. ABT; however, the difference did not reach significance ($P < 0.06$).

Glycerol. Arterial [glycerol] increased significantly over time from resting values of 0.07 mM for all groups to a 60-min exercise value of 0.20 mM for all but the 45% pretraining trials (Fig. 3A). Mean exercise [glycerol] for 45% pretraining was significantly lower than the other three exercise intensities. Glycerol v-a differences during exercise were significantly lower than at rest, falling from a value of 0.035 mmol to close to zero during exercise (Fig. 3B). Although there were no significant changes over time during exercise, glycerol

Table 3. Vastus lateralis muscle triglyceride concentrations before and after exercise in each experimental condition

IMTG, nmol/g wet weight	45% Pretraining	65% Pretraining	65% Old (ABT)	65% New (RLT)
Rest	524.38 ± 113.86	524.38 ± 113.86	282.17 ± 32.97*	282.17 ± 32.97*
Postexercise	908.78 ± 171.25†	1,095.52 ± 430.70	640.55 ± 152.44	818.32 ± 318.22
Delta	384.40 ± 109.20	571.15 ± 399.95	358.39 ± 164.74	536.16 ± 327.15

Values are means ± SE; $n = 9$ subjects. IMTG, intramuscular triglyceride. * Significantly different between resting conditions at $P < 0.05$. † Significantly different from rest at $P < 0.05$.

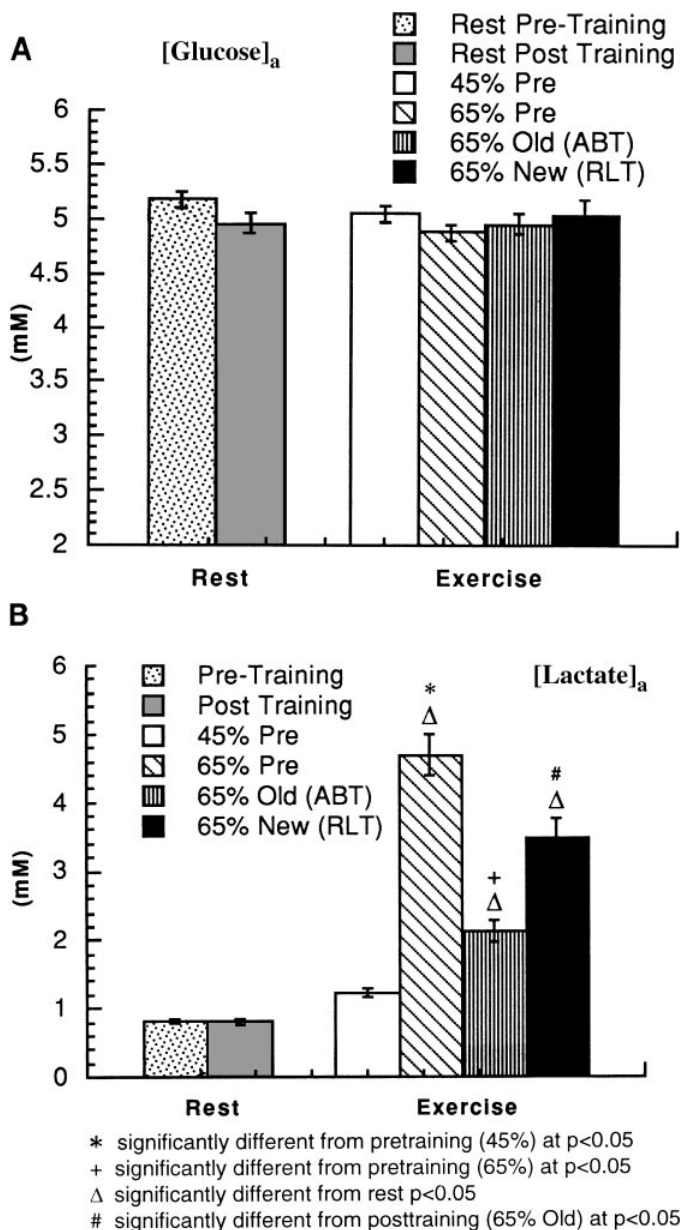


Fig. 1. Arterial glucose (A) and lactate (B) concentrations before and after 9 wk of training in men. Values are mean \pm SE for 8 subjects. Exercise data are means of last 30 min of exercise. Pre, pretraining; ABT, absolute workload [65% of pretraining peak rate of oxygen consumption ($\dot{V}O_{2\text{peak}}$)]; RLT, relative workload (65% of posttraining $\dot{V}O_{2\text{peak}}$).

v-a during the 65% pretraining trial decreased to negative values such that the mean v-a difference was significantly lower than 45% pretraining and ABT. Net glycerol release did not change significantly from rest throughout exercise, resulting in little net glycerol release during any of the pretraining or posttraining trials (Fig. 3C). After training, mean exercise net glycerol release at RLT was significantly lower than during ABT, with values from the last 30 min of exercise averaging from -0.019 mmol/min for RLT to 0.047 mmol/min for ABT. Net glycerol release data are consistent with literature values as shown in Fig. 4.

Leg RQ. As reported by others (20, 28, 34), leg RQ varied more than pulmonary RER. Leg RQ was not significantly different at rest before or after training (Fig. 5). Average exercise RQ was not different from rest at 45% $\dot{V}O_{2\text{peak}}$ but was increased significantly at 65% $\dot{V}O_{2\text{peak}}$ pretraining. Exercise RQ was also not significantly different from rest after training at either intensity. At the same absolute or relative exercise intensity after training, there were no differences in leg RQ. However, both posttraining exercise leg RQ values were significantly higher than 45% pretraining.

One-hour averages of leg FFA oxidation calculated from leg RQ and whole body FFA oxidation calculated from pulmonary RER are shown in Fig. 6. There were no significant differences in leg FFA oxidation during the two pretraining intensities (Fig. 6A). Posttraining, leg FFA oxidation was significantly lower (72%) at RLT compared with ABT (Fig. 6A). Whole body FFA oxidation at 65% pretraining was significantly lower than 45% pretraining (45%) and ABT (58%; Fig. 6B). Figure 6 also shows mean net FFA uptake and IMTG lipolysis calculated from net glycerol release data as described in METHODS. There was a tendency for greater mean net FFA uptake with increased exercise intensity pre- and posttraining (Fig. 6C); however, the differences did not reach significance ($P < 0.06$). Mean net FFA uptake was significantly greater at the same relative exercise intensity posttraining ($P < 0.05$). IMTG lipolysis was significantly decreased posttraining at RLT compared with ABT ($P < 0.05$; Fig. 6D).

DISCUSSION

We evaluated the hypothesis that endurance training increases reliance on IMTG at given absolute and relative exercise intensities; our results do not support the hypothesis. Working limb net glycerol release, and, therefore, intramuscular (intralimb) lipolysis, were unaffected at either absolute or relative workloads after training. Furthermore, no other data source (i.e., whole body RER, leg RQ, net FFA uptake, or change in muscle triglyceride content) indicated blood-borne FFA or IMTG to be major energy sources during 1 h of sustained exercise regardless of training state or exercise intensity.

Training Adaptations

Our training program was successful in promoting significant metabolic adaptations (Tables 1 and 2, Fig. 1B). During our 9-wk training program, subjects significantly increased $\dot{V}O_{2\text{peak}}$ (15%), decreased RER at a given absolute workload (3.2%), increased lactate threshold (60.9% pre- vs. 65.4% $\dot{V}O_{2\text{peak}}$ posttraining), and decreased arterial lactate concentration at the same relative (26%) and absolute (55%) exercise intensities.

Nutritional Controls

Our experiments were designed to reveal effects of exercise and training on substrate utilization. We employed experimental and nutritional controls appropriate to represent practices and conditions typical in the

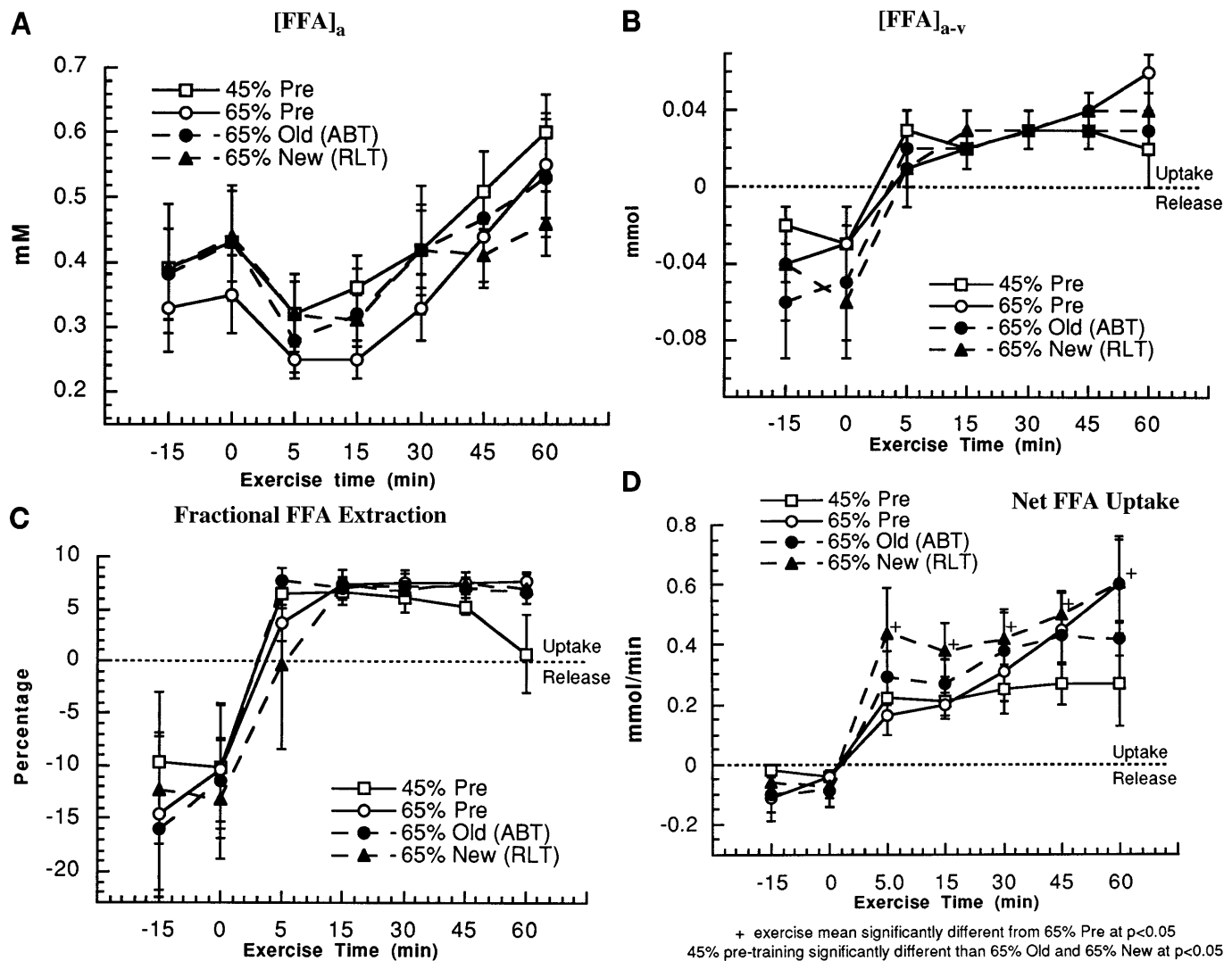


Fig. 2. Effect of exercise intensity and training on arterial free fatty acid (FFA) concentration (A), FFA arteriovenous difference (B), FFA fractional extraction (C), and net FFA uptake (D). Values are means \pm SE for 8 subjects in A-C and for 6 subjects in D.

population at large. For these reasons, we fed subjects to be weight stable and rested them the day before experimentation. Furthermore, we fed standardized, low-glycemic-index meals 4.5–5.5 h before exercise studies. Those efforts produced stable blood glucose levels during exercise, suggesting that subjects commenced exercise with normal liver glycogen reserves.

After resting and feeding subjects before experiments, arterial [FFA] and [glycerol] rose during exercise (Figs. 2A and 3A). In parallel studies on different subjects, we measured glycerol and FFA kinetics in men and women during rest and exercise both before and after training and found increased FFA and glycerol rates of appearance in women (12) and men (Friedlander, A.L., G.A. Casazza, M.A. Horning, and G.A. Brooks, unpublished observations). These results are consistent and show that our experimental design permitted lipolysis to occur during exercise and made FFAs available to working muscle in excess of demand. However, glycogen, glucose, and lactate were preferred to FFAs and IMTG when blood glucose was stable and

liver and muscle glycogen contents replete, as determined by leg RQ and mass balance. Thus the effects on substrate utilization that we observed are attributable to exercise intensity and endurance training and are not confounded by undernutrition, liver glycogen depletion, or hypoglycemia.

IMTG Concentration

Values for IMTG concentration are low compared with those of others (18, 22, 23). In this regard, we refer to the recent paper of Wendling et al. (36) who showed great interindividual variability in IMTG values. More important, degradation rates during exercise are similar to those reported by others (18, 22, 23). Similar to Kiens and Richter (23) and Kiens et al. (22), IMTG concentration did not decrease in our study after 1 h of exercise (Table 3), which corroborates our net glycerol release data suggesting that IMTG is not an important substrate for exercise when subjects are fed a meal 4–5 h before exercise.

Glycerol Exchange

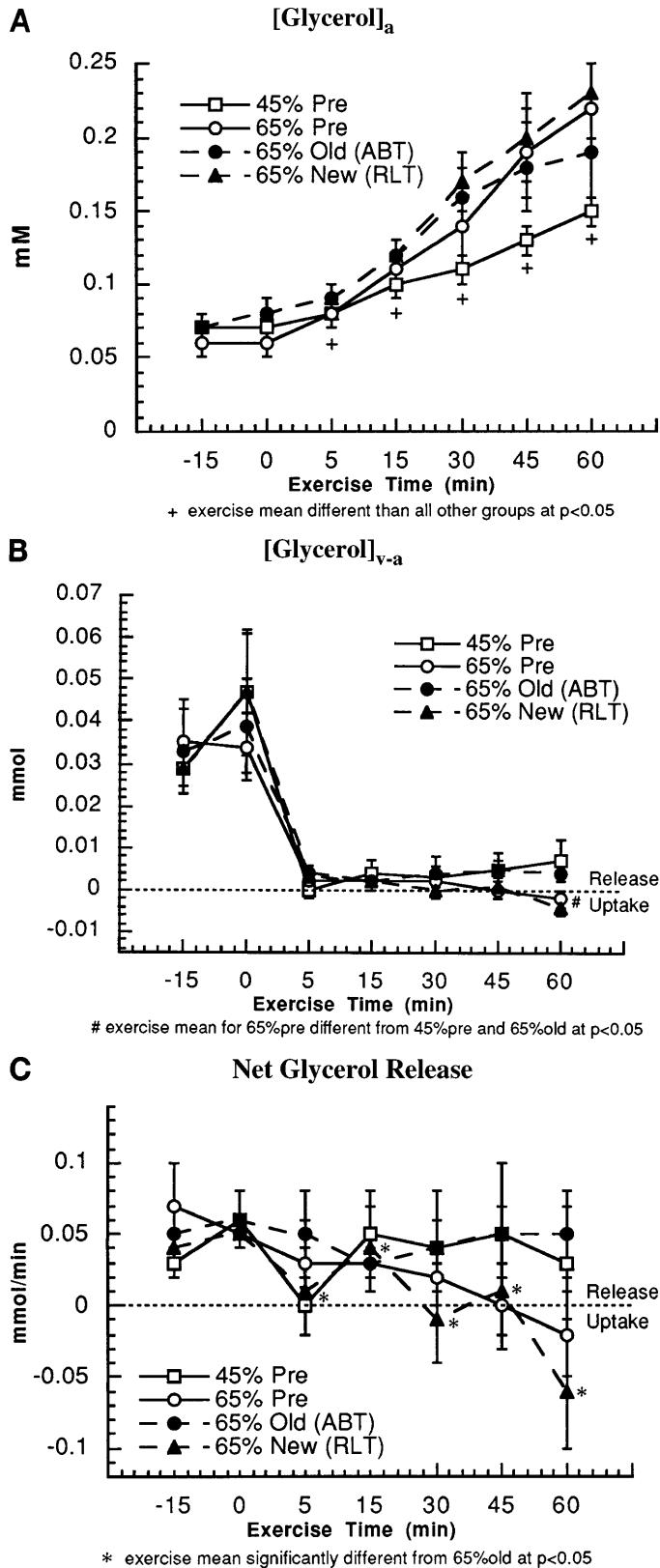


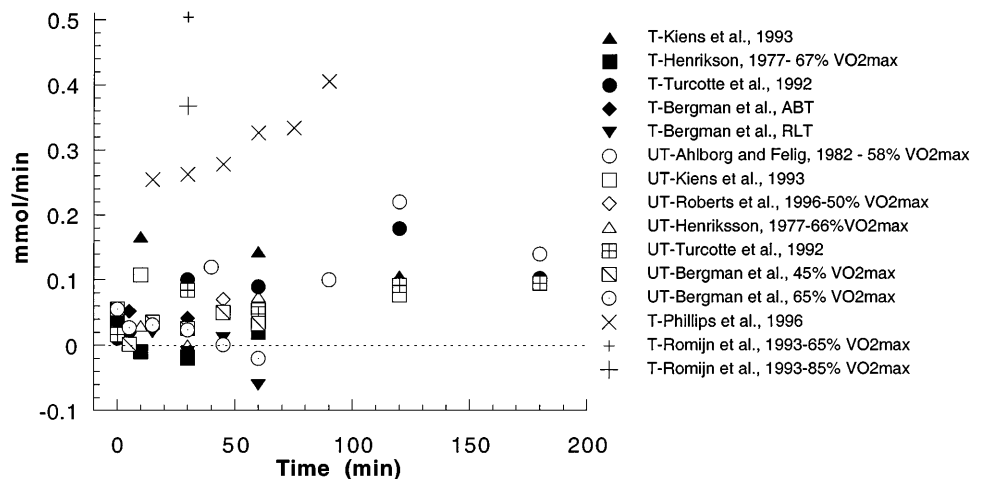
Fig. 3. Effect of exercise intensity and training on arterial glycerol concentration (A), glycerol venous-arterial difference (B), and net glycerol release (C). Values are mean \pm SE for 8 subjects in A and B and for 6 subjects in C.

Our results of resting net limb glycerol release (0.05 ± 0.01 mmol/min) are similar to previously reported values that range from 0.01 to 0.041 mmol/min (1, 22, 31, 32; Fig. 4). Unlike our results, however, some investigators have found increased net glycerol release during exercise compared with rest (1, 32). Our results are similar to those of Henriksson (17) who found unchanged glycerol release during exercise in trained and untrained working legs. Only one study reported increased net glycerol release after training at the same absolute workload, but 2 h of exercise were required to produce the effect (22). Although in the report of Kiens et al. (22) increased net limb glycerol release implicated increased intramuscular lipolysis, muscle biopsies did not reveal significantly lower IMTG stores after one leg knee extension exercise. Therefore, increased net glycerol release after 2 h of exercise may not have represented degradation of only IMTG but rather may have included FFA from other adipose tissue stores. Moreover, Phillips et al. (29) and we (12 and Friedlander et al., unpublished observation) found unchanged whole body glycerol turnover at the same absolute and relative workload after training, consistent with unaltered IMTG utilization. Thus, in the aggregate, glycerol tracer (29), limb balance (Fig. 3C and Refs. 17, 22, 35), and muscle biopsy data for IMTG (Table 3 and Refs. 22, 23) are interpreted to mean training does not increase IMTG use during exercise.

In contrast to results of net glycerol release and glycerol turnover studies, data from FFA turnover and indirect calorimetry, as well as muscle biopsies measuring IMTG, suggest that training may increase reliance on IMTG at a given absolute workload (18, 25, 29). Martin et al. (25) used the difference between whole body FFA oxidation calculated from pulmonary RER and isotopically determined FFA rate of disposal (R_d) to indirectly estimate IMTG utilization. Assuming the difference between these parameters represented intramuscular metabolism, they concluded that subjects utilized significantly more intramuscular lipid when evaluated at the same absolute workload after training.

To mimic the calculations of Martin et al. (25) and Phillips et al. (29), we used whole body fat oxidation (from RER) and limb net FFA uptake (from a-v difference) to estimate IMTG oxidation. Like them, our calculations also produced the apparent result of significantly increased (116%) IMTG oxidation at the same absolute intensity after training. Yet, using working limb net glycerol release to directly measure lipolysis, we found unchanged IMTG utilization after training at given absolute and relative exercise intensities. Our data provide evidence to suggest that methodologies employing whole body RER and FFA R_d overestimate IMTG oxidation (Fig. 6). Moreover, working muscle glycerol release rates calculated from indirect measures (29, 33) are 5- to 10-fold higher than directly measured net glycerol release rates reported for working limbs (Fig. 4). The indirect estimation of IMTG

Fig. 4. Literature values for net glycerol release determined by venous-arterial differences and intramuscular glycerol rate of appearance estimated from whole body fat oxidation and FFA rate of disposal. Mean net glycerol release at 60 min of exercise from all venous-arterial difference values including the present study is 0.05 ± 0.06 mmol/min. T, trained, UT, untrained.



oxidation (29, 33) relies on the assumption that the difference between a parameter measured in breath (i.e., total lipid oxidation) and a parameter measured in blood (i.e., FFA R_d) represents exclusively events occurring in intracellular triglyceride depots in contracting muscle. This assumption lacks experimental basis and is at variance with our results. Thus disagreement over interpretation of results obtained in various investigations regarding intramuscular lipolysis may be due to assumptions inherent in the methodologies employed.

FFA Exchange

On resting subjects following a 12- to 14-h fast, most investigations report the legs to release FFA (2, 17, 22, 31, 32). Net FFA uptake during exercise is a consistent finding (2, 14, 15, 20, 22, 30, 32, 35), with increased net FFA uptake in trained subjects only after 2 (22) and 3 h

(35) of leg kicking at the same absolute and relative intensity, respectively. Thus our limb FFA exchange data agree with literature values, as we found net limb FFA release at rest. During exercise, FFA were extracted on a net basis, with no effect of training at the same absolute workload during 1 h of cycling. Although it is an underestimate of fractional FFA extraction determined with tracers (35), fractional FFA extraction averaged 6.0% for untrained and 6.4% for trained subjects during exercise in our study, comparable to Kiens et al. (22) who observed 6–7% fractional extraction for the first hour of exercise in both trained and untrained subjects. Although there were trends suggesting elevated net FFA uptake with increasing exercise intensity ($P < 0.06$), similar to Hagenfeldt and Wahren (15), we did not find a significant difference in net FFA uptake when increasing exercise intensity before or after training (Figs. 2D and 6). In contrast to Jansson and Kaijser (20) who also compared trained and untrained subjects using cycle ergometry, we found increased net FFA uptake at the same relative intensity after training. Thus we report the only limb balance data using longitudinal comparisons indicating training-enhanced net FFA uptake at the same relative exercise intensity during cycle ergometry. Moreover, our and previous results from net limb balance (22) suggest that, after training, an exercising leg does not extract more FFA on a net basis after 1 h of exercise at a given workload.

Only one report utilized a longitudinal design to compare FFA R_d at the same absolute and relative exercise intensity before and after training (12). Friedlander et al. (12) found slightly increased tracer-measured FFA R_d for a given power output and increased FFA R_d at the same relative exercise intensity after training. If whole body FFA kinetics respond similarly to working limb net FFA uptake, one might expect increased net FFA uptake at both the same absolute and relative exercise intensity after training. However, although we found increased net FFA uptake at RLT, we did not observe significantly different net FFA uptake or leg FFA oxidation at ABT compared with 65% pretraining (Fig. 6 and Table 2), suggesting that

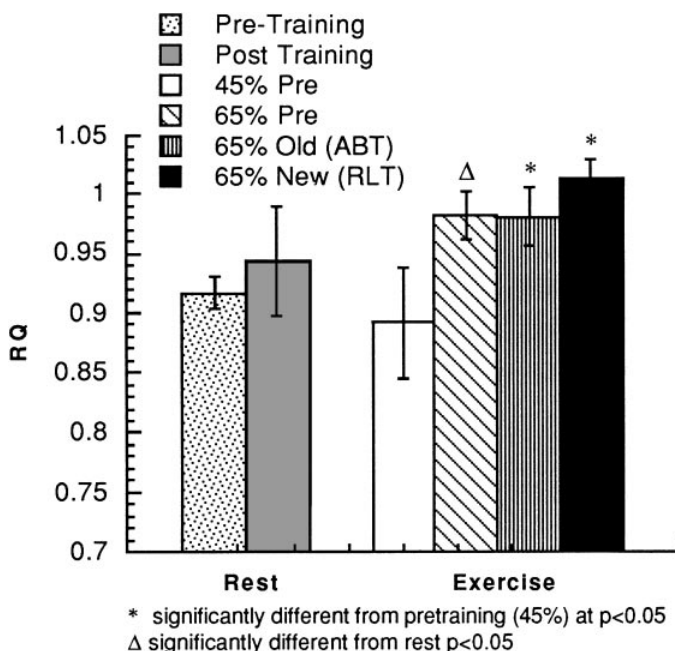


Fig. 5. Leg respiratory quotient (RQ) during rest and exercise before and after training. Exercise values are means of 1 h of exercise. Values are means \pm SE; $n = 6$ subjects.

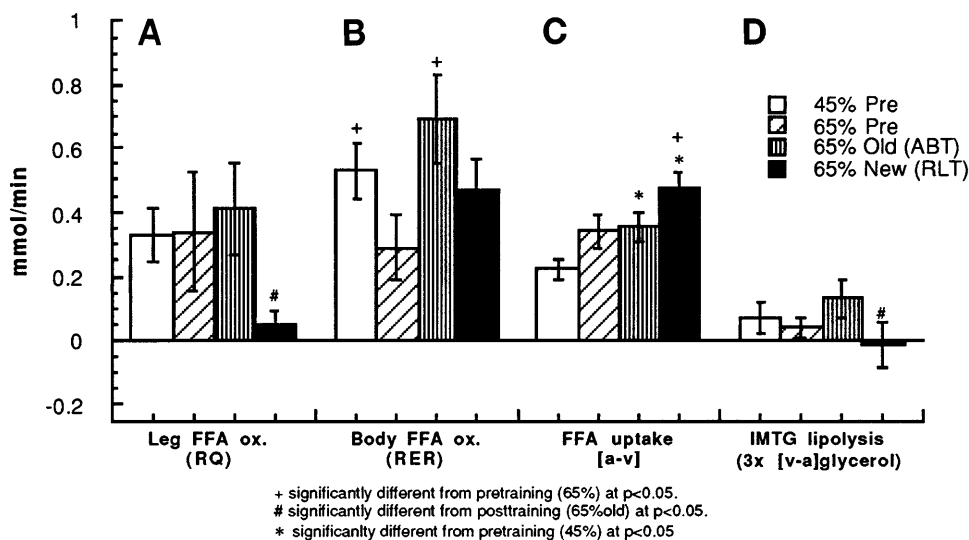


Fig. 6. Leg and whole body FFA metabolism determined from leg RQ, whole body respiratory exchange ratio (RER), and net FFA uptake. Values are means \pm SE; $n = 6-9$ subjects. RER, leg RQ, net FFA uptake, and net glycerol release are averages of the entire exercise bout. Leg (A) and whole body (B) FFA oxidation (ox) calculated from stoichiometric equations (10). FFA uptake (C) calculated as in METHODS. Intramuscular triglyceride (IMTG) lipolysis (D) calculated from the product of 3 times net glycerol difference. Please see text for details. [a-v], Arteriovenous difference; [v-a], venous-arterial difference.

whole body tracer kinetics do not always parallel FFA metabolism of the working limb.

In our report, we were careful to state that we measured leg net metabolite exchange, although we made repeated inference to muscle metabolism. Although it is true that muscle comprised most of the tissue and during exercise received most blood flow in the limbs studied, possible contributions of other tissues cannot be ignored. For instance, subcutaneous and intermuscular adipose tissue could have and probably did experience lipolysis during limb exercise. Release of glycerol and FFA from nonmuscle adipose tissue could have minimized the possibility of measuring muscle FFA uptake. However, our results are likely due to intramuscular rather than subcutaneous adipose tissue effects because muscle mass predominates in the leg. Furthermore, the anatomy of venous drainage in the leg would result in glycerol and FFA from subcutaneous fat entering the femoral venous blood without opportunity for muscle exchange. Because glycerol release was close to nil during rest and exercise, we can tentatively conclude that lipolysis in both subcutaneous and intramuscular fat depots was negligible. Additionally, neither adipocytes nor myocytes are known to contain glycerol kinase (26); thus, it is unlikely that reesterification accounted for minimal leg net glycerol release. Moreover, we overlooked possible contributions of lipid delivery via lipoproteins. Although largely unstudied, lipoproteins could deliver significant quantities of fatty acid during exercise. However, for the present, we discount this possibility, because leg RQ was high, glycerol release was small, and glycerol [v-a] declined during exercise. Thus we conclude that no lipid stores (IMTG, lipoproteins, interstitial or subcutaneous adipose tissue) underwent active lipolysis during exercise. Because future work is necessary to confirm the validity of our technical approaches and assumptions, we have emphasized interpretation of results from several sources, including whole body RER, leg RQ, glycerol, and FFA exchange. To reiterate, none of these experimental approaches indicate increased IMTG

utilization at either the same absolute or relative exercise intensity after training.

Leg RQ

There have been few studies investigating training and leg RQ, with divergent results. Henriksson (17) and Kiens et al. (22) found lower leg RQ after training at the same relative intensity during cycle ergometry, and absolute intensity during leg kicking, respectively. However, our results are similar to those of Saltin et al. (34) and Jansson and Kaijser (20) who found unchanged leg RQ's after training at a given workload during cycle ergometry. Reported differences may be associated with the mode of exercise, as power output during leg cycling is much greater than during kicking. Additionally, leg kicking does not alter the hormonal milieu characteristic of whole body exercise, which will influence substrate utilization. Thus our data, in addition to RQ values reported by others (20, 34), suggest that, unlike the whole body, leg substrate utilization does not change appreciably during cycle ergometry at a given workload after training.

Although our results of leg RQ are consistent with literature values, they do not match the pattern of limb FFA uptake observed during posttraining trials at 65% new $\dot{V}O_{2\text{peak}}$ (Fig. 6). The discrepancy may be attributed to the assumption of complete FFA oxidation or algorithms for computing leg RQ from blood gases. However, despite differences in the pattern of leg FFA oxidation determined from leg RQ and net FFA uptake, it remains that both methods yield low estimates of FFA oxidation during exercise. Additionally, after storage of FFA uptake as IMTG has been accounted for (Table 3), oxidation of the remaining FFA taken up during 65% posttraining $\dot{V}O_{2\text{peak}}$ would cause the limb RQ to decrease only from 1.00 to 0.96. Thus our conclusion of small fat oxidation by the exercising leg before and after training is supported by both FFA and glycerol a-v differences and RQ measurements.

Tracers vs. Net Balance Studies

We interpret our results of lower whole body RER but unchanged leg RQ at a given exercise intensity to mean that inactive tissue and not active skeletal muscle increases fat oxidation after training (Table 2). Similarly, Odland et al. (28) studied subjects 2–3 h after a meal during cycle ergometry at 65% $\dot{V}O_{2\max}$ and found lower values for whole body RER compared with active leg RQ (RER of 0.94 and leg RQ of 1.0). Although the authors do not discuss the point, their data also suggest that the majority of fat oxidation occurs in inactive tissue, with the active leg dependent almost exclusively on CHO as a fuel. Indirect support for the notion of inactive tissue as an important site of increased FFA oxidation after training comes from studies of glucose metabolism. Although glucose R_d decreases during exercise at the same absolute intensity (6, 7, 11), it is less certain that exercising muscle is the site of decreased glucose uptake. Results of studies showing decreased glucose R_d after training (6, 7, 11) may be reconciled with those showing no effect of training on working limb glucose uptake (17, 35) if glucose uptake by inactive tissue decreases (1) with concomitant increased FFA uptake and there occurs “shunting” of blood glucose to active tissues after training (4). Thus, if one accepts data from indirect calorimetry (28) and tracer-measured FFA R_d (11, 12, 25) as well as the current data as correct, then a conclusion that emerges is that, in well-nourished subjects exercising for periods of ~1 h, working skeletal muscle depends on CHO-derived energy sources (glycogen, glucose, lactate), whereas the remainder of the body switches to predominant lipid oxidation.

Differences between the present investigation showing a training effect increasing working limb FFA uptake (Figs. 2D and 6) and previous studies on men using tracers to estimate FFA R_d may be related more to the subject populations studied and details of protocol rather than physiology. For instance, in their report, Martin et al. (25) observed training to decrease FFA R_d for exercise at a given power output. However, in their study, circulating [FFA] were lower after training, whereas in our study arterial [FFA] rose to the same extent under all exercise conditions (Fig. 2A), and we measured increased net FFA uptake in working limbs at a given relative power output. Based on extensive biochemical studies demonstrating training to increase muscle capillarity, mitochondrial, and fatty acid binding protein contents, if blood flow and arterial [FFA] are maintained to working trained muscles, increased net FFA uptake is the expected result (J. O. Holloszy, personal communication).

In conclusion, results of the present study suggest that training does not increase reliance on IMTG stores at either the same absolute or relative exercise intensity when subjects are tested in energy and nitrogen balance. Even though we observed a small increase in working limb FFA at a power output eliciting 65% of $\dot{V}O_{2\text{peak}}$ after training, the balance of muscle substrate utilization was changed little by 9 wk of endurance

cycle training. CHO-derived energy sources were the main fuel sources for working skeletal muscle during prolonged (1-h) moderate-intensity exercise, both before and after training. Furthermore, accepting the present data using the mass balance technique, along with other data obtained using indirect calorimetry and isotope tracers, we conclude that, during exercise, CHO-derived energy sources sustain working muscle while other tissues depend on lipid oxidation.

The investigators thank the subjects for participating in this study and complying with the training program. The assistance of the nursing staff and dietitians at the General Research, Education, and Clinical Center in the Palo Alto Veterans Affairs (VA) Health Care Center is appreciated. We also thank David Guido for performing muscle biopsies and Jacinda Mawson and for blood gas analysis. We thank the student trainers who were vital in subject training and transport. We greatly appreciate the help of Barry Braun in blood sampling during the VA trials. We also thank Champion Nutrition for the generous gift of Cytomax consumed by subjects after training.

This work was supported by National Institute of Health Grants AR-42906 and DK-19577.

Address for reprint requests: G. A. Brooks, Exercise Physiology Laboratory, Dept. of Integrative Biology, 5101 Valley Life Sciences Bldg., Univ. of California, Berkeley, CA 94720-3140.

Received 23 February 1998; accepted in final form 28 September 1998.

REFERENCES

1. **Ahlborg, G., and P. Felig.** Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged exercise. *J. Clin. Invest.* 69: 45–54, 1982.
2. **Ahlborg, G., P. Felig, L. Hagenfeldt, R. Hendler, and G. Wahren.** Substrate turnover during prolonged exercise in man: splanchnic and leg metabolism of glucose, free fatty acids and amino acids (Abstract). *J. Clin. Invest.* 53: 1080, 1974.
3. **Bender, P. R., B. M. Groves, R. E. McCullough, R. G. McCullough, S. Y. Huang, A. J. Hamilton, P. D. Wagner, A. Cymerman, and J. T. Reeves.** Oxygen transport to exercising leg in chronic hypoxia. *J. Appl. Physiol.* 65: 2592–2597, 1988.
4. **Brooks, G. A.** Physical activity and carbohydrate metabolism. In: *Physical Activity, Fitness, and Health*, edited by C. Bouchard, R. J. Shepard, and T. Stephens. Champaign, IL: Human Kinetics, 1994, p. 401–416.
5. **Carraro, F., C. A. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolfe.** Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 259 (Endocrinol. Metab. 22): E470–E476, 1990.
6. **Coggan, A., W. Kohrt, R. Spina, D. Bier, and J. Holloszy.** Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. *J. Appl. Physiol.* 68: 990–996, 1990.
7. **Coggan, A., S. Swanson, L. Mendenhall, D. Habash, and C. Kien.** Effect of endurance training on hepatic glycogenolysis and gluconeogenesis during prolonged exercise in men. *Am. J. Physiol.* 268 (Endocrinol. Metab. 31): E375–E383, 1995.
8. **Douglas, A., N. Jones, and J. Reed.** Calculation of whole blood CO_2 content. *J. Appl. Physiol.* 65: 473–477, 1988.
9. **Duncombe, W. G.** The colorimetric micro-determination of long-chain fatty acids. *Biochem. J.* 88: 7–10, 1963.
10. **Frayn, K.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* 55: 628–634, 1983.
11. **Friedlander, A., G. Casazza, M. Huie, M. Horning, and G. Brooks.** Endurance training alters glucose kinetics in response to the same absolute, but not the same relative workload. *J. Appl. Physiol.* 82: 1360–1369, 1997.
12. **Friedlander, A. L., G. A. Casazza, M. A. Horning, T. F. Buddinger, and G. A. Brooks.** Effects of exercise intensity and training on lipid metabolism in young women. *Am. J. Physiol.* 275 (Endocrinol. Metab. 38): E853–E863, 1998.

13. **Gutmann, I., and A. Wahlefeld.** L(-)-Lactate determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis* (2nd ed.), edited by H. Bergmeyer. New York: Academic, 1974, p. 1464–1468.
14. **Hagenfeldt, L., and J. Wahren.** Human forearm muscle metabolism during exercise II uptake, release and oxidation of individual FFA and glycerol. *Scand. J. Clin. Lab. Invest.* 21: 263–276, 1968.
15. **Hagenfeldt, L., and J. Wahren.** Human forearm muscle metabolism during exercise VII. FFA uptake and oxidation at different work intensities. *Scand. J. Clin. Lab. Invest.* 30: 429–436, 1972.
16. **Hagenfeldt, L., J. Wahren, B. Pernow, and L. Raf.** Uptake of individual free fatty acids by skeletal muscle and liver in man. *J. Clin. Invest.* 51: 2324–2330, 1972.
17. **Henriksson, J.** Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J. Physiol. (Lond.)* 270: 661–675, 1977.
18. **Hurley, B., P. Nemeth, W. H. Martin III, J. Hagberg, G. Dalsky, and J. Holloszy.** Muscle triglyceride utilization during exercise: effect of training. *J. Appl. Physiol.* 60: 562–567, 1986.
19. **Jackson, A., and M. Pollock.** Generalized equations for predicting body density of men. *Br. J. Nutr.* 40: 497–504, 1978.
20. **Jansson, E., and L. Kaijser.** Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men. *J. Appl. Physiol.* 62: 999–1005, 1987.
21. **Kelman, G.** Digital computer procedure for the conversion of Pco₂ into blood content. *Respir. Physiol.* 3: 111–115, 1967.
22. **Kiens, B., B. Essen-Gustavsson, N. Christensen, and B. Saltin.** Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training. *J. Physiol. (Lond.)* 469: 459–478, 1993.
23. **Kiens, B. K., and E. A. Richter.** Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E332–E337, 1998.
24. **Lopaschuk, G. D., C. A. Hansen, and J. R. Neely.** Fatty acid metabolism in hearts containing elevated levels of CoA. *Am. J. Physiol.* 250 (*Heart Circ. Physiol.* 19): H351–H359, 1986.
25. **Martin, W., G. Dalsky, B. Hurley, D. Matthews, D. Bier, J. Hagberg, M. Rogers, D. King, and J. Holloszy.** Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E708–E714, 1993.
26. **Newsholme, E., and A. Leech.** *Biochemistry for the Medical Sciences.* New York: Wiley, 1983.
27. **Nunn, J. F.** *Nunn's Applied Respiratory Physiology.* Boston, MA: Butterworth-Heinemann, 1993.
28. **Odland, L. M., G. J. Heigenhauser, D. Wong, M. G. Hollidge-Horvat, and L. L. Spriet.** Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R894–R902, 1998.
29. **Phillips, S., H. Green, M. Tarnopolsky, G. Heigenhauser, R. Hill, and S. Grant.** Effects of training duration on substrate turnover and oxidation during exercise. *J. Appl. Physiol.* 81: 2182–2191, 1996.
30. **Richter, E., B. Kiens, B. Saltin, N. Christensen, and G. Savard.** Skeletal muscle glucose uptake during dynamic exercise in humans: role of muscle mass. *Am. J. Physiol.* 254 (*Endocrinol. Metab.* 17): E555–E561, 1988.
31. **Richter, E., L. Turcotte, P. Hespel, and B. Kiens.** Metabolic responses to exercise. Effects of endurance training and implications for diabetes. *Diabetes Care* 15: 1767–1776, 1992.
32. **Roberts, A., G. Butterfield, A. Cymerman, J. Reeves, E. Wolfel, and G. Brooks.** Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate. *J. Appl. Physiol.* 81: 1762–1771, 1996.
33. **Romijn, J., E. Coyle, L. Sidossis, A. Gastaldelli, J. Horowitz, E. Enderet, and R. Wolfe.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E380–E391, 1993.
34. **Saltin, B., K. Nazar, D. Costill, E. Stein, E. Jansson, B. Essen, and P. Gollnick.** The nature of the training response: peripheral and central adaptations to one-legged exercise. *Acta Physiol. Scand.* 96: 289–305, 1976.
35. **Turcotte, L., E. Richter, and B. Kiens.** Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. *Am. J. Physiol.* 262 (*Endocrinol. Metab.* 25): E791–E799, 1992.
36. **Wendling, P. S., S. J. Peters, G. J. Heigenhauser, and L. L. Spriet.** Variability of triacylglycerol content in human skeletal muscle biopsy. *J. Appl. Physiol.* 81: 1150–1155, 1996.
37. **Wolfel, E., B. Groves, G. Brooks, G. Butterfield, R. Mazzeo, L. Moore, J. Sutton, P. Bender, T. Dahms, R. McCullough, R. McCullough, S.-Y. Huang, S. Sun, R. Grover, H. Hultgren, and J. Reeves.** Oxygen transport during steady-state submaximal exercise in chronic hypoxia. *J. Appl. Physiol.* 70: 1129–1136, 1991.