Substantial working muscle glycerol turnover during two-legged cycle ergometry

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Wallis GA, Friedlander AL, Jacobs KA, Horning MA, Fattor JA, Wolfel EE, Lopaschuk GD, Brooks GA. Substantial working muscle glycerol turnover during two-legged cycle ergometry. Am J Physiol Endocrinol Metab 293:E950–E957, 2007. First published July 10, 2007; doi:10.1152/ajpendo.00099.2007.—We combined tracer and arteriovenous (a-v) balance techniques to evaluate the effects of exercise and endurance training on leg triacylglyceride turnover as assessed by glycerol exchange. Measurements on an exercising leg were taken to be a surrogate for working skeletal muscle. Eight men completed 9 wk of endurance training (5 days/wk, 1 h/day, 75% peak oxygen consumption (Vo2peak), with leg glycerol turnover determined during two pretraining trials [45% and 65% Vo2peak (45% Pre and 65% Pre, respectively)] and two posttraining trials [65% of pretraining Vo2peak (ABT) and 65% of posttraining Vo2peak (RLT)] using [1H3]glycerol infusion, femoral a-v sampling, and measurement of leg blood flow. Endurance training increased Vo2peak by 15% (45.2 ± 1.2 to 52.0 ± 1.8 ml·kg⁻¹·min⁻¹, P < 0.05). At rest, there was tracer-measured leg glycerol uptake (41 ± 8 and 52 ± 15 μmol/min for pre- and posttraining, respectively) even in the presence of small, but significant, net leg glycerol release (~68 ± 19 and ~50 ± 13 μmol/min, respectively; P < 0.05 vs. zero). Furthermore, while there was no significant net leg glyceral exchange during any of the exercise bouts, there was substantial tracer-measured leg glycerol turnover during exercise (i.e., simultaneous leg muscle uptake and leg release) (uptake, release: 45% Pre, 194 ± 41, 214 ± 33; 65% Pre, 217 ± 79, 201 ± 84; ABT, 275 ± 76, 312 ± 87; RLT, 282 ± 83, 424 ± 75 μmol/min; all P < 0.05 vs. corresponding rest). Leg glycerol turnover was unaffected by exercise intensity or endurance training. In summary, simultaneous leg glycerol uptake and release (indicative of leg triacylglyceride turnover) occurs despite small or negligible net leg glyceral exchange, and furthermore, leg glycerol turnover can be substantially augmented during exercise.

Subjects

Eight healthy, nonsmoking, sedentary men aged 18–32 yr were recruited from the University of California, Berkeley, by posted notices. Participants were considered untrained if they had engaged in no more than 2 h of physical activity per week for the previous year and had peak oxygen consumptions (Vo2peak) of <50 ml·kg⁻¹·min⁻¹. They were also required to be diet and weight

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METHODS

Descriptions of the experimental protocols employed have been presented in previous reports (7, 16). However, pertinent methodological details relevant to the focus on exercise and training effects on leg and whole body glycerol metabolism are reiterated.

Subjects

Eight healthy, nonsmoking, sedentary men aged 18–32 yr were recruited from the University of California, Berkeley, by posted notices. Participants were considered untrained if they had engaged in no more than 2 h of physical activity per week for the previous year and had peak oxygen consumptions (Vo2peak) of <50 ml·kg⁻¹·min⁻¹. They were also required to be diet and weight.

WHOLE BODY LIPOLYTIC RATE is typically assessed using the isotope tracer dilution technique (35), and there has been extensive application of this approach to study the response of systemic glycerol turnover to exercise. Systemic glycerol turnover has been shown to increase several-fold in response to exercise (6, 25, 28), although the effects of whole body exercise on glycerol metabolism in different body segments and particularly in the working limbs and muscles of those limbs have received limited attention. In fact, our understanding of the response of whole working limb glycerol metabolism to exercise (e.g., the legs during 2-legged cycle ergometry) is largely limited to the interpretation of net limb glycerol balance data (i.e., arteriovenous concentration difference × blood flow) (1, 13). Previously, we reported essentially zero net leg glycerol balance during two-legged cycle ergometry regardless of exercise intensity or endurance training status, findings that, buoyed by the absence of net reductions in muscle biopsy triacylglyceride content and a leg respiratory quotient (RQ) of ~1.0, were interpreted to mean that working muscle lipolysis is minimal during whole body exercise (1). However, reports of significant limb glycerol turnover (i.e., simultaneous uptake and release) at rest (4, 5, 17, 18, 22, 26) and during low power output muscle contractions (10, 11, 34) raised concern that the response of limb glycerol metabolism to whole body exercise could be greater than previously appreciated.

Therefore, the purpose of the present study was to use a longitudinal study design and combine isotope tracer infusion ([1H3]glycerol) with measurements of femoral blood flow and arteriovenous blood sampling to fully address the scope of variation in leg glycerol metabolism in response to exercise and endurance training. Measurements on an exercising leg were taken to be a surrogate for working skeletal muscle. Importantly, we used the two-legged cycle ergometer exercise model, such that our findings would reflect metabolic responses to the whole body exercise tasks typically encountered by individuals engaged in regular physical activity.

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stable, have a body fat percentage of <25%, and deemed injury and disease free as determined by a health history questionnaire and physical examination. Participants provided written, informed consent, and the study was approved by the Committee for the Protection of Human Subjects at Stanford University and the University of California (CPHS 2002-3-21).

Experimental Design

Following preliminary testing, participants completed two experimental trials consisting of 90 min of rest followed by 60 min of two-leg cycle ergometry before and after 9 wk of endurance training. Before training, the exercise component of the experimental trials was performed at an intensity that corresponded to 45% (45% Pre) and 65% (65% Pre) VO2peak, and these trials were randomly allocated and separated by 1 wk. After training, the exercise component of the trials corresponded to 65% of pretraining VO2peak [same absolute intensity (ABT)] and 65% posttraining VO2peak [same relative intensity (RLT)]. Again, these trials were randomly allocated and separated by 1 wk, during which exercise training continued.

Fitness Assessments

VO2peak was determined before training, after 4–5 wk of training, and at the end of the 9-wk training period, using an incremental cycle test performed to volitional exhaustion on an electromagnetically braked cycle ergometer (Monark Ergometric 839E, Vansbro, Sweden), as previously described (16). Respiratory gas exchange measurements during assessments of VO2peak were made using an online automated gas analysis system (ParvoMedics TrueMax 2400, Sandy, UT). Body composition was determined from skinfold thickness measurements (15). Three-day diet records were collected to obtain the subjects’ habitual dietary composition and caloric intake before training, with records analyzed using the Nutritionist III software (N-Squared Computing, Salem, OR).

Experimental Trials

Participants arrived at the Clinical Studies Unit at the Veterans Affairs Palo Alto Health Care System before 8 PM on the evening before experimental trials and were fed a standard meal of 1,800 kcal, with 69% of calories as carbohydrate (CHO), 21% as fat, and 10% as protein. Experimental trials were performed the following morning or afternoon (2 subjects/day, consistent between trials) in the postabsorptive state. Morning subjects were fed a standard pretrial meal (434 kcal; 74% CHO, 10% fat, 16% protein) at ~5 AM, 1 h before procedures started and 3 and 4.5 h before the resting and exercise components of trials commenced, respectively. Afternoon subjects were fed a standard breakfast (661 kcal; 55% CHO, 34% fat, 11% protein) and the identical pretrial meal as the morning subjects 1 h (~12 PM) before procedures started.

One hour after the pretrial meal, the femoral artery and vein of the same leg were catheterized for blood sampling under local anesthesia using standard percutaneous techniques, as previously described (16), with catheter placement localized using vascular ultrasound (Site-Rite 3, Bard Access Systems, Dymax, Miami, FL). Alternate legs were used for the two trials during both pre- and posttraining testing. A venous catheter was placed in one arm for tracer infusion. Following a background blood sample collection, a primed continuous infusion of [6,6-2H2]glycerol (prime, 11.54 ± 0.25 μmol/kg; continuous, 0.05 ± 0.00 μmol·kg⁻¹·min⁻¹) was commenced for 90 min of semi-supine rest and for the duration of the 60-min exercise period. The tracer infusion rate was increased and maintained at exercise onset to 0.10 ± 0.00 μmol·kg⁻¹·min⁻¹ for 45% Pre, 65% Pre, and ABT and to 0.15 ± 0.01 μmol·kg⁻¹·min⁻¹ for RLT.

Training Protocol

Participants completed 9 wk of unsupervised endurance training performed on stationary leg cycle ergometers. Supervised training was performed for 1 h each day on 5 days/wk, with an additional hour of unsupervised exercise performed on the weekend. During the first 3 wk of training, exercise intensity was gradually increased from 50 to 75% of each individual’s VO2peak. After 6 wk of continuous cycle training, two sessions per week were replaced by interval training. Exercise intensity was monitored by heart rate. Workload was adjusted accordingly to maintain the required relative intensity as aerobic capacity, assessed by periodic evaluations of VO2peak, improved. Throughout training, participants were weighed daily and instructed to increase energy intake to maintain weight during the training program without changing dietary macronutrient composition. Concordance with dietary requests was confirmed by analysis of 3-day diet records collected over the course of the study.

Blood Sampling and Analysis

During the experimental trials, arteriovenous (a-v) femoral blood samples were drawn simultaneously and anaerobically at 0, 75, and 90 min of rest and after 30, 45, and 60 min of exercise. Blood samples were transferred into prechilled tubes containing 8% perchloric acid and stored on ice until centrifugation. Samples were then centrifuged (20 min at 3,000 g at 4°C), and perchloric extracts were transferred to storage tubes and frozen at −20°C until analysis. Immediately after each blood sample collection, leg venous blood flow was determined using the thermodilution technique as previously described (16).

Whole blood glycerol concentration and enrichment were determined in neutralized perchloric extracts using gas chromatography-mass spectrometry (GC-MS) of the triacetate derivative (GC, model 5890, series II; MS, model 5989A; both Hewlett-Packard), using [U-13C]glycerol as an internal standard for concentration measurement. Briefly, neutralized perchloric extracts were passed through cation (AG 50W-X8, 50–100 mesh H+ resin) and anion (AG 1-X8, 200–400 mesh formate resin) exchange columns, and glycerol was eluted with deionized water. Thereafter, samples were lyophilized, transferred to GC-MS vials using methanol, and evaporated under a stream of N2. The samples were subsequently derivatized with 100 μl of an acetic anhydride-pyridine mixture (2:1). Chemical ionization (methane gas) was used with selected ion monitoring for mass-to-charge ratios of 159 (unlabeled glycerol) and 162 (M+3) or 159 and 164 (M+5) for determination of blood glycerol concentration and enrichment, respectively.

Calculations

Leg glycerol flux. Net leg glycerol balance, glycerol tracer leg fractional extraction, tracer-measured leg glycerol uptake, and total leg glycerol release were determined as follows

\[
\text{net leg balance (μmol/min) = 2 (1} g \times C_e - C_v) \\
\text{fractional extraction (%) =} \frac{E \times C_e - E \times C_v}{E \times C_v} \times 100 \\
\text{leg uptake (μmol/min) = 2 (1} g \times C_e) \\
\text{Total leg release (μmol/min) = leg uptake} - \text{net balance}
\]

where Q represents leg blood flow, and C and E are glycerol concentration and enrichment in arterial (a) and venous (v) blood, respectively.

Whole body glycerol kinetics. Whole body measurements of the rates of appearance (Ra) and disappearance (Rd) of glycerol were calculated using the single-pool non-steady-state model modified for use with stable isotopes (32, 35).
GLYCEROL TURNOVER AND TRAINING

\[
R_a = \frac{F - V[(C_2 + C_1)/2](E_2 - E_1)(t_2 - t_1)}}{(E_2 + E_1)/2}
\]

\[
R_d = R_a - V[(C_2 - C_1)(t_2 - t_1)]
\]

where \(F\) is the tracer infusion rate (\(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\)), \(V\) is the volume of distribution (270 ml/kg), \(C_2\) and \(C_1\) are the arterial glycerol concentrations at times 2 and 1 (i.e., \(t_2\) and \(t_1\)), respectively, and \(E_2\) and \(E_1\) are the arterial glycerol isotopic enrichments at times 2 and 1, respectively. Glycerol metabolic clearance rate (MCR) was calculated as \(R_d\) of glycerol divided by the mean arterial glycerol concentration over that time period.

The contribution (%) of leg glycerol turnover to whole body glycerol turnover was calculated as 100·[leg glycerol uptake or release/\(R_a\) or \(R_d\) (in \(\mu\)mol/min)], while the fraction (%) of systemically released glycerol cleared by the legs was calculated as 100·[leg glycerol uptake/\(R_a\) (in \(\mu\)mol/min)].

**Statistics**

Data are presented as means ± SE. Paired-sample \(t\)-tests were used to determine changes in subject characteristics with training. A two-way repeated-measures analysis of variance (RANOVA) was used to determine differences in blood glycerol concentration over time and between trials. For each trial, differences between the increase in glycerol concentration ([glycerol]) in arterial and venous blood over time during the exercise period were assessed using 95% confidence intervals (95% CI) (e.g., change in arterial [glycerol] vs. change in venous [glycerol] from 30–45 and 45–60 min). Changes in blood glycerol enrichment over time were investigated by one-way RANOVA for arterial and venous blood in each individual trial. Unless stated otherwise, remaining results were averaged for the 75- to 90-min period at rest and the 30- to 60-min period during exercise, and mean differences were assessed by one-way RANOVA. For all RANOVA tests, significant effects were followed by pairwise comparisons using Fishers least significant difference test. Additionally, leg a-v [glycerol] difference and leg balance data were assessed using 95% CI; trials that did not contain zero were deemed to have reached significant levels of uptake or release. Statistical significance was accepted at \(P < 0.05\).

**RESULTS**

All eight subjects completed the training program. One subject experienced pain related to the femoral catheter placement during exercise in 45% Pre, and the catheter was removed. Another subject was deemed a statistical outlier following analysis of exercise responses in RLT, as his net glycerol balance values were greater than the mean ± 3 standard deviations. Therefore, for leg-specific responses, comparisons including both 45% Pre and RLT were made with six subjects, while seven subjects were included for comparisons involving the three 65% trials (65% Pre, ABT, RLT). Accordingly, analysis of whole body responses included seven subjects involving the three 65% trials (65% Pre, ABT, RLT). Power outputs required to elicit 45% Pre and 65% Pre were 99 ± 4 and 156 ± 6 W, respectively. The endurance training program elicited significant increases in \(V_{\text{O}_2}\text{peak}\), such that exercise bouts performed at ABT elicited only ~55% of the new posttraining \(V_{\text{O}_2}\text{peak}\), and 193 ± 10 W was required to achieve 65% of \(V_{\text{O}_2}\text{peak}\) in RLT.

**Subject Characteristics**

Participants remained stable in body weight and composition during the training period (Table 1). Power outputs required to elicit 45% Pre and 65% Pre were 99 ± 4 and 156 ± 6 W, respectively. The endurance training program elicited significant increases in \(V_{\text{O}_2}\text{peak}\), such that exercise bouts performed at ABT elicited only ~55% of the new posttraining \(V_{\text{O}_2}\text{peak}\), and 193 ± 10 W was required to achieve 65% of \(V_{\text{O}_2}\text{peak}\) in RLT.

![Table 1. Subject characteristics before and after 9 wk of endurance training](image-url)

Other specific cardiovascular and metabolic adaptations at the regional (leg/muscle) and whole body level are reported in previous studies (7, 16).

**Blood [Glycerol], a-v [Glycerol] Difference, and Net Leg Glycerol Balance**

In all trials, femoral arterial and venous [glycerol] remained stable during the resting period but was significantly elevated above resting values after 30 min of exercise (30 min vs. −15 and 0 min, \(P < 0.05\)) and continued to rise with exercise duration (30 < 45 < 60 min, \(P < 0.05\); Fig. 1). In addition, the increase in arterial and venous glycerol concentration was similar during exercise from 30 to 60 min. Despite similar patterns in response to exercise, arterial and venous [glycerol] was systematically higher during RLT compared with 65% Pre. A significant negative a-v [glycerol] difference was observed at rest, although the a-v [glycerol] difference was not significantly different from zero during exercise in any trial (Fig. 2A). Accordingly, during rest, a significant net leg glycerol release (Fig. 2B) was observed both before (−68 ± 19 \(\mu\)mol/min) and after (−50 ± 13 \(\mu\)mol/min) training. Furthermore, although subject to considerable individual variability, there was no significant net leg glycerol exchange during exercise in any trial, nor was leg glycerol balance significantly influenced by exercise, exercise intensity, or training.

**Blood Glycerol Enrichment and Leg Glycerol Turnover**

Our efforts to minimize changes in arterial blood glycerol enrichment during exercise were successful, reducing exercise in all trials and during the entire exercise period in 45% Pre and RLT (Fig. 3). Moreover, arterial glycerol enrichments were stable during the 45- to 60-min and 30- to 45-min exercise period in 65% Pre and ABT, respectively. Venous glycerol enrichments were stable at rest and increased significantly during exercise in all trials as blood flow increased (16), remaining stable during the exercise period in 45% Pre, ABT, and RLT. In 65% Pre, venous glycerol enrichment did not change between 30 and 45 min of exercise but was reduced on average by 0.37% (\(P < 0.05\)) between 45 and 60 min. Nonetheless, as can be seen from the individual trials in Fig. 3, blood glycerol enrichment in the femoral venous effluent was considerably lower than the arterial enrichment at rest (~52% of the arterial enrichment) and remained lower during exercise (~86% of arterial enrichment).
Calculations of glycerol kinetics were limited to periods of steady-state arterial isotope enrichments, vide supra. Fractional extraction of glycerol by the legs was 41% at rest and was reduced significantly during exercise (to 10%) because of the five- to eightfold increase in leg blood flow associated with exercise (Fig. 4A) (16). Tracer-measured leg glycerol uptake (Fig. 4B) was unchanged at rest before and after training and significantly increased from rest to exercise in all trials. Tracer-determined leg glycerol release (Fig. 4C) was also unchanged at rest before and after training and increased significantly from rest to exercise in all trials. Neither tracer-measured leg glycerol uptake nor release was significantly affected by exercise intensity or training.

Whole Body Glycerol Turnover and Relative Leg Contributions

Responses for systemic glycerol R_s and R_d are similar and thus described collectively (Fig. 5). Compared with the corresponding values during rest, exercise resulted in a significant (~2- to 3-fold) increase in whole body glycerol turnover. A significant effect of exercise intensity was observed after (RLT > ABT) but not before (45% Pre vs. 65% Pre) training. Furthermore, endurance training resulted in a modest but significant increase in whole body glycerol turnover during exercise performed at the same relative power output (RLT > 65% Pre). Glycerol MCR was unaffected by exercise, exercise intensity, or training (rest, 26 ± 1 ml·kg⁻¹·min⁻¹; exercise, 24 ± 1 ml·kg⁻¹·min⁻¹).

Under resting conditions, the legs accounted for ~63% of systemic glycerol release and ~28% of systemic glycerol

Fig. 1. Femoral artery and vein blood glycerol concentration ([glycerol]) at rest and during exercise before and after 9 wk of endurance training. Time 0 denotes the onset of exercise. For statistical analysis, arterial or venous data from each trial were considered as a whole, although for clarity, each trial is reported separately. A, B, C, and D: experimental trials for 45% Pre, 65% Pre, ABT, and RLT, respectively. Pretraining trials: 45% Pre and 65% Pre, 45 and 65% peak oxygen consumption (VO_2peak), respectively. Posttraining trials: ABT-Post, 65% of pretraining VO_2peak (absolute intensity); RLT-Post, 65% of posttraining VO_2peak (relative intensity). Values are means ± SE. For arterial values, n = 7 for comparisons involving 45% Pre, and n = 8 for comparisons involving 65% Pre, ABT, and RLT; for venous values, n = 6 for comparisons involving 45% Pre, and n = 7 for comparisons involving 65% Pre, ABT, and RLT. *Significantly different from corresponding rest value; #significantly different from corresponding value in 65% Pre, P < 0.05.

Fig. 2. Leg arteriovenous (a-v) [glycerol] difference (A) and leg glycerol net balance (B) at rest and during exercise before and after 9 wk of endurance training. Values are means ± SE; n = 6 for comparisons involving both 45% Pre and RLT, and n = 7 for comparisons involving 65% Pre, ABT, and RLT. *Significantly different from zero, P < 0.05.
disappearance (Table 2). Accordingly, ∼31% of systemically released glycerol was cleared by the legs at rest. The proportion of systemic glycerol Ra accounted for by the legs tended to increase during exercise (to ∼88%, 1.4-fold increase). Also, a marked increase in mean contribution of the legs to whole body glycerol Rd and in the clearance of systemically released glycerol was observed during exercise (to 68–96%, 2- to 3-fold increase). Despite the observed average increases due to exercise, intersubject variability limited the detection of statistical significance.

**DISCUSSION**

In the present study, stable isotope dilution and arteriovenous difference techniques were combined to assess the response of leg glycerol turnover to exercise and endurance training. As demonstrated previously, the resting legs displayed a small net glycerol release, while there was no net leg glycerol exchange during exercise (1). However, we observed substantial dilution of blood glycerol enrichment across the legs at rest and also during exercise. Our results confirm reports that significant limb glycerol turnover (i.e., simultaneous uptake and release) can occur (4, 5, 10, 11, 17, 18, 22, 26, 34), and furthermore, we provide novel data showing that leg glycerol turnover is substantially increased during whole body two-legged cycle ergometer exercise.

![Fig. 3. Femoral artery and vein blood glycerol enrichment at rest and during exercise before and after 9 wk of endurance training. Time 0 denotes the onset of exercise. For statistical analysis, arterial or venous data from each trial were considered separately. A, B, C, and D: experimental trials for 45% Pre, 65% Pre, ABT, and RLT, respectively. IE, isotopic enrichment; MPE, mole percent excess. Values are means ± SE; n = 7 in 45% Pre and RLT venous, and n = 8 for all other trials. *Significantly different from rest (venous only); #significantly different from preceding exercise time point (arterial only), P < 0.05.](image)

![Fig. 4. Leg glycerol kinetics at rest and during exercise before and after 9 wk of endurance training. Values are means ± SE; n = 6 for comparisons involving both 45% Pre and RLT, and n = 7 for comparisons involving 65% Pre, ABT, and RLT. *Significantly different from corresponding rest value, P < 0.05.](image)
As noted above, the present data showing significant leg muscle glycerol uptake during postabsorptive rest (fractional extraction, ~41%) are consistent with previous reports that have documented limb glycerol uptake in humans. Additionally, we demonstrate substantial leg muscle glycerol uptake (fractional extraction, ~10%) during exercise (~4.5-fold above that observed at rest). To our knowledge, we are the first to report muscle glycerol uptake during whole body exercise (2-legged cycle ergometry). Previously, the one- or two-legged knee extension model has been used to study the response of thigh muscle glycerol uptake to muscle contraction per se (11, 34). The two-legged cycle ergometry model used in the present investigation allows the study of metabolic responses to exercise involving high power outputs characterized by large increases in muscle recruitment, whole body metabolic demand, and autonomic neuroendocrine response. Also, our findings have external relevance in that the whole body exercise tasks described herein are typical of those performed by individuals engaged in regular physical activity. Remarkably, despite discrepancies in the experimental approach, our findings of exercise-associated augmented leg muscle glycerol uptake during two-legged cycle ergometry are entirely consistent with the several-fold increase in thigh muscle glycerol uptake observed using the knee extension model (11, 34).

Additionally, our data confirm that there can be substantial extrahepatic and extrarenal glycerol clearance during resting conditions (4, 22), and furthermore, this appears to be increased during dynamic and intense whole body exercise (~31 and ~77% of systemic glycerol Ra was cleared by the legs during rest and exercise, respectively). The observation of glycerol uptake in resting and working leg muscle represents a considerable departure from the traditional understanding that the liver and kidneys predominate in glycerol clearance (14). In part, thinking has been dominated by findings of low glycerol kinase expression and/or activity (and thus low potential for glycerol utilization) in peripheral mammalian tissues (24, 27, 29, 30). However, experiments performed on laboratory rats have demonstrated direct and substantial incorporation of blood glycerol into skeletal muscle triacylglyceride in the quadriceps, soleus, and gastrocnemius in vivo (9). Furthermore, substantial tracer-determined glycerol uptake at rest and during muscle contraction has been demonstrated in human models, interpreted to reflect predominantly skeletal muscle metabolism (4, 5, 10, 11, 22, 34). Thus our data showing substantial leg muscle glycerol uptake is consistent with the concept that tissue heterogeneity in glycerol kinase function is a relative limitation offset by the mass of peripheral tissue such as skeletal muscle in the leg in humans (9, 22).

We interpret the observed glycerol uptake as leg muscle glycerol uptake, although we acknowledge that, because our measurements were made across the whole leg, we cannot categorically attribute uptake to a specific tissue compartment (e.g., skeletal muscle or adipose tissue). However, both human and animal studies show skeletal muscle glycerol uptake, with the latter defining glycerol incorporation into muscle triacylglyceride as a major metabolic fate, vide supra. In contrast, observations of glycerol uptake into subcutaneous adipose tissue are inconsistent and controversial (4, 5, 21, 33). In one study where subcutaneous adipose tissue glycerol uptake was observed, total adipose tissue uptake was estimated to represent a minor portion (~3%) of whole body glycerol uptake during rest and exercise (33). With the assumption that 25–30% of the body’s adipose tissue mass is located in the legs (19), at most, leg adipose tissue would still only contribute <1% to the tracer-measured leg glycerol uptake we observed during exercise in the present study. Therefore, we are confident in interpreting the leg glycerol uptake in the present study as reflecting predominant clearance by noncontracting and contracting skeletal muscle during rest and exercise, respectively. Because working muscle RQ, particularly during exercise at high relative power output (i.e., 65% VO2peak), is close

Table 2. Leg contribution (expressed as a percentage) to systemic glycerol turnover during rest and exercise before and after 9 wk of endurance training

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Values are means ± SE; n = 6 for comparisons involving both 45% Pre and RLT, and n = 7 for comparisons involving 65% Pre, ABT, and RLT. Pre, pretraining; ABT, absolute intensity; RLT, relative intensity; Ra, rate of appearance; Rd, rate of disappearance.
to unity (1, 7), and since the combustion coefficient of glycerol is ~0.86 (i.e., significantly lower than the observed muscle RQ), we have indirect evidence that the tracer-measured glycerol uptake was directed to nonoxidative disposal. In the absence of net reductions in intramuscular [triacylglyceride] under the conditions we studied (1), our data are consistent with direct glycerol incorporation into muscle triacylglyceride (9) giving rise to turnover of the muscle triacylglyceride pool during exercise (8).

As noted, leg glycerol release occurred simultaneously with leg muscle glycerol uptake (i.e., leg glycerol turnover) at rest and showed several-fold increases during exercise. While leg glycerol uptake may predominantly reflect uptake into skeletal muscle, the source of leg glycerol release is less certain. For example, endogenous leg glycerol production may reflect lipolysis and subsequent glycerol drainage into the femoral vein from triacylglyceride in leg subcutaneous adipose tissue or intra- or extracellular triacylglyceride depots in skeletal muscle or following lipoprotein lipase-mediated lipoprotein triacylglyceride hydrolysis. Because we did not observe changes in blood triacylglyceride balances in the legs before or after training (16), our release data most likely reflect adipose tissue or skeletal muscle lipolysis. In support of this assertion, thigh subcutaneous adipose tissue lipolysis (inferred from microdialysis measurements) can be increased by exercise (2, 31), and, as noted above, skeletal muscle triacylglyceride turnover (which could lead to muscle glycerol release) has been demonstrated during exercise (8). Nonetheless, it is clear that during exercise, the substantial and simultaneous uptake of glycerol into leg muscle and release of glycerol from the leg were balanced, such that net leg glycerol balance did not differ significantly from zero during exercise.

Our data showing that a large portion of resting systemic glycerol turnover can be accounted for by lower extremity limbs are consistent with the observations of others. For example, Jensen (17) reported that resting legs could account for ~40% of systemic glycerol Ra and ~16% of systemic Rg, while we report ~63 and ~28%, respectively. To reiterate, the latter (leg muscle glycerol uptake) confirms the contention that, during resting conditions, the liver and kidneys are not exclusive sites of glycerol metabolism, vide supra. In addition, our data indicate that the legs have increased and seemingly high contributions to the systemic response during whole body exercise (the legs accounted for ~86% of systemic glycerol Ra and ~77% of systemic glycerol Rd during exercise). Our exercise values for each of the components estimating limb glycerol turnover [e.g., arterial [glycerol], blood flow (16), net balance, and fractional extraction] are consistent with values in the literature (1, 11, 33, 34), as are our systemic glycerol flux rates (6). Also, the up to fivefold increases above rest in absolute and relative leg glycerol turnover during exercise are consistent, and if anything lower, than those reported during low power output knee extension (11, 34). Therefore, despite the considerable analytic complexity associated with measuring limb blood flow rate and arterial and venous [glycerol] and isotopic enrichments during high power output whole body exercise, we are confident that our data can be interpreted to mean that leg glycerol turnover and its contribution to systemic glycerol flux can be substantial, especially during physical activity. Finally, our data allowed evaluation of the independent effects of exercise intensity and endurance training on leg glycerol flux. In this regard, we confirm our previous report (1) that net working leg glycerol balance did not differ significantly from zero during exercise regardless of intensity or training state. However, we do note dissociations between intensity and training effects on leg substrate oxidation and the response of leg glycerol turnover to exercise. As predicted (3) and in our previous report (7), we showed predominant carbohydrate oxidation in working skeletal muscle at high relative exercise intensities, downregulation of lipid oxidation, and training-associated increases in working muscle lipid oxidation for a given absolute but not relative exercise intensity (lipid oxidation in μmol/min: 45% Pre, 464 ± 132; 65% Pre, 187 ± 94; ABT, 730 ± 163; RLT, 254 ± 146). However, despite the large magnitude and adaptability of leg lipid oxidation to intensity and training, we observed remarkably consistent responses of leg glycerol turnover to whole body exercise stress with no or borderline differences detected between trials. Helge et al. (11) also reported dissociation in response to increases in contraction intensity during two-legged knee extensions, with working thigh lipid oxidation remaining stable but thigh glycerol turnover augmented. Thus the significance of leg glycerol turnover, particularly in the context of working limb and whole body energy substrate partitioning, remains to be established. Nonetheless, a high turnover of endogenous triacylglyceride pools within working limbs may provide an environment that is highly sensitive and responsive to changes in muscle lipid oxidation requirements (23). Collectively (1, 7, 16), our results suggest that working muscle lipid oxidation provides only a minor fraction of total energy substrate provision during strenuous exercise, but increases in intralimb triacylglyceride turnover elicited by such exercise may provide a segue to the postexercise recovery period when lipid oxidation predominates in muscle (34) and at the whole body level (Henderson GC, Fattor JA, Horning MA, Faghihnia N, Johnson M, Mau TL, Luke-Żeitoun M, and Brooks GA, unpublished data; and Ref. 20).

In summary, the data indicate a need for glycerol tracer data to appreciate that limb turnover (i.e., uptake and release) of glycerol can be substantial, despite seemingly small or insignificant net glycerol exchange. We showed significant leg glycerol turnover at rest that was substantially increased by moderate and high relative intensity exercise, before and after endurance training. Although novel for whole body high power output exercise, our results of working muscle glycerol uptake and simultaneous leg glycerol release are consistent with previous reports on the muscles of laboratory animals and untrained humans engaged in low power output, hyperemic muscle activity. Our results contribute to a growing literature that can be interpreted to mean that peripheral tissues such as skeletal muscle can contribute to glycerol turnover in the body, and furthermore, that the role(s) of working limbs and contracting muscles is substantial during physical activity.

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REFERENCES


