Kinetics of intramuscular triglyceride fatty acids in exercising humans

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Guo, ZengKui, Bartolome Burguera, and Michael D. Jensen. Kinetics of intramuscular triglyceride fatty acids in exercising humans. J Appl Physiol 89: 2057–2064, 2000.—A pulse ([14C]palmitate)-chase ([3H]palmitate) approach was used to study intramuscular triglyceride (imTG) fatty acid and plasma free fatty acid (FFA) kinetics during exercise at 45% peak O2 consumption in 12 adults. Vastus lateralis muscle was biopsied before and after 90 min of bicycle exercise; [3H2O production, breath [14CO2 excretion and lipid oxidation (indirect calorimetry) rates were measured during exercise. Results: during exercise, 8.2 ± 1.2 and 8.4 ± 0.7 mmol·kg−1·min−1 of imTG fatty acids and plasma FFA, respectively, were oxidized according to isotopic measurements. The sum of these two values was not different (P = 0.6) from lipid oxidation by indirect calorimetry (15.4 ± 1.6 mmol·kg−1·min−1); the isotopic and indirect calorimetry values were correlated (r = 0.79, P < 0.005). During exercise, imTG turnover rate was 0.32 ± 0.07%/min (6.0 ± 2.0 μmol of imTG·kg wet muscle−1·min−1) and plasma FFA were incorporated into imTG at a rate of 0.7 ± 0.1 μmol·kg wet muscle−1·min−1. The imTG pool size did not change during exercise. This pulse-chase, dual tracer appears to be a reasonable approach to measure oxidation and synthesis kinetics of imTG.

[14C]palmitate; body composition; free fatty acids

Intramuscular triglyceride (imTG) fatty acids are an important energy source during exercise (21, 30) and possibly at rest (4). Thus far, however, imTG oxidation in vivo has only been assessed using an indirect approach: plasma free fatty acid (FFA) oxidation (isotopic measures) is subtracted from total fatty acid oxidation (indirect calorimetry) (38). Uncertainties with respect to isotopic measures of FFA oxidation (14, 31, 32, 37) and the unreliability of indirect calorimetry at times of changing acid-base status (9) could limit the confidence in quantitative estimates of imTG oxidation using this approach. Another concern is that plasma FFA incorporated into imTG (4) during the preexercise tracer infusion interval might be oxidized during exercise, resulting in an overestimate of plasma FFA oxidation. This possibility does not appear to have been evaluated to date. Therefore, a method that can measure imTG oxidation more directly should advance our understanding of imTG metabolism under different nutritional and physical conditions. The dual-tracer, pulse-chase technique has been used to study intramuscular fatty acid metabolism in isolated muscles ex vivo (5, 6). We wished to determine whether this approach would also be applicable to in vivo conditions to study intramuscular fatty acid kinetics within the normal neural and hormonal milieu.

In the present study, we use this pulse-chase approach to measure imTG fatty acid oxidation in healthy men and women during moderate exercise. The method combines prelabeling imTG by a [14C]-FFA tracer infusion before exercise with infusion of a [3H]-FFA tracer during the exercise. Muscle biopsies were performed before and after the exercise to determine turnover of the imTG pool. The turnover rate of imTG was determined by analyzing the decay of [14C]-fatty acids from the imTG pool. We related the [14CO2 production to the imTG [14C]-fatty acid specific activity (SA) to estimate the oxidation rate of imTG fatty acids. The [3H]-FFA tracer was infused during exercise to allowed measurement of plasma FFA oxidation and incorporation of plasma FFA into imTG during exercise. We wished to test the hypothesis that the combined isotopic measures of fatty acid oxidation rates (imTG plus circulating FFA) would equal total fatty acid oxidation as determined by indirect calorimetry. The results suggest that this pulse-chase, dual-tracer and two-point muscle biopsy technique is useful for investigation of skeletal muscle lipid metabolism in vivo in humans.

METHODS

Subjects

Written, informed consent was obtained from 12 healthy, nonobese adults (6 men). The women were premenopausal and were studied in the follicular phase of their menstrual cycle. All participants consumed an isocaloric diet in the Mayo Clinic General Clinical Research Center (GCRC) for 7 days before this study. The diet provided 35% of calories as fat, 15% as protein, and 50% as carbohydrate. All subjects were moderately active physically and maintained their usual level of physical activity during the week before the study. Weight stability was achieved before the study. These volunteers are the same individuals participating in a recently published study of leg lipolysis during exercise (1). 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.

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additional \([^{14}C]\)palmitate tracer and muscle biopsies were included with the intention of acquiring pilot data with respect to the feasibility of using this approach to study imTG kinetics; the results were sufficiently encouraging to warrant dissemination.

**Protocol**

Body and leg fat and fat-free mass (FFM) were measured within 3 days of the study using dual-energy X-ray absorptiometry (model DPX-IQ, Lunar Radiation, Madison, WI). Each subject was scanned for 20 min, and whole body and regional analysis was performed by using software version 4.1. Total body water was measured \(~1\) wk before the study using the \(^3\)H\(_2\)O technique (18). Each participant’s peak \(O_2\) consumption (\(V\dot{O}_2\) peak) was measured with a continuous bicycle exercise test. Exercise was initiated at 25 and 50 W for women and men, respectively, and increased by 25 or 30 W, respectively, every 2 min until exhaustion. Breath-by-breath \(O_2\) consumption (\(V\dot{O}_2\)) and \(CO_2\) production (\(V\dot{CO}_2\)) were measured throughout the test by indirect calorimetry (29).

Figure 1 provides an overview of the study protocol. The participants were admitted to the GCRC the evening before the study, and an 18-gauge intravenous infusion catheter was placed in a forearm and kept patent with a controlled infusion of 0.45% NaCl. At 0400 the next day, a continuous infusion of \([\begin{array}{c}1 \\ -\end{array}^{14}C]\)palmitate was initiated at 0.3 mCi/min to prelabel the imTG pool. Resting energy expenditure was measured by indirect calorimetry (DeltaTrac Metabolic Cart, Sensor Medics, Yorba Linda, CA) at 0700, after which a radial artery catheter was placed under local anesthesia for blood sampling. Femoral artery and vein catheters were also placed (1). At 0830, an infusion of \([\begin{array}{c}9,10 \end{array}^{3}H]\)palmitate (0.6 mCi/min) was started. After 30 min for isotopic equilibration, a series of four blood samples was obtained over a 30-min period for measurement of plasma palmitate and FFA concentration and SA. The tracer infusions were then stopped, and the participants were prepared for the first biopsy from vastus lateralis muscle using local anesthesia and sterile techniques. The muscle samples (200–300 mg) were washed of blood in ice-cold normal saline solution, dissected of all adipose tissue under a stereomicroscope, further rinsed of lipid droplets, and saved immediately in liquid N\(_2\). We discontinued the \([\begin{array}{c}1 \end{array}^{14}C]\)palmitate infusion at least 30 min before the first biopsy to minimize the possibility that additional tracer would be incorporated into imTG after the biopsy. Immediately after the biopsy, the participants were transferred to the Integrative Physiology Core of the GCRC for the exercise study. The \([\begin{array}{c}9,10 \end{array}^{3}H]\)palmitate infusion was restarted as soon as the participant arrived in the exercise study room. After \(\sim30\) min, the participants began to exercise on a bicycle ergometer at 45% of \(V\dot{O}_2\) peak continuously for 90 min. Blood samples were collected at 15-min intervals during the exercise, and heart rate was monitored throughout the study. After the completion of the exercise, a second muscle biopsy was taken from the contralateral leg as quickly as possible by the same procedures. The catheters were removed after completion of the study, and the volunteers remained under observation in the GCRC until the following morning.

During the exercise, systemic \(V\dot{O}_2\) and \(V\dot{CO}_2\) were measured at 15-min intervals by using the same breath-by-breath mass spectrometry system used for the fitness test (29). Breath samples for measurement of \(^{14}CO_2\) SA (10) were collected before and at 15-min intervals during exercise.

**Materials**

\([\begin{array}{c}1 \end{array}^{14}C]\)palmitate, \([\begin{array}{c}9,10 \end{array}^{3}H]\)palmitate and \(^3\)H\(_2\)O were obtained from Amersham (Arlington Heights, IL). The FFA were prepared for intravenous infusion as a solution of 0.3% albumin in 0.9% NaCl.

**Assays and Measurements**

**Plasma FFA and triglycerides.** Plasma total FFA and palmitate concentrations, as well as the isotopic purity of radioactive tracers, were measured by HPLC (19, 26). Plasma triglyceride concentrations were measured by a microfluorometric method (15), and plasma triglyceride SA was measured on an HPLC-purified (3) Folch extract of plasma.

**Muscle lipids.** The frozen muscle samples were pulverized into fine powder by using a stainless steel mortar and pestle on dry ice. The powder was extracted for total lipids by the method of Folch and colleagues (8), from which triglyceride was purified by HPLC (3). The majority of the purified muscle triglyceride was counted on a liquid scintillation counter for total \(^3\)H and \(^{14}\)C radioactivity [disintegrations/min (dpm)]. A small aliquot of the triglyceride was directly transmethylated by using 2.5% H\(_2\)SO\(_4\) in methanol at 70°C for 2 h (20). The concentration and composition of imTG palmitate were determined by gas chromatography/combustion/isotope ratio Fig. 1. Outline of study protocol. REE: rest energy expenditure.
mass spectrometry with heptadecanoic acid as an internal standard (12), and the $^{14}$C and $^3$H SA of imTG-palmitate (dpm/$\mu$mol) were then calculated.

$^{3}$H$_2$O production. Plasma water $^{3}$H$_2$O concentrations were determined as previously described by using blood samples obtained before and during exercise (14). The slope of the increase in $^{3}$H$_2$O in total body water was used to calculate the $^{3}$H$_2$O production rate for each participant.

Urinary nitrogen was measured using an Analox GM7 fast enzymatic metabolite analyzer (Analox Instruments, Lunenburg, MA).

Calculations

Rate of net imTG turnover during exercise. imTG turnover rate was calculated by using the $^{14}$C imTG-palmitate data. The $^{14}$C imTG-palmitate SA values before and after exercise were transformed by natural logarithm. The decrease in the transformed value from the beginning to the end of exercise was used to calculate the fractional turnover rate (expressed as % decrease per minute). The appendix provides the theory and process of this approach. The net turnover rate of imTG (µmol imTG-kg wet muscle$^{-1}\cdot$min$^{-1}$) was calculated from the imTG-palmitate concentration data, corrected by its abundance in the imTG fatty acid pool (28%).

Rate of imTG fatty acid oxidation during exercise. During the initial stages of exercise, the $^{14}$CO$_2$ expired may reflect a rapid washout of $^{14}$CO$_2$ produced and accumulated in the body bicarbonate pool during the prolonged $^{14}$C-palmitate infusion. We reasoned that, after 45 min of exercise, $^{14}$CO$_2$ excretion should occur almost exclusively from the immediate oxidation of $^{14}$C-labeled imTG. Therefore, imTG fatty acid oxidation rates were calculated for the last 45 min of exercise. The $^{14}$CO$_2$ production rates at 45, 60, 75, and 90 min of the exercise were determined by multiplying the $^{14}$CO$_2$ SA of breath by the $^{14}$CO$_2$ production rate measured by indirect calorimetry. The $^{14}$CO$_2$ production was corrected for CO$_2$ fixation on the basis of a previously published CO$_2$ fixation-exercise intensity relationship derived by Sidossis et al. (32). The corrected $^{14}$CO$_2$ production rate was divided by the imTG fatty acid SA estimated to be present at that time to calculate the rate ($\mu$mol-$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) of imTG fatty acid oxidation during that interval. Because there were only two muscle biopsies, one before (~0 min) and one after exercise (~90 min), it was necessary to estimate the SA of imTG-palmitate at 45, 60, and 75 min. These values were calculated for each individual by using the initial SA value of imTG-palmitate corrected for decay over time by the determined fractional imTG turnover rate. In doing so, we assumed that the decay in SA of imTG $^{14}$C-fatty acids was at a constant rate during the course of exercise (see APPENDIX).

Plasma FFA oxidation during exercise. The rate of whole body $^3$H$_2$O production (dpm/min) was determined by linear regression analysis of the concentration of $^3$H$_2$O in body water vs. time. The rate of $^3$H$_2$O production was divided by the average plasma $^{3}$H-FFA SA during the same period to calculate plasma FFA oxidation rates during that period. The plasma palmitate SA value was converted to FFA SA on the basis of plasma FFA composition.

The "indirect" oxidation of plasma fatty acids via imTG (esterification into imTG before being oxidized) was estimated as follows: the increment in imTG $^{3}$H-fatty acid SA and the fractional turnover rate of imTG were used to determine the rate of $^3$H$_2$O production resulting from indirect oxidation of plasma $^{3}$H-fatty acids via imTG pool of leg skeletal muscle (22% and 27% of body weight for men and women, respectively, as determined by dual-energy X-ray absorptiometry). The rate of $^3$H$_2$O production estimated from this indirect pathway was subtracted from whole body $^3$H$_2$O production in the calculation of plasma fatty acid oxidation.

Whole body fat oxidation and total energy expenditure during exercise. Because plasma lactate concentrations were stable during exercise (1), we calculated fatty acid oxidation rates by using $V_{O2}$, $V_{CO2}$, and urinary nitrogen excretion rates with published formulas (9).

imTG synthesis during exercise. The increment in imTG $^3$H fatty acid SA from the beginning to the end of exercise was considered to represent the rate of net incorporation of plasma FFA during $[^3]$H-palmitate infusion. This rate was divided by the average plasma $[^3]$H-FFA SA to derive the amount of FFA incorporated into imTG pool from plasma fatty acid pool. Palmitate value was converted to that for all fatty acids on the basis of plasma fatty acid composition (28%).

Statistics. All values are means ± SE except as indicated. For comparisons of imTG pool size before and after exercise, and between the rates of fatty acid oxidation measured isotopically with those measured by indirect calorimetry, a paired Student's t-test was employed. Total fatty acid oxidation (indirect calorimetry) was related to the sum of isotopically measured fatty acid oxidation (plasma FFA + imTG) for each individual using linear regression analysis.

RESULTS

Subject Characteristics and FFA Kinetics

The characteristics of the subjects participating in this study have been previously published (1). The subjects were 31 ± 2 yr old, with a mean body mass index of 25.0 ± 1.2 kg/m$^2$ and peak $V_{O2}$ of 52 ± 2 ml·kg$^{-1}$·min$^{-1}$. Basal FFA flux was 406 ± 43 µmol/min (5.8 ± 0.7 µmol·kg$^{-1}$$\cdot$min$^{-1}$) and increased (P < 0.005) to an average of 881 ± 64 µmol/min (12.3 ± 1.2 µmol·kg$^{-1}$$\cdot$min$^{-1}$) during the last 45 min of exercise.

imTG Synthesis and Turnover Kinetics During Exercise

Figure 2 shows simultaneous turnover and synthesis of imTG in vastus lateralis muscle during exercise. The preexercise imTG $^{14}$C-fatty acid SA was 447 ± 69 dpm/µmol. After 90 min of exercise, the imTG $^{14}$C fatty acid SA decreased (P = 0.04) to 197 ± 54 dpm/µmol. The fractional turnover rate of imTG was 0.32 ± 0.07%/min, and the absolute turnover rate of imTG was 6.0 ± 2.0 µmol of imTG-kg wet muscle$^{-1}$$\cdot$min$^{-1}$. Plasma FFA were actively incorporated into the imTG pool during exercise as indicated by the increase in the imTG fatty acid $^{3}$H SA (from 52 ± 21 to 146 ± 44 dpm/µmol, P = 0.004). The rate of incorporation of plasma fatty acids into the imTG pool was 0.7 ± 0.1 µmol·kg wet muscle$^{-1}$$\cdot$min$^{-1}$. Despite the apparent differences between uptake and disappearance of fatty acids from imTG, we did not find differences in the content of imTG fatty acids (palmitate) before and after exercise (2.0 ± 0.5 vs. 2.4 ± 0.8 mmol imTG/kg wet muscle before vs. after exercise, respectively, P = 0.7).
imTG Fatty Acid Oxidation During Exercise: Isotopic Approach

As expected, there was virtually no [14C]palmitate in arterial or femoral venous plasma (Fig. 3) at the beginning of exercise because its infusion had been discontinued for >60 min. Despite this, considerable 14CO2 was expired throughout exercise (Fig. 4). With the estimated imTG-fatty acid SA used as the precursor for imTG oxidation during the last 45 min of exercise, the average imTG fatty acid oxidation rate was 8.2 ± 1.2 μmol·kg⁻¹·min⁻¹. This represents 49 ± 11% of whole body lipid oxidation and 24 ± 4% of total energy expenditure during exercise.

Plasma Fatty Acid Oxidation During Exercise

Figure 5 depicts the cumulative production of 3H2O during exercise. With the generation of [3H2O] used as a means of measuring FFA oxidation, 8.4 ± 0.7 μmol·kg⁻¹·min⁻¹ of plasma FFA were oxidized during exercise. The indirect oxidation of plasma fatty acids that had traversed imTG fatty acid pool before being oxidized was estimated to be negligible (0.12 ± 0.2 μmol·kg⁻¹·min⁻¹, or 1.2 ± 0.3%).

Systemic Energy Expenditure and Fatty Acid Oxidation During Exercise

The total energy expenditure during the last 45 min of exercise was 29.9 ± 3.0 kJ/min. Systemic fatty acid oxidation...
oxidation (indirect calorimetry) during exercise was $15.4 \pm 1.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

**Fatty Acid Oxidation Determined by Isotopic Vs. Indirect Calorimetry Approaches**

Isotopically measured total fatty acid oxidation during exercise ($\text{imTG} + \text{plasma FFA}$) was strongly correlated with that determined by indirect calorimetry (Fig. 6; $r = 0.79, P < 0.005$). In addition, the sum of isotopically measured rates of fatty acid oxidation ($16.3 \pm 1.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during exercise was not different ($P = 0.6$) from fatty acid oxidation rates measured with indirect calorimetry.

imTG fatty acid oxidation rates calculated by subtracting plasma FFA oxidation from total fatty acid oxidation (indirect calorimetry) were $7.2 \pm 1.1 \mu\text{mol} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$, compared with the isotopically measured rate of $8.2 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($P = 0.4$).

**Plasma Triglyceride Concentration, SA, and Leg Balance**

During exercise, the arterial plasma triglyceride concentrations averaged $284 \pm 37 \mu\text{mol/l}$. The net balance across the leg was $-26 \pm 18 \mu\text{mol/min}$ for both legs ($P$ = not significant (NS) vs. 0). The arterial plasma triglyceride SA averaged $2,481 \pm 315 \text{dpm/\mu mol}$, indicating that substantial incorporation of $[14\text{C}]$palmitate had occurred during the isotope infusion interval. We were unable to detect leg uptake of $[14\text{C}]$triglyceride during exercise; the leg $[14\text{C}]$triglyceride balance was $-49 \pm 39 \text{dpm/triglyceride per ml plasma}$ ($P$ = NS vs. 0).

**DISCUSSION**

imTGs are an important energy source (4, 21, 30) and, when present in excess, they are associated with greater risk of adverse metabolic consequences of obesity (7, 17, 23, 24, 27, 28, 34, 36). Both highly trained athletes (22) and insulin-resistant obese individuals (23, 27) have increased imTG content, however, suggesting that the kinetics of imTG may be more important than the amount of imTG. In this study, the pulse-chase, dual-isotope approach, previously used to study rat muscle ex vivo (6), was adapted to study the in vivo kinetics of imTG in humans. We chose to study exercise because imTG are thought to be an especially important fuel source during exercise (16). imTG were readily labeled with the exogenous FFA tracer (e.g., $[14\text{C}]$palmitate) in humans, similar to our findings in rats (12). Furthermore, the excretion of $^{14}\text{CO}_2$ relative to the change in imTG $^{14}\text{C}$ SA that occurred during exercise provided estimates of imTG oxidation that were consistent with the previously applied model using indirect calorimetry and $[13\text{C}]$palmitate (38). The rate of circulating FFA oxidation, as determined by the generation of $^3\text{H}_2\text{O}$, was similar to that reported by Sidossis et al. (32) using $[13\text{C}]$FFA tracers. The sum of isotopically measured fatty acid oxidation (imTG plus plasma FFA) was comparable to total fatty acid oxidation measured with indirect calorimetry, suggesting that the isotopic measures are credible. Finally, plasma FFA were incorporated into imTG during exercise, which could account for the stable imTG pool size. We conclude that the pulse-chase, dual-isotope, muscle biopsy approach to studying imTG metabolism is a potentially valuable approach for studying imTG kinetics in vivo.

Exercise was chosen as a means of examining the dual-isotope, pulse-chase, muscle biopsy approach to studying imTG kinetics because high rates of fatty acid oxidation could be expected (16, 25). This allowed us to compare the traditional approach of calculating imTG oxidation (total fatty acid oxidation by indirect calorimetry-FFA oxidation by isotopic measures) with the new approach under high-flux conditions. Comparing these two methods of measuring imTG oxidation may not have been possible in resting volunteers because non-skeletal muscle fatty acid oxidation contributes a sufficiently large portion of total fatty acid oxidation that the results would be difficult to interpret.

We did not attempt to take more than two muscle biopsy samples (one just before and one just after exercise) during these studies, making it necessary to estimate the imTG $^{14}\text{C}$ SA corresponding to the breath $^{14}\text{CO}_2$ production rate at each time point during exercise. To do this, we employed a mathematical model using the two-point muscle biopsy data to determine the imTG turnover rate. This approach seems reasonable, considering that the pool size of imTG did not change appreciably during exercise. In addition, studies using a similar pulse-chase approach to examine isolated rat muscle (6) have indicated a linear or log-linear accumulation of exogenous fatty acids into imTG.
over time. In order for the imTG pool size to remain stable during exercise, the accumulation of imTG from plasma FFA would likely need to mirror imTG fatty acid oxidation. Finally, the good agreement between the combined fatty acid oxidation estimated isotopically (plasma FFA and imTG) and total fatty acid oxidation as determined by indirect calorimetry is reassuring that the model is reasonable. Although it would be a considerable experimental design challenge, a study that included more than two biopsies (at least one during exercise) would be a welcome test of the assumption that imTG decay is log-linear during exercise.

Another means of examining the internal consistency of our data is to compare the leg plasma fatty acid uptake (measured using arterial-venous $[^{3}H]$palmitate kinetics across legs) during exercise (1) with the sum of oxidation and esterification of plasma FFA. We reasoned that leg FFA uptake during exercise should be similar to, although somewhat less than, FFA oxidation + reesterification because the majority of FFA oxidation during exercise should be within leg muscle (leg $O_{2}$ uptake during exercise was $65-70\%$ of total $O_{2}$ consumption; Ref. 1). Leg total FFA uptake during exercise in these volunteers (1) averaged $7.1 \pm 0.4 \mu mol\cdot kg^{-1}\cdot min^{-1}$. Using the incorporation of $[^{3}H]$palmitate into imTG, the average $[^{3}H]$palmitate SA during exercise, the imTG concentration, and leg muscle mass, we estimated that $\approx 20\%$ of leg FFA uptake during exercise was esterified into imTG. As expected, the sum of plasma FFA oxidation rates ($8.4 \pm 0.7 \mu mol\cdot kg^{-1}\cdot min^{-1}$) and leg incorporation of $[^{3}H]$palmitate into imTG ($0.12 \pm 0.2 \mu mol\cdot kg^{-1}\cdot min^{-1}$) is greater (by 1.5 $\mu mol\cdot kg^{-1}\cdot min^{-1}$) than leg FFA uptake rates measured isotopically. This is consistent with some FFA oxidation also occurring in tissues other than leg skeletal muscle during exercise (heart, liver, etc.). We are reassured that these two independent measures of leg muscle FFA metabolism are internally consistent.

Previous studies of isolated rat muscle using the pulse-chase, dual-isotope approach yielded results that were interpreted as providing evidence for rapidly turning over "subpools" of imTG (6); a $32\%$ loss of $[^{14}C]$ in imTG was observed without loss of imTG. If the $[^{14}C]$FFA tracer in the present study was incorporated into small, rapidly turning over subpools (6), our isotopic estimates of imTG oxidation should have greatly exceeded the values derived by indirect calorimetry. Instead, the isotopic estimates were in good agreement with the indirect calorimetry-plasma FFA oxidation approach. Thus our results do not support the concept that distinct, intracellular subpools of rapidly turning over imTG are present in vivo in humans.

We did not observe significant decreases in imTG content in response to exercise. This is perhaps not surprising given the reciprocal losses of $[^{14}C]$ and simultaneous gains in $[^{1}\text{H}]$ in imTG (Fig. 2). The lack of change in imTG content after exercise is consistent with some (22, 35) but not other (2) studies. Carlson et al. (2) observed a $20\%$ decline in imTG content after an intensive exercise ($65\%$ maximal $V_{O2}$) for 100 min. Higher exercise intensities produce the combination of greater imTG utilization and lesser FFA availability (Ref. 21; to provide “replacement” imTG fatty acids) and should make it easier to detect changes in imTG content. Our results suggest that during moderate, short-term exercise, imTG undergoes simultaneous hydrolysis and esterification such that the pool size is relatively well maintained.

Another potential source of fatty acids for muscle is circulating triglycerides, primarily those contained in very-low-density lipoprotein (VLDL) particles. Although we could not detect leg triglyceride uptake during exercise, the high rates of blood flow would make it difficult to detect modest rates of VLDL-triglyceride uptake. Consistent with the findings of Havel et al. (13), the plasma triglycerides of our volunteers were labeled with the $[^{14}C]$palmitate that was infused to label imTGs. In fact, the SA of plasma triglyceride was $\sim 10\text{-fold}$ greater than imTG, which could have effects on calculated imTG oxidation rates if VLDL-triglyceride fatty acids are used for fuel instead of being stored in adipose tissue. VLDL-triglyceride production rates average $0.35 \mu mol\cdot kg^{-1}\cdot min^{-1}$ in healthy adults (33) and, if maintained at this rate during exercise, would deliver a substantial amount of $[^{14}C]$ triglyceride fatty acids to the circulation in this study. Wolfe et al. (39) have reported that $\sim 40\%$ of VLDL fatty acids are oxidized in healthy dogs. If the same is true in humans, we estimated that as much as $13\%$ of $[^{14}C]CO_{2}$ production could originate from this source in our subjects, causing us to overestimate imTG oxidation. In addition, if VLDL-triglyceride fatty acids were taken up and esterified by exercising muscle, this would cause us to underestimate imTG turnover. The potentially confounding variable of isotopically labeled VLDL-triglyceride that occurs with this experimental design can be addressed using the approach of Sidossis et al. (33). Measurement of VLDL-triglyceride fatty acid kinetics and oxidation during...
exercise would help determine the relative contribution of plasma FFA vs. imTG vs. VLDL-triglyceride to lipid fuel metabolism. Although VLDL-triglyceride are not thought to contribute to muscle fuel metabolism during exercise (11), confirmation of this with isotopic tracers would simplify the model needed to understand imTG kinetics. Consistent with the results of pulse-chase studies of fatty acid metabolism in isolated rat muscle (6), we found no evidence of FFA release from imTG during exercise, a condition that should result in high rates of imTG hydrolysis (16). There was no increase in the level of $[^{14}C]$palmitate in plasma (either arterial or femoral venous) with exercise despite the massive increase in $^{14}CO_2$ production resulting from the oxidation of intracellular imTG $[^{14}C]$palmitate. We conclude that intramuscular triacylglycerol stores are used primarily, if not solely, as local oxidative fuel and are not exported. Given the relatively high plasma $[^{14}C]$triacylglyceride SA, the failure to find significant $^{14}C$ in plasma free palmitate also suggests that lipoprotein lipase-mediated clearance of VLDL-triglyceride fatty acids does not result in significant entrance of triglyceride fatty acids into the plasma FFA pool.

In summary, the present studies have examined the use of a dual-isotope, pulse-chase experimental design to evaluate imTG metabolism during exercise. By measuring the imTG $^{13}C$ SA before and after exercise, and relating these changes to $^{14}CO_2$ production, we more directly assessed imTG kinetics. imTG oxidation as determined using this isotopic approach agreed well with the more traditional means of determining imTG oxidation. Moreover, our results suggest that human imTG is a more homogeneous pool than ex vivo studies of rat muscle have indicated. The lack of change in imTG content during moderate exercise appears to relate at least partly to simultaneous esterification of plasma FFA within muscle. If VLDL-triglyceride fatty acids are not a significant source of lipid fuel in humans, this approach to the study of imTG metabolism may offer promise in the study of intracellular triglyceride fatty acid kinetics in vivo.

APPENDIX

Determination of Turnover Rate of imTG Fatty Acids and Its Use for the Prediction of Fatty Acid Label in the Triglyceride Pool

During the turnover of imTG-$[^{14}C]$palmitate, the labeled palmitate is replaced by unlabeled palmitate so that the imTG-palmitate pool size remains unchanged, whereas the absolute amount of the label continuously diminishes (Fig. 7). At a constant turnover rate $k$, the amount of $[^{14}C]$palmitate remaining in the imTG pool at any point of time can be described as following

$$Q = I \times e^{-kt} \quad (1)$$

where $Q$ is the absolute amount of $[^{14}C]$palmitate remaining in the imTG pool at time $t$, and $I$ is its initial amount at time 0. $Q$ is depicted in Fig. 7 as an exponential curve over time. In the figure, $I$ is arbitrarily set at 100 (it can be proved that the value can be of any quantity and the calculated $k$ is identical) and turnover rate $k$ at 20% for simplicity. In present studies, $I$ is the determined amount (dpm) of imTG-$[^{14}C]$palmitate in the first muscle biopsy. Because of the nonlinear nature, $k$ calculated from the original data varies depending on the time points used (not shown). When $Q$ is transformed into natural logarithm, $\ln(Q)$, a linear curve is obtained (Fig. 7). The transformed linear curve provides a single, constant $k$ no matter how many (>1) and which data points are used. Here, only a two-point model is presented. The equation for determining turnover rate using the two-point model is

$$k = \frac{\ln(Q)_1 - \ln(Q)_0}{t} \quad (2)$$

where $\ln(Q)_0$ and $\ln(Q)_i$ are the logarithm of the amounts of imTG-$[^{14}C]$palmitate label at time 0 and $t$, respectively.

For data presented in Fig. 7, $\ln(Q)_0$ is 3.6 and $\ln(Q)_i$ is 4.6. The value of $k$ is thus calculated, by Eq. 2, to be $(3.6 - 4.6)/5 = -0.2$ (the negative sign indicates removal of $[^{14}C]$palmitate from imTG pool). The same $k$ value can be obtained in the same way with any two or more data points. The data treatment is necessary for a two-point approach such as that used in present study, in which only two muscle biopsies were available. On the other hand, the two-point approach should provide a $k$ value that is identical to that obtained by a multiple-point approach.

The assumptions for this model are 1) a homogeneous pool of stable size and 2) a constant turnover rate $k$. These assumptions satisfy a single-pool model. Therefore, in using this model, it is assumed that imTG is a metabolically homogeneous pool turning over at a constant rate during the exercise.

For calculation of remaining amount of $[^{14}C]$palmitate in imTG pool at any time point during the exercise, Eq. 1 is directly used with appropriate substitutions of the $k$ for the determined turnover rate and time variable $t$.

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REFERENCES


