A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men

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Magkos F, Patterson BW, Mohammed BS, Mittendorfer B. A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men. Am J Physiol Endocrinol Metab 292:E1568–E1574, 2007. First published January 30, 2007; doi:10.1152/ajpendo.00636.2006.—Our group (Magkos F, Wright DC, Patterson BW, Mohammed BS, Mittendorfer B, Am J Physiol Endocrinol Metab 290: E355–E362, 2006) has recently demonstrated that a single, prolonged bout of moderate-intensity cycling (2 h at 60% of peak oxygen consumption) in the evening increases basal whole-body free fatty acid (FFA) flux and fat oxidation, decreases hepatic VLDL-apolipoprotein B-100 (apoB-100) secretion, and enhances removal efficiency of VLDL-triglyceride (TG) from the circulation the following day in untrained, healthy, lean men. In the present study, we investigated the effect of a single, shorter-duration bout of the same exercise (1 h cycling at 60% of peak oxygen consumption) on basal FFA, VLDL-TG, and VLDL-apoB-100 kinetics in seven untrained, healthy, lean men by using stable isotope-labeled tracer techniques. Basal FFA rate of appearance in plasma and plasma FFA concentration were ~55% greater (P < 0.05) the morning after exercise than rest, whereas resting metabolic rate and whole-body substrate oxidation rates were not different after rest and exercise. Exercise had no effect on plasma VLDL-TG and VLDL-apoB-100 concentrations, hepatic VLDL-TG and VLDL-apoB-100 secretion rates, and VLDL-TG and VLDL-apoB-100 plasma clearance rates (all P > 0.05). We conclude that in untrained, healthy, lean men 1) the exercise-induced changes in basal whole-body fat oxidation, VLDL-TG, and VLDL-apoB-100 metabolism during the late phase of recovery from exercise are related to the duration of the exercise bout; 2) single sessions of typical recreational activities appear to have little effect on basal, fasting plasma TG homeostasis; and 3) there is a dissociation between systemic FFA availability and VLDL-TG and VLDL-apoB-100 secretion by the liver. hepatic lipid metabolism; lipoprotein; stable isotope; tracer

INCREASED PLASMA TRIGLYCERIDE (TG) concentration is an important risk factor for cardiovascular disease (6, 31). Regular endurance exercise is associated with lower plasma TG concentrations (15). The cardioprotective nature of regular endurance-type physical activity (22, 36) is therefore likely, at least in part, to be linked to the hypotriglyceridemic effect of endurance exercise. Little, however, is known about the effect of exercise on plasma TG metabolism.

It has long been recognized that the TG-lowering effect of exercise manifests in response to a single bout of aerobic activity with little, if any, evidence for chronic physiological adaptations after repeated exercise sessions (i.e., exercise training) (14, 32). The exercise-induced reduction in fasting plasma TG concentrations in human subjects is typically not evident immediately after a single bout of exercise but occurs with a delay of several hours and lasts for ~1–2 days after completion of the exercise bout (3, 10, 13, 16, 18, 58). Similarly, plasma TG concentration is lower in endurance-trained compared with untrained individuals when measured within 48 h of exercise cessation but not thereafter (7, 19, 27, 29, 30, 32). There is some evidence that the TG-lowering capacity of exercise may depend on the amount of energy expended during the exercise session. Prolonged moderate-intensity exercise bouts (≥2-h duration) reduce fasting plasma TG concentration by ~30% (3, 10, 16, 21, 58, 59), whereas shorter bouts of similar exercise (≤30-min duration) have no effect of plasma TG concentration (1, 9). Exercise intensity, on the other hand, appears to be less important than exercise duration because the plasma TG response is not affected by changes in exercise intensity when total energy expenditure is maintained constant (7, 8, 11, 60). However, the effect of exercise, such as recommended for cardiovascular and metabolic health [i.e., 30–60 min at moderate intensity (2)], on plasma TG concentration remains uncertain (8, 16, 24, 57, 64, 65), and the mechanisms by which exercise regulates fasting plasma TG concentration are not well understood.

Our group (43) has previously demonstrated that fasting hypotriglyceridemia in response to a single, prolonged bout of moderate-intensity exercise (2 h of cycling at 60% of peak oxygen consumption, VO2peak) reduces the secretion of VLDL-apolipoprotein B-100 (apoB-100), an index for the secretion of VLDL particles from the liver, and enhances the efficiency of VLDL-TG removal from plasma; the latter phenomenon has also been demonstrated in endurance-trained individuals within 16–20 h after the end of exercise (44). The lipid metabolism response to exercise that conforms with current guidelines for a healthy lifestyle (2), however, is not known. Therefore, we evaluated plasma free fatty acid (FFA), VLDL-TG, and VLDL-apoB-100 kinetics after a single 60-min bout of cycling at 60% of VO2peak by using stable isotope labeled tracer techniques.
MATERIALS AND METHODS

Subjects and preliminary testing. Seven lean, recreationally active but untrained, healthy young men (age 30 ± 3 yr, body weight 75 ± 2 kg, body mass index 23 ± 1 kg/m², body fat 15 ± 2%; means ± SE) participated in the study; one of the seven subjects had also taken part in our previous study (43), in which we evaluated the effect of a prolonged (2-h) bout of moderate intensity exercise on basal lipid metabolism. All subjects were considered to be in good health after they completed a medical evaluation, which included a history and physical examination and standard blood tests. All had normal fasting plasma glucose and TG concentrations. None were smoking or taking medications known to affect lipid metabolism. Written, informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Advisory Committee at Washington University School of Medicine (St. Louis, MO).

Approximately 2 wk before the beginning of the experiment, during the screening visit or on a separate outpatient visit to the GCRC, body composition was assessed by dual-energy X-ray absorptiometry (Delphi-W densitometer; Hologic, Waltham, MA). 

\( V_{O2\text{peak}} \) was determined after subjects were familiarized with breathing through the apparatus used for metabolic measurements and had warmed up with cycling (Ergoline 800S ergometer; SensorMedics, Yorba Linda, CA) at 50 W for 4 min. After the warm-up session, the work rate was increased by 25 W/min until volitional exhaustion or until a plateau in oxygen consumption \( (V\dot{O}_2) \) was reached, despite the work rate was increased by 25 W/min until volitional exhaustion or until a plateau in oxygen consumption \( (V\dot{O}_2) \) was reached, despite the increasing workload, and a respiratory exchange ratio \( \geq 1.1 \) over at least 1 min was achieved. \( V_{O2} \) and carbon dioxide production \( (V\dot{CO}_2) \) were measured continuously by online expiratory gas-exchange analysis (TrueOne 2400 Metabolic Measurement System; ParvoMedics, Salt Lake City, UT).

Experimental protocol. Each subject completed two stable isotope-labeled tracer infusion studies within 3 wk, in randomized order: once after resting and once after cycling on the preceding afternoon. Subjects were instructed to adhere to their regular diet and to refrain from exercise for a minimum of 3 days before being admitted to the GCRC, the afternoon before each isotope infusion study (after rest and after exercise). For the exercise study, subjects cycled on a semi-recumbent cycle ergometer (EC-C400R Ergometer; CatEye Fitness, Source Distributors, Dallas, TX) for 1 h starting at 0800; the workload was set to elicit a \( V_{O2\text{peak}} \) equivalent to 60% of \( V_{O2\text{peak}} \). 

\( V_{O2\text{peak}} \) was measured (TrueOne 2400 metabolic measurement system; ParvoMedics) for 5 min every 10 min during exercise, and the workload was adjusted as necessary to maintain the desired \( V_{O2} \) (within ± 5%). After completion of the exercise, subjects took a shower and then rested in a chair. At ≈1930, they consumed a standard meal containing 15 kcal/kg body wt (57% of total energy from carbohydrate, 28% from fat, and 15% from protein) and then fasted (except for water) and rested in bed until completion of the study the next day.

At 0530 the following morning, one catheter was inserted into a forearm vein to administer stable isotope-labeled tracers, and a second catheter was inserted into a vein in the contralateral hand, which was heated to 55°C with a thermostatically controlled box, to obtain arterialized blood samples. Catheters were kept open with slow, controlled infusions of 0.9% NaCl solution (30 ml/h). At 0700 [time 0 (t = 0)], blood samples for the determination of plasma substrate and hormone concentrations and background glycerol, palmitate, and leucine tracer-to-tracee ratios (TTR) in plasma and VLDL-TG and apoB-100 were obtained. A bolus of [1,1,2,3-3H2]glycerol (75 \( \mu \text{mol/kg} \), dissolved in 0.9% NaCl solution, was then administered through the catheter in the forearm vein, and constant infusions of [2,2-2H2]palmitate (0.03 \( \mu \text{mol-kg}^{-1} \text{-min}^{-1} \), dissolved in 25% human albumin solution, and [5,5,5-3H]leucine (0.12 \( \mu \text{mol-kg}^{-1} \text{-min}^{-1} \), priming dose: 7.2 \( \mu \text{mol/kg} \), dissolved in 0.9% NaCl solution, were started and maintained for 12 h. Blood samples were collected at 5, 15, 30, 60, 90, and 120 min and then every hour for 10 h to determine glycerol and palmitate TTR in plasma and VLDL-TG and leucine TTR in plasma and VLDL-apoB-100. 

Sample collection and analyses. To determine glucose concentration, blood was collected in tubes containing heparin; plasma was separated by centrifugation and analyzed immediately. All other blood samples were collected in chilled tubes containing sodium EDTA. Samples were placed on ice, and plasma was separated by centrifugation within 30 min of collection. Aliquots of plasma were kept in the refrigerator for immediate isolation of VLDL and measurement of plasma apoB-100 concentration. The remaining plasma samples were stored at −80°C until final analyses were performed.

The VLDL fraction was prepared as previously described (49). Briefly, ~1.5 ml of plasma was transferred into OptiSeal polyallomer tubes (Beckman Instruments, Palo Alto, CA), overlaid with a NaCl-EDTA solution (1.006 g/ml), and centrifuged at 100,000 g for 16 h at 10°C in an Optima LE-80K preparative ultracentrifuge equipped with a type 50.4 Ti rotor (Beckman Instruments). The top layer, containing VLDL, was removed by tube slicing (CenTtube slicer; Beckman Instruments). Aliquots of the VLDL fraction were set aside for measurement of VLDL-apoB-100 concentration immediately after collection; the remaining samples were stored at −80°C until final analyses were performed.

Plasma glucose concentration (\( t = 0 \)) was determined on an automated glucose analyser (YSI 2300 STAT plus; Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin concentration (\( t = 0 \)) was measured by RIA (Linco Research, St. Louis, MO). Plasma FFA concentrations (\( t = 0 \)) were quantified by gas chromatography (HP 5890 Series II GC; Hewlett-Packard, Palo Alto, CA) after addition of heptadecanoic acid to plasma as an internal standard (52). Total plasma TG and VLDL-TG concentrations were determined with a colorimetric enzymatic kit (Sigma, St. Louis, MO). Total plasma apoB-100 and VLDL-apoB-100 concentrations were measured with a turbidimetric immunoassay (Wako Pure Chemical Industries, Osaka, Japan). We used the average VLDL-TG and VLDL-apoB-100 concentrations during the 12-h time period for calculating VLDL-TG and VLDL-apoB-100 kinetics because these concentrations were steady during the time frame of the isotope infusion study (\( P = 0.929 \) and 0.499, respectively, for the effect of time, by repeated-measures ANOVA).

Plasma free glycerol, palmitate, and leucine TTRs, the TTRs of glycerol and palmitate in VLDL-TG, and the TTR of leucine in VLDL-apoB-100 were determined by gas chromatography-mass spectrometry (Agilent Technologies/HP 6890 series GC system-5973 mass selective detector; Hewlett-Packard) (49, 52). The heptadecanoic acid to plasma as an internal standard (52). Total plasma TG and VLDL-TG concentration immediately after collection; the remaining samples were stored at −80°C until final analyses were performed.

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Calculations. Palmitate rate of appearance (\( R_{pal} \)) in plasma was calculated by dividing the palmitate tracer infusion rate by the average plasma palmitate TTR value between 60 and 240 min during physiological and isotopic steady state; total FFA \( R_{FFA} \) (in \( \mu \text{mol/min} \)) was derived by dividing palmitate \( R_{pal} \) by the proportional contribution of palmitate to total plasma FFA concentration (48). Basal whole-body carbohydrate and fat oxidation rates and resting metabolic rate were calculated based on \( V_{O2} \) and \( V_{CO2} \) measurements (17). The homeostasis model assessment index for whole-body insulin resistance was...
calculated as the product of plasma insulin (in μU/ml) and glucose (in mmol/l) concentrations divided by 22.5 (47).

A metabolic steady state existed with regard to VLDL-TG and VLDL-apoB-100 kinetics because plasma VLDL-TG and VLDL-apoB-100 concentrations remained constant throughout the 12-h sampling period. The fractional turnover rate (FTR) of VLDL-TG was determined by fitting the TTR time courses of free glycerol in plasma and glycerol in VLDL-TG to a compartmental model (51). The rate of VLDL-TG secretion (in μmol/min), which represents the total amount of VLDL-TG secreted by the liver, was calculated by multiplying the FTR of VLDL-TG (in pools/min) by the concentration of VLDL-TG in plasma (in μmol/l) and the plasma volume (in liters); plasma volume was assumed to be equal to VLDL-TG volume of distribution and was calculated as 0.055 l/kg fat-free mass (43). We also calculated VLDL-TG secretion rate per unit of plasma (μmol·l plasma⁻¹·min⁻¹) by multiplying the FTR of VLDL-TG by the concentration of VLDL-TG in plasma; this represents the relationship between VLDL-TG secretion and its distribution space and is not affected by potential changes in plasma volume. The results were the same as when expressed in micromoles per minute and are therefore not shown. The plasma clearance rate of VLDL-TG (in ml plasma/min) was calculated as the production rate divided by the plasma concentration. The mean residence time (MRT) of VLDL-TG (in minutes) was calculated as 1/FTR. The MRT indicates the average time that VLDL-TG circulates in the bloodstream; a short VLDL-TG MRT indicates a quick removal of TG from circulating VLDL particles, whereas a long MRT suggests that VLDL-TG, after being secreted by the liver, remains in plasma without being hydrolyzed for a considerable amount of time.

The relative contribution of systemic plasma FFA to VLDL-TG fatty acids was calculated based on isotopic dilution and fitting the palmitate TTR in plasma and VLDL-TG to a compartmental model (49, 51). These fatty acids represent FFA from the systemic circulation that are taken up by the liver and directly incorporated into VLDL-TG or temporarily incorporated into rapidly turning over intrahepatic and intraportal TG stores before incorporation into VLDL-TG. The remaining fatty acids in VLDL-TG (nonsystemic) are derived from pools of fatty acids that are not labeled with tracer during the infusion period; this includes 1) fatty acids released from preexisting, slowly turning over lipid stores in the liver and tissues draining directly into the portal vein, 2) fatty acids resulting from lipolysis of plasma lipoproteins that are taken up by the liver, and 3) fatty acids derived from hepatic de novo lipogenesis (41).

The FTR of VLDL-apoB-100 was calculated by fitting the TTR time courses of free leucine in plasma and leucine in VLDL-apoB-100 to a compartmental model (49). The rate of VLDL-apoB-100 secretion (in mg/min), the plasma clearance rate of VLDL-apoB-100 (in ml plasma/min), and the MRT (in minutes) of VLDL-apoB-100 (indexes of the secretion rate, plasma clearance rate, and MRT of VLDL particles) were calculated from plasma VLDL-apoB-100 concentration and VLDL-apoB-100 FTR as described above for VLDL-TG. The MRT of VLDL-apoB-100 indicates the time that the VLDL particle remains in the circulation after being secreted by the liver.

Statistical analysis. All data sets were tested for normality according to Kolmogorov-Smirnov. Normally distributed variables are presented as means ± SE, and paired Student’s t-test was used to compare data from studies after rest and after exercise. Variables that were not normally distributed (VLDL-TG secretion rate and VLDL-apoB-100 MRT) are presented as medians and quartiles, and Wilcoxon’s signed ranks test was used to compare data from studies after rest and after exercise. P ≤ 0.05 was considered statistically significant.

RESULTS

\( \dot{V}O_2 \text{ peak}, \ exercise \ workload, \ and \ intensity. \) The average \( \dot{V}O_2 \text{ peak} \) achieved during preliminary testing was 3.2 ± 0.2 l/min. Absolute power output and \( \dot{V}O_2 \) during the 60-min exercise session were constant between 5 and 60 min of exercise: subjects exercised at an average of 137 ± 12 W, which elicited 60 ± 1% of their \( \dot{V}O_2 \text{ peak} \).

\( \text{Plasma insulin and substrate concentrations.} \) Plasma glucose concentration was moderately, but significantly, reduced the morning after evening exercise compared with rest, whereas plasma FFA concentration was significantly increased after exercise (Table 1). Plasma insulin, total plasma TG and VLDL-TG, and total plasma apoB-100 and VLDL-apoB-100 concentrations were not different after rest and exercise (Table 1).

\( \text{Substrate oxidation and FFA} \) \( R_a. \) Basal \( \dot{V}O_2 \) and \( VCO_2 \), and thus respiratory exchange ratio, resting metabolic rate, and whole-body carbohydrate and fatty acid oxidation rates, were not affected by evening exercise (Table 2 and Fig. 1). Plasma FFA \( R_a \) was 53 ± 20% greater after evening exercise than rest (\( P = 0.013; \) Fig. 1).

\( \text{VLDL-TG} \) and \( \text{VLDL-apoB-100} \) kinetics. The FTR of VLDL-TG was 0.42 ± 0.03 pools/h after rest and 0.52 ± 0.05 pools/h after exercise (\( P = 0.094 \)). Exercise had no effect on hepatic VLDL-TG secretion rate (Fig. 2) but tended to increase plasma VLDL-TG clearance rate (\( P = 0.090; \) Fig. 3) and decrease VLDL-TG MRT (\( P = 0.094; \) Fig. 4). The relative contribution of systemic plasma FFA to all fatty acids in VLDL-TG was not different after evening exercise and rest (80 ± 4% and 72 ± 6%, respectively; \( P = 0.335 \)); accordingly, nonsystemic fatty acids accounted for 28 ± 6% of all fatty acids in VLDL-TG after rest and 20 ± 4% after exercise.

The FTR of VLDL-apoB-100 was 0.34 ± 0.05 pools/h after rest and 0.35 ± 0.03 pools/h after exercise (\( P = 0.921 \)). Exercise had no effect on hepatic VLDL-apoB-100 secretion rate (Fig. 2), plasma VLDL-apoB-100 clearance rate (Fig. 3), and VLDL-apoB-100 MRT (Fig. 4).

The molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates, reflecting the average TG content of the newly secreted VLDL particles, was not different after rest and exercise (11,975 ± 2,518 and 12,414 ± 1,040, respectively; \( P = 0.820 \)).

DISCUSSION

In this study, we investigated the effect of a single, 1-h bout of moderate-intensity evening exercise on basal plasma FFA, VLDL-TG, and VLDL-apoB-100 concentrations and kinetics in untrained, healthy, lean men. Our findings demonstrate that such exercise, which is recommended for maintenance of metabolic and cardiovascular health (2), induces a marked increase in basal FFA \( R_a \) but has no significant effect on whole-body carbohydrate oxidation and FFA oxidation rates. Indeed, the average FFA oxidation rate decreased slightly, whereas the average carbohydrate oxidation rate increased slightly, after evening exercise (Table 2 and Fig. 1). This is in agreement with a recent study in untrained, healthy, lean men which showed that evening exercise increased lipolysis and decreased carbohydrate oxidation (11).
Table 2. Respiratory gas exchange measurements, resting metabolic rate, and whole-body substrate oxidation rates after evening rest and evening exercise (1 h at 60% peak oxygen consumption)

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen consumption, ml/min</td>
<td>235±8</td>
<td>245±7</td>
<td>0.114</td>
</tr>
<tr>
<td>Carbon dioxide production, ml/min</td>
<td>195±7</td>
<td>201±4</td>
<td>0.158</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.83±0.01</td>
<td>0.82±0.01</td>
<td>0.573</td>
</tr>
<tr>
<td>Resting metabolic rate, kcal/min</td>
<td>1.13±0.04</td>
<td>1.17±0.03</td>
<td>0.109</td>
</tr>
<tr>
<td>Carbohydrate oxidation, mg/min</td>
<td>108±16</td>
<td>106±10</td>
<td>0.864</td>
</tr>
<tr>
<td>Fat oxidation, mg/min</td>
<td>53±7</td>
<td>59±5</td>
<td>0.383</td>
</tr>
</tbody>
</table>

Values are means ± SE.

body fatty acid oxidation and plasma VLDL-TG and VLDL-apoB-100 concentrations and kinetics. This response is different from the effect of a single, prolonged (2-h) session of the same exercise on lipid metabolism, which markedly increases basal FFA Ra, whole-body fat oxidation, and VLDL-TG plasma clearance rate and reduces VLDL-apoB-100 secretion rate (43). These findings suggest that the effects of evening exercise on basal whole-body fat oxidation, VLDL-TG, and VLDL-apoB-100 metabolism are dependent on exercise duration and thus probably directly related to the exercise-induced energy expenditure. Single sessions of typical recreational activities appear to have little effect on basal plasma TG homeostasis. Furthermore, our data illustrate that, during prolonged recovery from exercise, there is a clear dissociation between systemic FFA availability and VLDL-TG and VLDL-apoB-100 secretion by the liver.

The acute effects of exercise on adipose tissue lipolysis and FFA Ra are well described (33). Lipolysis increases severalfold immediately after the onset of exercise (33) and then diminishes significantly on cessation of the exercise to approximately twofold resting values for at least 3 h during recovery (61). However, little is known about the longer-term effect of exercise on FFA Ra and the mechanisms regulating lipolysis during recovery from exercise. Resting, basal FFA Ra was found to be greater (almost double) in endurance-trained than in sedentary subjects (53). This was probably a consequence of the last exercise bout because increased adipose tissue lipolysis and FFA Ra cannot be demonstrated 2–4 days after training cessation (35, 46). In sedentary subjects, we have previously found that 2 h of moderate intensity cycling in the evening increased basal FFA Ra by ~65% on the following morning (43), a response very similar in magnitude to the increase observed in the present study (~55%) after only 1 h of the same exercise. This is consistent with the results from a study in trained athletes by Romijn et al. (53) who found that a single exercise bout increases basal FFA flux the following morning; the extent to which exercise stimulated lipolysis and thus FFA flux was independent of the intensity and duration of exercise (i.e., energy expenditure). Insulin is the major regulator of basal FFA Ra (34, 39) and could have contributed to the higher FFA Ra in endurance-trained athletes (53) and the increase in FFA Ra after 2 h of exercise in our previous study (43); however, we did not observe a change in fasting plasma insulin concentration after evening exercise in the present study. Catecholamines are largely responsible for the acute stimulation of lipolysis during exercise (37), but their concentration returns to baseline levels within ~2 h after the end of exercise (12). Moreover, basal, resting catecholamine concentrations are not different in highly trained athletes and sedentary controls (53). Insulin and catecholamines are therefore unlikely a major regulatory factor responsible for increased FFA flux during the late phase of recovery from exercise. Enhanced adipose tissue blood flow is also unlikely to be responsible for this phenomenon because, although it is acutely increased by exercise (61), there is no evidence that this effect persists into the prolonged recovery period (e.g., 12–16 h postexercise) (44). Other hormonal, paracrine, and/or autocrine signals [e.g., atrial natriuretic peptide and interleukin-6 (55)] are therefore likely responsible for increased, basal FFA Ra after a single bout of endurance exercise.

In the present study, unlike our previous one (43) in which the effect of a single, prolonged (2-h) bout of moderate intensity cycling was investigated, VLDL-TG and VLDL-apoB-100 concentrations and metabolism were not altered by exercise. But we noted a trend toward increased VLDL-TG plasma clearance and therefore decreased VLDL-TG MRT because VLDL-TG plasma clearance rate increased in three of the seven subjects. However, it did not change or even decreased in the other four. Because our study had enough power (80%) to detect a ≥25% difference in VLDL-TG plasma clearance rate (at α = 0.05), we believe our findings most probably reflect true differences in the response to 1 and 2 h of exercise. The reasons for the heterogeneous response to the 1-h bout cannot be determined from our data. It could reflect a gradual return to baseline values, since increased VLDL-TG plasma clearance, although no change in concentration, has been demonstrated immediately after 45 min of moderate-intensity cycling (50). It therefore appears that basal VLDL-TG and VLDL-apoB-100 metabolism after exercise are directly related to exercise duration and thus probably the energy expended during exercise. On the basis of this and our previous study (43), in which subjects performed at the upper end of moderate-intensity exercise, the threshold amount of exercise required to lower fasting plasma TG concentration in a single session appears to be >500 kcal. The same exercise, however, can reduce postprandial triglyceridemia even if it does not affect fasting plasma TG concentrations (57, 65). Because the exercise-induced hypotriglyceridemia cannot be replicated by
an equivalent energy deficit incurred by caloric restriction (20), nor can it be abolished by compensatory hypercaloric feeding (25), the mechanisms involved in mediating the hypotriglyceridemic effect of exercise likely relate to muscular contraction per se and not to the negative energy balance.

Our data indicate that FFA $R_a$ is either more sensitive to exercise than VLDL-TG and VLDL-apoB-100 metabolism or that exercise produces a more prolonged response in FFA $R_a$ than in VLDL-TG and VLDL-apoB-100 metabolism. This clearly highlights a dissociation between plasma FFA availability and hepatic VLDL-TG and VLDL-apoB-100 secretion that is particularly evident in the present study in which fat oxidation was not altered by exercise. This finding is consistent with the results from recent studies (5, 23) but appears to contradict the widely accepted notion that plasma FFA concentration regulates hepatic VLDL-TG and VLDL-apoB-100 secretion, presumably by increasing FFA delivery to the liver (42). It is unlikely that we were unable to detect an effect of increased FFA availability on VLDL-TG and VLDL-apoB-100 secretion. Even if only a small fraction, e.g., 10–30% (4, 38, 54, 61), of the additional fatty acids (~77 umol/min) were directed toward the liver and VLDL-TG synthesis and secretion, exercise would have caused a 25–60% increase in hepatic VLDL-TG secretion rate, which was not the case. Studies that found an in vivo effect of FFA on VLDL-TG and VLDL-apoB-100 secretion involved direct manipulation of plasma FFA concentration by infusing a lipid emulsion plus heparin to increase plasma FFA availability (42) or by administering nicotinic acid (63) or acipimox (45) to decrease plasma FFA availability; therefore, these results were likely confounded by the effects of heparin (28), nicotinic acid (63), and acipimox (26) on VLDL-TG turnover that occur independently of plasma FFA concentration. The dissociation between FFA availability and VLDL-TG and VLDL-apoB-100 secretion suggests that intrahepatic channeling of fatty acids toward storage or secretion is tightly regulated by yet largely unknown mechanisms. The fate of the excess FFA after exercise is uncertain. Most likely, FFA are directed toward storage to replenish tissue TG stores because whole-body fatty acid oxidation was not affected by exercise in the present study. Muscle (40, 62) and probably also liver (56) TG concentrations decrease after exercise and return to baseline levels 18–24 h postexercise (40), a time frame consistent with our experiment.

In summary, we found that a single 60-min evening cycling session had no effect on basal, postabsorptive VLDL-TG and VLDL-apoB-100 metabolism and whole-body fat oxidation but markedly stimulated FFA $R_a$ in untrained, healthy, young men. In view of our previous findings concerning the effect of a single prolonged (2-h) bout of the same exercise, we suggest
that exercise affects basal VLDL-TG and VLDL-apoB-100 metabolism in a dose-dependent manner, and the threshold for any exercise-mediated effect on basal VLDL-TG and VLDL-apoB-100 metabolism lies above 60 min of moderate-intensity exercise.

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