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# A single bout of brisk walking increases basal very low-density lipoprotein triacylglycerol clearance in young men

Tsekouras E. Yiannis, Yanni E. Amalia, Bougatsas Dimitrios, Kavouras A. Stavros, Sidossis S. Labros*

*Department of Nutrition and Dietetics, Laboratory of Nutrition and Clinical Dietetics, Harokopio University, 176-71 Athens, Greece*

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**Abstract**

Very low-density lipoprotein triacylglycerol (VLDL-TG) turnover rate was evaluated in the morning, 12 hours after a single bout of brisk walking (90 minutes at approximately 60% of $V_{O_2}$max; EXE), compared to a resting control period (CON) in 10 recreationally active men. VLDL-TG fractional catabolic rate was calculated from the decay in isotopic enrichment after a bolus injection of [$^2H_5$]glycerol. Plasma VLDL-TG concentration was 24% lower in the morning after the EXE trial compared to control (0.47 ± 0.04 and 0.36 ± 0.04 mmol $L^{-1}$, for CON and EXE, respectively; $P < .01$). Serum insulin (7.4 ± 0.7 and 5.6 ± 0.4 mIU $L^{-1}$, CON and EXE, respectively; $P < .05$) and plasma glucose (5.6 ± 0.1 and 5.4 ± 0.1 mmol/L, CON and EXE, respectively; $P < .05$) concentrations were also significantly lower in the EXE trial. Insulin sensitivity (homeostasis model assessment [HOMA] index) was improved by 27% in EXE compared with the CON trial ($P < .05$).VLDL–apolipoprotein B-100 and plasma fatty acid concentrations were similar in the two trials. Hepatic VLDL-TG secretion rates were not significantly affected by exercise (13.1 ± 1.2 and 13.2 ± 1.6 $\mu$mol·$min^{-1}$ for the CON and EXE trials, respectively), whereas VLDL-TG clearance rate increased by 36% (28.1 ± 1.3 and 38.1 ± 3.5 mL·$min^{-1}$ for the CON and EXE trials, respectively; $P < .05$). It is concluded that the decrease in fasting plasma VLDL-TG concentration observed 12 hours after brisk walking is related mainly to increased VLDL-TG clearance from plasma.


## 1. Introduction

Hypertriglyceridemia is considered to be an independent risk factor for coronary heart disease [1]. It is accompanied by low levels of high-density lipoprotein cholesterol (HDL-C) [2], presumably mediated by increased transfer of cholesterol esters from HDL to the very low-density lipoprotein (VLDL) particles [3], leading to high levels of low-density lipoprotein cholesterol (LDL-C) [4]. This lipid profile appears early in adolescence and is associated with low cardiorespiratory fitness and physical inactivity [5]. Thus, physical activity has been recommended as a powerful means for regulating blood lipid levels [6] not only in hypertriglyceridemics [7], but also in healthy sedentary individuals [8]. However, the exact mechanisms by which physical activity mediates the hypotriglyceridemic effect have not been determined yet.

Isolated exercise bouts produce transient metabolic responses that result in decreased plasma triacylglycerol (TG) levels, with the greatest responses appearing 12 to 24 hours in the postexercise period [9]. It is possible that these changes are related to the energy deficit resulting from exercise [10,11]. However, it has recently been demonstrated that resistance and aerobic exercise bouts of equivalent energy cost result in different lipidemic responses [12]. Furthermore, endurance-trained athletes have lower blood lipid levels compared with sedentary subjects [13], reflecting either a cumulative training effect or simply the effect of the previous exercise bout. Short-term detraining has been shown to increase both fasting TG levels and postprandial lipemia in athletes [14]. In any case, the mechanisms for the hypotriglyceridemic effect of exercise remain speculative when based on lipid concentrations without considering lipid turnover rates.

Prolonged brisk walking for 90 minutes at 60% of $V_{O_2}$max is associated with decreases in total and VLDL-TG levels [15]. This change could be related to increased VLDL-TG clearance, resulting from the up-regulation of lipoprotein lipase (LPL) activity in muscle [16,17] and/or to reduced

* Corresponding author. Tel.: +30 210 954 9154; fax: +30 210 954 9141.
*E-mail address:* lsidossis@hua.gr (S.S. Labros).

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hepatic VLDL-TG production [18]. Reduced VLDL-TG secretion in response to exercise has been reported previously in rats [19-21] and has been attributed to increased hepatic fatty acid oxidation.

Magkos et al [22] recently reported that 2 hours of cycling at 60% of $V_{O_2}$max increased VLDL-TG clearance with no effect on hepatic VLDL-TG secretion rate. These findings have important clinical implications because they support the recommendations for exercise in the treatment of hypertriglyceridemia. However, few people use cycling in their exercise routine. Most people walk, as walking is considered a safe, convenient form of exercise that almost everyone can practice. Unfortunately, we cannot safely assume that the effects of cycling on VLDL-TG kinetics apply during walking. This is because walking and cycling, although both mainly aerobic-type activities, differ significantly in the muscle fiber recruitment pattern, a difference that has been found to affect substrate metabolism [23]. Specifically, fat oxidation was significantly higher during walking compared to cycling in moderately trained men [23]. This difference may significantly affect the metabolic pathways involved in VLDL secretion and clearance, as free fatty acid (FFA) kinetics and oxidation can modify both hepatic and peripheral VLDL-TG metabolism [24,25].

Therefore, the aim of the present study was to investigate the effect of a single, prolonged bout of brisk walking (90 minutes at 60% of $V_{O_2}$max) on the relative contribution of VLDL-TG secretion and clearance rate on the observed hypotriglyceridemic response in young men.

## 2. Methods

### 2.1. Subjects and preliminary testing

Ten healthy men (age, 26 ± 1 years; body weight, 86 ± 3 kg; body mass index [BMI], 25 ± 0.4 kg $m^{-2}$; means ± SEM) participated in the study. Subjects were considered untrained, but recreationally active (participated in moderate intensity physical activities only once a week), after completing a physical activity questionnaire. All subjects signed a written informed consent before their participation in the study, which was approved by the Human Studies Committee of Harokopion University.

All subjects were nonsmokers, were considered to be in good health, and were not taking any medication. Their body weight was stable for at least 2 months before the study. A complete physical examination, including preliminary blood screening, was carried out to determine whether all volunteers were normolipidemic and normoglycemic. They were instructed to refrain from vigorous exercise for at least 4 days before the tracer infusion studies and from alcohol or caffeine consumption 2 days before tracer infusion.

Anthropometric and body composition measurements were carried out approximately 1 week before the studies. Body weight and height were measured to the nearest 0.1 kg and 0.5 cm, respectively. Body mass index was obtained by the formula BMI = weight (kg)/height$^2$ ($m^2$). Total body measurements of fat mass and lean mass were made by dual-energy x-ray absorptiometry (model DPX, Lunar, Madison, WI, software version 3.6) [26].

Peak oxygen consumption ($V_{O_2}$max) was determined by a submaximal incremental brisk walking test (modified Balke treadmill protocol) [27]. Subjects warmed up for 5 minutes and were familiarized with the treadmill (Technogym Runrace, Gambettola, Italy). Expiratory gases were measured continuously with a breath-by-breath system by a gas analyzer (Vmax229, Sensormedics, Yorba Linda, CA). After warm-up, treadmill speed was kept constant and treadmill inclination was increased by 2% every 3 minutes. The test was terminated at 80% of heart rate reserve, and $V_{O_2}$max was predicted from the $V_{O_2}$-heart rate relationship.

### 2.2. Experimental protocol

Each subject completed 2 stable isotope–labeled tracer infusion studies 1 week apart, in a randomized order (Fig. 1). During the exercise, trial volunteers were admitted to the laboratory the evening before the isotope infusion and exercised for 90 minutes on a treadmill. The speed and the gradient of the treadmill were set accordingly so that each participant exercised at approximately 60% of $V_{O_2}$max. At 30-minute intervals during exercise, a 5-minute gas sampling was performed to measure steady-state $V_{O_2}$ at the preset level. In the resting trial, participants were requested to refrain from any form of physical activity and to remain rested at home.

### 2.3. Diet assessment and control

Instructions were provided to all participants on how to record food and beverage intake. Specifically, participants recorded all food and drinks consumed during the 3 days immediately preceding the first trial and they replicated this diet during the 3 days before the second trial, a week later. Food journals were analyzed using Food Processor nutrition analysis software (Nutritionist Five, v2.2, San Bruno, CA). The mean diet composition of the day immediately preceding the kinetic studies, for both trials, was 47% ± 3% carbohydrates, 37% ± 3% fat, and 16% ± 2% protein. The last meal was taken 10 to 12 hours before tracer infusion.

### 2.4. Stable isotope infusion

The morning after the exercise trial or the rest day, subjects arrived at the laboratory in the fasting state. One

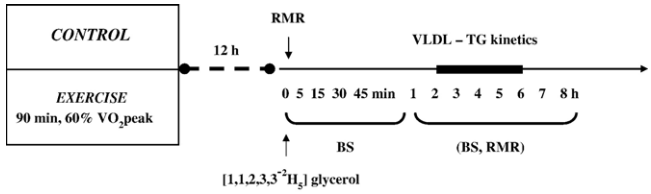


Fig. 1. Graphical representation of the experimental protocol. RMR indicates resting metabolic rate; BS, blood sampling.

catheter was inserted into a forearm vein to administer stable isotope–labeled tracers, and a second catheter was inserted into a contralateral hand vein for blood sampling. The latter was kept warm with a heating pad. Catheters were washed after blood sampling by infusion of 0.9% NaCl solution. Subjects were allowed to relax and get used to the catheters for approximately 1 hour. At 12 ± 1 hours after the completion of the exercise trial the previous evening, a baseline blood sample (time = 0) was taken to determine resting plasma glucose, total TG, cholesterol, HDL-C, free fatty acids, serum insulin concentration, and background glycerol enrichment. Then, a bolus of [1,1,2,3,3-$^2H_5$] glycerol (Goss Scientific Instruments, Essex, UK) (~75 μmol/kg body weight) dissolved in 0.9% NaCl solution was administered through the catheter in the forearm vein and blood samples were obtained at 5, 15, 30, 45, and 60 minutes and then every hour for 8 hours to determine glycerol tracer-tracee ratio (TTR) in VLDL-TG.. Resting energy expenditure was measured with indirect calorimetry (Vmax229, Sensormedics) for 15 minutes before catheter insertion and for every hour after bolus injection to determine resting metabolic rate. During this period, all subjects remained fasted in the laboratory in a sitting position. Water consumption was allowed ad libitum.

### 2.5. Sample collection and storage

Blood samples were collected in precooled tubes containing EDTA as anticoagulant to determine substrate concentrations. Samples were immediately placed on ice and plasma was separated by centrifugation within 30 minutes of collection. Aliquots of plasma (~3 mL) were transferred into plastic culture tubes and kept in the refrigerator for immediate isolation of VLDL. The remaining plasma samples were stored at −80°C for determination of total TG, total cholesterol, HDL-C, glucose, and free fatty acid concentration using commercially available enzymatic kits (Wassermann Diagnostics, NJ, USA) in an automated analyzer (Sciapparelli Biosystems, USA). A separate blood sample portion was transferred into tubes with no additives for serum preparation. Serum was stored at −80°C until analysis of insulin (immunofluorescence enzymatic assay, ST AIA pack IRI, TOSOH AIA System Analyzers, South San Francisco, CA). All samples from each subject were analyzed in the same batch. A small aliquot from baseline blood samples of each trial was used for determination, in triplicates, of hematocrit by microhematocrit and hemoglobin concentration by cyanomethemoglobin (Boehringer Mannheim Diagnostics, Mannheim, Germany) assays. Changes in plasma volume between trials were estimated by the Dill and Costill equation [28].

### 2.6. VLDL fraction preparation and analyses

Very low-density lipoprotein fraction was prepared as previously described [29] with minor modifications. Briefly, 2 mL of plasma was transferred into Quick Seal Centrifuge Polyallomer Tubes (Beckman Instruments, Palo Alto, CA), overlaid with a NaCl/EDTA solution (1.006 kg/L), and centrifuged in a TLN-100 rotor (Beckman Instruments) at 90,000 rpm for 3 hours at 4°C. The top layer containing the VLDL particles was removed by tube slicing (Beckman Instruments) and stored at −80°C until final analyses were performed by commercially available enzymatic kits (Wassermann Diagnostics) using an automated analyzer (Sciapparelli Biosystems). Apolipoprotein (apo) B-100 concentration in VLDL particles was determined by immunoturbidimetric immunoassay (RANDOX Ltd, Crumlin, CO. Antrim, UK).

### 2.7. Isolation of TG in VLDL particles

Aliquots (750 μL) of the isolated VLDL lipoprotein fractions were deproteinized with 3 mL acetone and centrifuged (3000 rpm for 15 minutes at 4°C), and the supernatant was dried under vacuum (SpeedVac, Thermo-Savant, Holbrook, NY) before isolating TG by thin-layer chromatography (TLC). Dried samples were resuspended in chloroform-methanol (3:1), spotted on an LK6D silica gel plate (6.0-nm silica gel, 250-μm layer thickness; Whatman, Maidstone, UK), and developed with heptane-diethyl ether-formic acid (80:20:2) solvent in an enclosed developing chamber. The TG band was visualized with 0.01% rhodamine 6G in the TLC lanes. These bands were scraped from the TLC plate and transferred into 13 × 159 mm test tubes. TGs were extracted from the silica gel with chloroform-methanol (3:1) and dried under vacuum. The dried samples were hydrolyzed by resuspension in 10% acetyl chloride-methanol and incubation for 30 minutes at 70°C and then dried under vacuum. Glycerol was derivatized by using heptafluorobutyric anhydride (30 minutes incubation at 70°C) and dried under vacuum for 150 minutes. Finally, dry glycerol was dissolved in heptane and stored at −40°C. The TTR of glycerol present in VLDL-TG was determined by gas chromatography-mass spectrometry (GC/MS; MSD 5973 system, Hewlett-Packard, Palo Alto, CA) by monitoring the ions at mass to charge ratios (*m*/*z*) 467 and 472. Calibration curve for standards with known isotopic enrichment was used.

### 2.8. Calculations

The fractional catabolic rate (FCR) of VLDL-TG was determined by using the monoexponential approach [29,30]. The absolute rate of VLDL-TG secretion was calculated as:

$$\text{VLDL–TG secretion rate } (\mu\ \text{molmin}^{-1}) = (\text{FCR}_{\text{VLDL–TG}} \times C_{\text{VLDL–TG}} \times \text{PV})/60,$$

where $C_{\text{VLDL-TG}}$ is the concentration of VLDL-TG in plasma, and PV is the plasma volume, which was estimated to be 0.055 L per kilogram fat-free mass [31]. It was assumed that the VLDL-TG volume of distribution is equal to PV because VLDL particles are restricted in plasma [32]. The rate of whole-body VLDL-TG clearance from plasma (an index of the efficiency of VLDL-TG removal) was calculated by dividing the rate of VLDL-TG disappearance from plasma (in micromoles per minute) by the plasma

Table 1
Substrate and hormone concentrations 12 hours postexercise

| | Control | Exercise |
|---|---|---|
| Insulin (mIU $L^{-1}$) | 7.4 ± 0.7 | 5.6 ± 0.4 * |
| Glucose (mmol $L^{-1}$) | 5.6 ± 0.1 | 5.4 ± 0.1 * |
| Free fatty acids (mmol $L^{-1}$) | 0.50 ± 0.06 | 0.57 ± 0.06 |
| Total TG (mmol $L^{-1}$) | 0.82 ± 0.06 | 0.70 ± 0.05 * |
| VLDL-TG (mmol $L^{-1}$) | 0.47 ± 0.04 | 0.36 ± 0.04 ** |
| VLDL–apo B-100 (mg $dL^{-1}$) | 2.99 ± 0.3 | 4.00 ± 0.5 |
| HDL-C (mg $dL^{-1}$) | 34.4 ± 1.2 | 33.0 ± 1.5 |
| LDL-C (mg $dL^{-1}$) | 124.2 ± 6.1 | 108.6 ± 9.3 * |
| VLDL-C (mg $dL^{-1}$) | 7.0 ± 0.7 | 5.5 ± 0.8 * |
| Total C (mg $dL^{-1}$) | 165.6 ± 6.2 | 147.1 ± 9.8 * |

Data are means ± SEM.
* $P < .05$, significant difference vs control.
** $P < .01$, significant difference vs control.

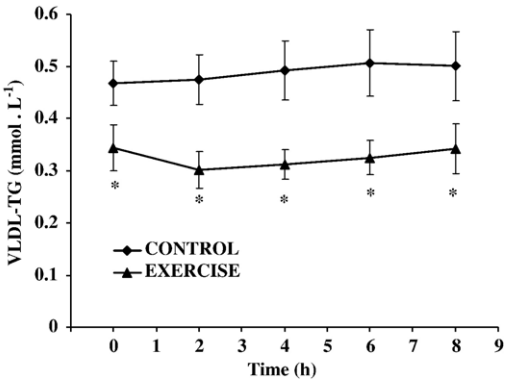


Fig. 3. VLDL-TG concentration throughout the 8-hour study period for the CON and EXE trials, respectively. *$P < .05$, significantly different from control.

VLDL-TG concentration (in micromoles per milliliter). Mean residence time of VLDL-TG was calculated as 1/FCR. Insulin resistance was assessed by HOMA index as follows: fasting serum insulin ($\mu$U/mL) × fasting plasma glucose (mmol/L)/22.5 [33].

### 2.9. Statistical analysis

Results are presented as means ± SEM. Comparisons between rest and exercise were made by Student paired *t* test. A repeated-measures analysis of covariance was performed to test the stability of total TG concentration and $V_{O_2}$ throughout the 8-hour kinetic study for both trials that took into account intercorrelation between time points. Covariates as potential cofounders were age, BMI, and $V_{O_2}$max. Data were tested for normality and statistical significance was set at $P \leq .05$.

## 3. Results

### 3.1. Body composition, $V_{O_2}$max, and exercise intensity

Average fat mass and lean mass were 17.4 ± 1.4 and 68.6 ± 2.9 kg, respectively. Absolute and relative $V_{O_2}$max were 3.3 ± 0.2 L·$min^{-1}$ and 39.0 ± 1.9 mL·$min^{-1}$·(kg body weight)$^{-1}$, respectively. Absolute $V_{O_2}$ was kept constant throughout the 90 minutes of exercise; subjects exercised at an average intensity of 62% ± 2.6% of their $V_{O_2}$max. Average oxygen consumption during 90 minutes of exercise was 2.1 ± 0.1 L·$min^{-1}$ with a mean respiratory quotient of 0.91 ± 0.01. Exercise gross energy expenditure was 3.96 ± 0.23 MJ with 71% ± 6% originating from carbohydrate oxidation (169 ± 18 g) and 29% ± 6% from fat oxidation (30 ± 6 g).

### 3.2. Substrate oxidation

After repeated-measures analysis of covariance, a metabolic steady state existed with regard to VLDL-TG kinetics, as indicated by the constant plasma VLDL-TG concentration

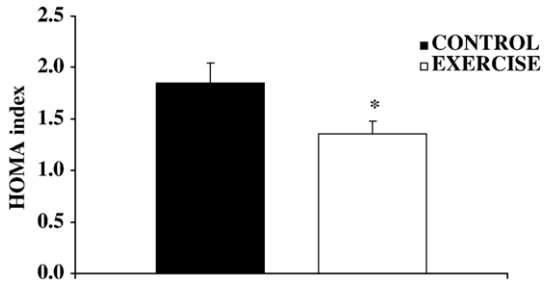


Fig. 2. Insulin sensitivity assessed by the HOMA index in the control (filled bars) and exercise trial (open bars). *$P < .05$, significantly different from control.

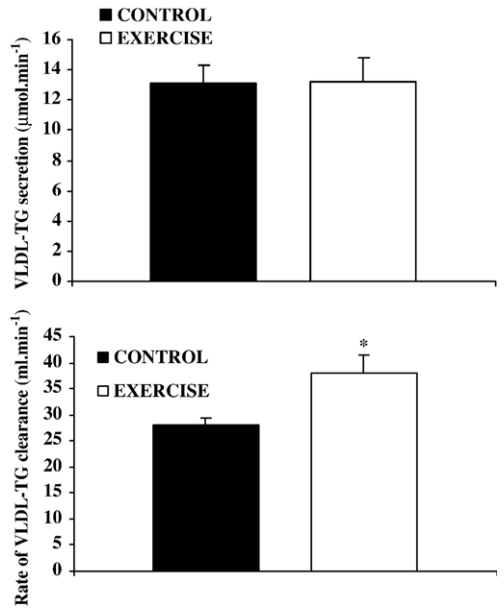


Fig 4. A, Rate of VLDL-TG secretion (top) into plasma in the control (filled bars) and exercise trial (open bars). B, VLDL-TG plasma clearance rate (bottom) in the control (filled bars) and exercise trial (open bars). *$P < .05$, significantly different from control.

throughout the 8-hour sampling period. Similarly, resting energy expenditure, estimated by indirect calorimetry, was not affected by the evening exercise session (192 ± 10 and 189 ± 13 mL oxygen per·minute for the CON and EXE trials, respectively). Respiratory exchange ratio was identical (0.79 ± 0.02) for both trials and, hence the rate of whole-body fat oxidation based on $V_{O_2}$ and $V_{CO_2}$ measurements remained the same.

### 3.3. Insulin and substrate concentrations

Changes in plasma volume between the CON and EXE trials were minor (4% ± 1%; *P* = not significant); therefore, no corrections have been made on substrate concentrations in the present study. Serum insulin, plasma glucose, total TG, and VLDL-TG concentrations were significantly lower in the EXE compared with the CON trial ($P < .05$) (Table 1). Insulin sensitivity, assessed by the HOMA index, was improved in the EXE trial by 27% ($P < .05$; Fig. 2). On the contrary, VLDL–apo B-100 and plasma free fatty acid concentrations were not different between the 2 trials (Table 1). In addition, in the EXE trial, total plasma cholesterol, LDL-C, and VLDL cholesterol (VLDL-C) were significantly lower compared with the CON trial ($P < .05$). HDL-C level, however, was not different between the CON and the EXE trials (Table 1). Plasma FFA levels were monitored throughout the 8-hour sampling period and concentrations remained constant for both the CON and the EXE trials.

### 3.4. VLDL-TG kinetics

VLDL-TG levels did not change significantly during the 8-hour study period, both in the CON and in the EXE trials (Fig. 3). VLDL-TG FCR was significantly higher in the EXE compared with the CON trial (0.61 ± 0.06 vs 0.45 ± 0.03 pools·per hour , EXE vs CON, respectively; $P < .05$). On the contrary, VLDL-TG rate of secretion into plasma was similar in the 2 trials (13.1 ± 1.2 and 13.2 ± 1.6 $\mu$mol·min$^{-1}$ for the CON and the EXE trials, respectively) (Fig. 4). The clearance rate of VLDL-TG, however, was 35% greater ($P < .05$) in the EXE trial (38.1 ± 3.5 mL·min$^{-1}$) compared with CON (28.1 ± 1.3 mL·min$^{-1}$). Typical data from a single subject are shown in Fig. 5.

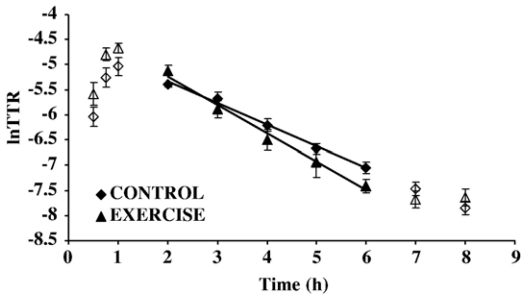


Fig 5. Mean plasma glycerol TTRs in the CON (diamonds) and EXE (triangles) trials. Solid diamonds and triangles represent selected time points for analysis. Open diamonds and triangles represent omitted time points for analysis.

## 4. Discussion

The aim of the present study was to investigate the mechanism(s) underlying the exercise-induced reduction in plasma VLDL-TG concentration. Our results showed a 24% decrease in VLDL-TG concentration 12 hours after brisk walking at 60% of $V_{O_2}$max for 90 minutes. This effect was related to a 35% increase in VLDL-TG clearance, whereas no difference was observed in VLDL-TG secretion rate.

Our results agree with those reported by Magkos et al [22], namely, that 2 hours of cycling at 60% of $V_{O_2}$max increased VLDL-TG clearance with no effect on hepatic VLDL-TG secretion rate, confirming that both forms of exercise (ie, prolonged walking and cycling) not only produce similar effects on total TG and VLDL-TG concentrations in plasma, but also appear to elicit this effect via similar mechanisms. This finding was somewhat unexpected, as these 2 forms of exercise differ significantly in the muscle fiber recruitment pattern and the rate of fat oxidation, being higher during walking compared to cycling in moderately trained men [23]. Altered fatty acid oxidation pattern could significantly affect VLDL-TG secretion and clearance, especially if this change reflects intrahepatic events [24,25]. We have thus confirmed that prolonged walking, which is considered a safe, convenient form of exercise, can elicit significant responses in VLDL-TG metabolism and presumably, in the long run, have a cardiovascular protective effect.

However, not everyone can practice 90 minutes of brisk walking daily. The design of the present study does not allow us to draw conclusions as to the effects of shorter duration exercise. Magkos et al [34] recently reported that in contrast to 2 hours of cycling at 60% of $V_{O_2}$max, 1 hour of cycling in the same intensity did not produce any effect on plasma TG concentration and hepatic VLDL-TG secretion and clearance rates. In the present study, significant effects on VLDL-TG secretion were observed after 90 minutes of exercise the previous day. However, more studies are required to determine the minimum walking duration to elicit a significant VLDL-TG response.

A monoexponential slope of the decline in VLDL-TG glycerol TTR could be reliably determined in all volunteers over a 2- to 6-hour period after peak enrichment. Therefore, we used the monoexponential decay of glycerol enrichment in VLDL-TG to quantify VLDL-TG turnover rates. This approach has been previously validated [29,30]. Data after the 6-hour point were not used to avoid tracer recycling, especially in the postexercise period. Furthermore, we used [1,1,2,3,3-$^2H_5$]glycerol instead of palmitate, as this tracer was found to recycle to a lesser degree [29]. It is expected though that some tracer recycling is likely to occur, especially in the postexercise period, thereby possibly

underestimating VLDL-TG FCR in the EXE trial. Despite this fact, a 35% difference in VLDL-FCR was obtained between the rest and the exercise trials, suggesting that this change reflects a real event and is not the result of an estimation error. Finally, total plasma TG and VLDL-TG concentrations remained stable during the 8-hour study period; hence, assumptions for a constant VLDL-TG pool size were presumably valid.

The observed decrease in plasma VLDL-TG concentration the day after the exercise trial (Table 1) could have resulted from a decrease in hepatic VLDL-TG secretion, an increase in VLDL-TG removal rate from plasma, or both. We did not observe any change in hepatic VLDL-TG production rate (Fig. 4A) as a result of exercise the previous day, although, theoretically, we could expect either an increase or a decrease in VLDL-TG rate of appearance in plasma. The exercise-induced decrease in hepatic glycogen availability the previous evening could facilitate the use of hepatic FFAs for oxidation. This in turn could limit FFA availability for TG synthesis, resulting in a decrease in hepatic TG synthesis and secretion [25]. In the present study, although 170 g of carbohydrate was used during 90 minutes of exercise at 60% of $Vo_2$max the previous evening, we did not observe any change in hepatic VLDL-TG production. This could be either due to low hepatic glycogen use during exercise or due to the rapid hepatic glycogen replenishment during the early 12-hour recovery period in the presence of increased insulin sensitivity [35]. In the later scenario, however, it can be argued that carbohydrate intake from the last meal (97 g) was not sufficient to completely compensate for carbohydrate oxidation during exercise. This is also supported by the lower blood glucose levels in the EXE compared with the CON trial, 12 hours after exercise, indicating a necessity to further replenish endogenous carbohydrate stores. On the other hand, exercise the day before could have increased VLDL-TG secretion rates. Although in our study we did not observe any change in plasma FFA concentration (Table 1), others have reported increased FFA availability to the liver the day after 2 hours of cycling [22]. Increased FFA availability to the liver could result in higher VLDL-TG production and secretion rates.

Our data suggest that increased VLDL-TG clearance may explain the observed decrease in VLDL-TG concentration the morning after exercise. VLDL-TG clearance was substantially increased 12-hours postexercise, probably in response to improved insulin sensitivity (Fig. 2) that up-regulates muscle LPL [36]. LPL activity in skeletal muscle has been closely associated with insulin sensitivity [37]. Although physical inactivity suppresses LPL activity, reducing TG uptake by hind limb muscles in the rat [38], exercise training even without weight loss improves insulin sensitivity and increases lipase activity in plasma [39]. Exercise could also enhance lipid catabolism by directly increasing LPL protein mass in the muscle [16,40], increasing LPL enzyme activity, or both [41,42]. Recent data do not support the increase in muscle LPL protein mass in response to a prolonged exercise bout [22] as the mechanism by which exercise could enhance lipid catabolism. Finally, it is possible that the exercise-induced effects on plasma VLDL-TG metabolism were due to increased direct uptake of VLDL-TG by liver and/or other tissues.

The evening exercise session resulted in lower total cholesterol, VLDL-C, and LDL-C with no changes in HDL-C. This is in agreement with previous studies that report cholesterol decrease, in response to exercise, in subjects with slightly elevated blood cholesterol levels [43]. It can be argued that exercise at 60% of $Vo_2$max mediates the reduction in cholesterol due to a short-term increase in plasma volume [13]. However, plasma cholesterol changes were evident in the present study even after correcting for changes in plasma volume.

In the present study, the exercise trial was associated with a considerable negative energy balance compared to rest. Therefore, one could argue that the observed increase in VLDL-TG clearance may be related to the energy deficit and not to muscle contraction per se. It has been suggested that both the energy deficit produced by exercise and a diet-induced deficit of the same magnitude will have an effect on the lipid profile, with the first one being more prominent [11]. Recently, the effect of exercise training on insulin sensitivity was counteracted by refeeding the energy expended during exercise [44] in insulin-resistant volunteers. Therefore, we cannot discount the possibility that the results of the present study may reflect the effect of the negative energy balance due to exercise, an effect mediated by the intense muscle contraction per se, or a combination of both. The possible effects of energy deficit per se should be further studied.

In conclusion, brisk walking for 90 minutes at 60% of $Vo_2$max decreases VLDL-TG concentration by 24%. This effect is not associated with reduced VLDL-TG production by the liver, but seems to be due to enhanced rate of VLDL-TG removal. Improved insulin sensitivity in response to exercise may be involved in the stimulation of LPL activity and the enhancement of VLDL-TG clearance.

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