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# High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men

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**Tsekouras YE, Magkos F, Kellas Y, Basioukas KN, Kavouras SA, Sidossis LS.** High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. *Am J Physiol Endocrinol Metab* 295: E851–E858, 2008. First published July 29, 2008; doi:10.1152/ajpendo.90545.2008.—A single bout of strenuous endurance exercise reduces fasting plasma triglyceride (TG) concentrations the next day (12–24 h later) by augmenting the efficiency of very low-density lipoprotein (VLDL)-TG removal from the circulation. Although much of the hypotriglyceridemia associated with training is attributed to the last bout of exercise, the relevant changes in VLDL-TG metabolism have never been investigated. We therefore examined basal VLDL-TG kinetics in a group of sedentary young men ( $n = 7$ ) who underwent 2 mo of supervised high-intensity interval training (3 sessions/wk; running at 60 and 90% of peak oxygen consumption in 4-min intervals for a total of 32 min; gross energy expenditure:  $446 \pm 29$  kcal) and a nonexercising control group ( $n = 8$ ). Each subject completed two stable isotope-labeled tracer infusion studies in the postabsorptive state, once before and again after the intervention ( $\sim 48$  h after the last exercise bout in the training group). Peak oxygen consumption increased by  $\sim 18\%$  after training ( $P \leq 0.05$ ), whereas body weight and body composition were not altered. Fasting plasma VLDL-TG concentration was reduced after training by  $\sim 28\%$  ( $P \leq 0.05$ ), and this was due to reduced hepatic VLDL-TG secretion rate (by  $\sim 35\%$ ,  $P \leq 0.05$ ) with no changes ( $<5\%$ ,  $P > 0.7$ ) in VLDL-TG plasma clearance rate and the mean residence time of VLDL-TG in the circulation. No significant changes in VLDL-TG concentration and kinetics were observed in the nonexercising control group (all  $P \geq 0.3$ ). We conclude that a short period of high-intensity interval aerobic training lowers the rate of VLDL-TG secretion by the liver in previously sedentary men. This is different from the mechanism underlying the hypotriglyceridemia of acute exercise; however, it remains to be established whether our finding reflects an effect of the longer time lapse from the last exercise bout, an effect specific to the type of exercise performed, or an effect of aerobic training itself.

isotope; triacylglycerol; endurance; physical activity; chronic exercise

DISTURBANCES IN LIPID METABOLISM leading to unfavorable alterations in the plasma lipid profile increase the risk for coronary heart disease (CHD) (42). Regular exercise training favorably modifies most of the lipid-related atherosclerotic risk factors (14), and this likely contributes to the much lower CHD risk in physically active individuals compared with their sedentary counterparts (4). Acute and chronic exercise substantially reduce plasma triglyceride (TG) concentrations by 15–50% (10). Most of the hypotriglyceridemic effect associated with training is attributed to the last bout of exercise (24) rather than being

the result of metabolic adaptations to repeated exercise sessions (22, 23).

A single bout of strenuous endurance exercise lowers fasting plasma TG concentrations  $\sim 12$ –24 h after its cessation, and this effect lasts for 2–3 days (9, 55), provided that sufficient energy be expended during exercise (2, 8, 58, 63). Exercise-induced TG-lowering predominantly reflects reduced very low-density lipoprotein (VLDL)-TG concentrations, in both the fasted and fed states (2, 20, 39). We have recently demonstrated that a single, prolonged bout of moderate-intensity endurance exercise (90–120 min at 60% of peak oxygen consumption,  $\dot{V}O_{2\text{peak}}$ ) lowers fasting plasma VLDL-TG concentrations the next morning by augmenting the removal efficiency of VLDL-TG from the circulation (38, 57). It is currently not known whether the same mechanism mediates the hypotriglyceridemia of chronic exercise. Data are available only in animals, and relevant studies indicate that strenuous aerobic exercise training is associated with reduced rate of VLDL-TG secretion by the liver in vivo (41); liver perfusion studies confirm this notion in situ and in vitro (17, 62).

To examine this possibility in humans, we measured basal VLDL-TG kinetics before and after a short period of high-intensity aerobic interval training or no exercise in healthy sedentary men, in a randomized controlled fashion.

## MATERIALS AND METHODS

**Subjects.** Sixteen young (age: 20–40 yr), nonobese (body mass index: 20–30 kg/m<sup>2</sup>) men volunteered for the study. Their body weight was self-reported stable for at least 2 mo before enrollment. Subjects were healthy, as indicated by comprehensive history, physical examination, and standard blood tests, and were recreationally active but untrained (participated in moderate-intensity physical activities  $\leq 2$  times/wk). None of them smoked tobacco or was taking medications known to affect lipid metabolism. The study protocol was approved by the Human Studies Committee of Harokopio University, Athens, Greece, and written informed consent was obtained from all participants. Subjects refrained from vigorous exercise for at least 1 wk before any of the study procedures took place; these are shown schematically in Fig. 1.

**Anthropometric, body composition, and cardiopulmonary assessment.** Physical testing was carried out  $\sim 1$  wk before the start of the 2-mo intervention and again 3 days after its completion. Body weight and height were measured to the nearest 0.1 kg and 0.5 cm, respectively, and body mass index was calculated. Total body fat mass and fat-free mass were determined by dual-energy X-ray absorptiometry (model DPX-MD; Lunar, Madison, WI).  $\dot{V}O_{2\text{peak}}$  was determined by a submaximal incremental brisk walking test (modified Balke treadmill protocol) (1). Subjects warmed up for 5 min and were familiar-

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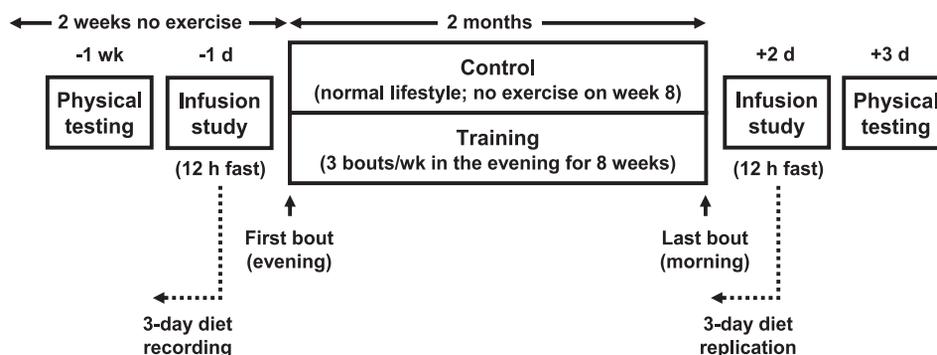


Fig. 1. Schematic representation of the study protocol.

ized with the treadmill (Technogym Runrace, Gambettola, Italy). After warm-up, treadmill speed was kept constant, and grade was increased by 2% every 3 min. Expiratory gases were collected continuously by using a breath-by-breath gas analyzer system (Vmax229D; Sensormedics, Yorba Linda, CA). The test was terminated at 80% of heart rate reserve, and  $\dot{V}O_{2peak}$  was predicted from the oxygen consumption/heart rate relationship (1).

**Experimental protocol.** Participants were randomly assigned to a nonexercising control group ( $n = 8$ ) and an exercise training group ( $n = 8$ ); one subject from the training group dropped off after the 2nd wk of training, and his data is thus excluded from all analyses. Subjects in the training group underwent 2 mo of supervised high-intensity interval training (31), consisting of three bouts of aerobic exercise per week for 8 wk. Each training session involved running on the treadmill (Technogym Runrace, Gambettola, Italy) at level grade and adjustable speed. After 5 min of warm-up, subjects alternated four times between 4 min at 60% of pretraining  $\dot{V}O_{2peak}$  and 4 min at 90% of pretraining  $\dot{V}O_{2peak}$  for a total of 32 min (i.e., 16 min at each intensity), with no rest in between. Appropriate speed for each intensity had been verified  $\geq 4$  days before the beginning of the intervention (2–3 days after the baseline  $\dot{V}O_{2peak}$  test). Heart rate was monitored continuously during each training session by using a telemetric heart rate monitor (Polar Accurex Plus) to estimate the energy expenditure of exercise (27). Exercise was always performed in the evening (1600–2000), at least 3 h after meal consumption, except from the last session, which was conducted after a light breakfast in the morning (~0900). Subjects in the control group were instructed to maintain their normal physical activity habits for the duration of the study but to completely refrain from exercise during the last week of the 2-mo period.

Upon entry in the study, subjects received instructions on how to record food and beverage intake and provided a detailed recording of all nutrient intake for the 3 days preceding the first infusion study. They were then instructed to reproduce the exact same diet for the 3 days leading up to the second infusion study. None of the subjects reported any deviation from the dietary plan. Subjects in the training group were instructed to self-regulate their dietary intake during the training period to avoid weight loss or gain, whereas those in the control group were instructed to maintain their normal dietary habits. All subjects abstained from alcohol and caffeine intake for 2 days before each isotope infusion study and consumed dinner by ~2100 on the previous evening. Thereafter, they remained fasted for ~12 h before starting the tracer infusion the next morning.

**Tracer infusion study.** Each subject underwent two stable isotope-labeled tracer infusion studies in the postabsorptive state in the morning, once before (1 day before the first exercise bout in the training group) and again after the intervention (2 days after the last exercise bout in the training group). Subjects arrived at the laboratory at 0800 with minimal physical activity and after having fasted overnight. One catheter was inserted in a forearm vein to administer stable isotope-labeled tracers, and a second catheter was inserted in a contralateral hand vein for blood sampling; the latter was kept warm

with a heating pad. Catheters were flushed with 0.9% NaCl solution to maintain patency. Subjects were allowed to relax and get used to the catheters for an additional hour [time ( $t$ ) = 0; ~48 h after completion of the last exercise bout in the training group] before a baseline blood sample was taken to determine fasting plasma lipid concentrations and background tracer-to-tracee ratio (TTR) of glycerol in VLDL-TG. Immediately after, a bolus of [1,1,2,3,3- $^2H_5$ ]glycerol (75  $\mu$ mol/kg body wt; Goss Scientific Instruments, Essex, UK), dissolved in 0.9% NaCl solution, was administered through the catheter in the forearm vein, and blood samples were obtained at 15 min and then every hour after tracer injection for 6 h, to determine glycerol TTR in VLDL-TG.  $\dot{V}O_2$  and  $\dot{V}CO_2$  were measured for 15 min by using a gas analyzer system equipped with a ventilated hood (Vmax229D; Sensormedics), once before and then hourly after tracer injection, and data were averaged. For the entire duration of the isotope infusion study, subjects remained fasted in the laboratory in a sitting position. Water consumption was allowed ad libitum.

**Sample collection and analysis.** Blood samples were collected in pre-cooled tubes containing EDTA as anticoagulant and placed on ice immediately, and plasma was separated by centrifugation within 30 min of collection. Aliquots of plasma (~3 ml) were transferred to plastic culture tubes and kept in the refrigerator for immediate isolation of VLDL. The remaining plasma samples were stored at  $-80^\circ C$  until further analyses.

The VLDL fraction was prepared as previously described (57). Briefly, ~2 ml of plasma were transferred to Quick Seal Centrifuge Polyallomer Tubes (Beckman Instruments, Palo Alto, CA), overlaid with a NaCl/EDTA solution ( $d = 1.006$  g/ml), and spun for 3 h at 90,000 revolutions/min at  $4^\circ C$ , in an Optima TLX ultracentrifuge equipped with the fixed-angle TLN-100 rotor (Beckman Instruments). The top layer, containing VLDL, was removed and collected quantitatively by tube slicing (CentriTube slicer; Beckman Instruments) and stored at  $-80^\circ C$  until analyses. VLDL-TG were isolated by thin-layer chromatography and hydrolyzed, and VLDL-TG-glycerol was derivatized with heptafluorobutyric anhydride (57). The TTR of glycerol in VLDL-TG was determined by gas chromatography-mass spectrometry (MSD 5973 system; Hewlett-Packard, Palo Alto, CA) by selectively monitoring the ions at mass-to-charge ratios 467 and 472 (43). Calibration curve for standards with known isotopic enrichment was used.

The concentrations of total TG, VLDL-TG, and free fatty acids (FFA) in plasma were determined by using commercially available enzymatic kits (Alfa Wassermann Diagnostics Technologies, West Caldwell, NJ) on an automated analyzer (ACE Schiapparelli Biosystems, Fairfield, NJ). Paired samples for each volunteer were analyzed in the same batch. Total plasma TG and FFA concentrations were measured at  $t = 0$ ; VLDL-TG concentration was measured throughout the 6-h sampling period, and data were averaged.

**Calculations.** Resting metabolic rate (RMR, kcal/min) and whole body substrate oxidation rates in the basal state were calculated based on respective  $\dot{V}O_2$  and  $\dot{V}CO_2$  measurements (12). The fractional turnover rate (FTR) of VLDL-TG was determined by using the

monoexponential approach (32). The total rate of VLDL-TG secretion ( $\mu\text{mol}/\text{min}$ ), which represents the total amount of VLDL-TG secreted by the liver, was calculated by multiplying the FTR of VLDL-TG (pools/min) by the steady-state concentration of VLDL-TG in plasma ( $\mu\text{mol}/\text{ml}$ ) and the plasma volume (ml); plasma volume was assumed to be equal to VLDL-TG volume of distribution and was calculated as 55 ml/kg fat-free mass (5). We also calculated hepatic VLDL-TG secretion rate per unit of plasma ( $\mu\text{mol}\cdot\text{l}^{-1}\cdot\text{min}^{-1}$ ), as FTR times concentration, to account for potential confounding due to body composition and plasma volume changes over the course of the intervention. The plasma clearance rate of VLDL-TG (ml/min), which is an index of the efficiency of VLDL-TG removal from the circulation via all possible routes, was calculated by dividing the rate of VLDL-TG disappearance from plasma, which equals total VLDL-TG secretion rate under steady-state conditions, by the concentration of VLDL-TG in plasma. The mean residence time (MRT) of VLDL-TG in the circulation (min) was calculated as  $1/\text{FTR}$ .

**Statistical analysis.** Analysis was carried out with SPSS version 16.0 (SPSS, Chicago, IL). All data sets were tested for normality according to Kolmogorov-Smirnov. Total plasma TG concentration and whole body carbohydrate and fat oxidation rates were not normally distributed and were thus log-transformed for analysis and back-transformed for presentation as means and 95% confidence intervals. Results for the remaining parameters are presented as means  $\pm$  SE. Data were analyzed by using repeated-measures ANOVA, with time as within-subject factor (before vs. after the intervention) and group as between-subject factor (control vs. training). When significant interactions between time and group emerged (i.e., for  $\dot{V}\text{O}_{2\text{peak}}$ , plasma VLDL-TG concentration, and hepatic VLDL-TG secretion rates), further analysis was carried out with Student's paired *t*-test to evaluate the effect of time within each group and Student's independent *t*-test (preceded by Levene's test to assess the equality of group variances on each dependant variable) to evaluate differences between the training and control groups before and after the intervention. Statistical significance was set at  $P \leq 0.05$ . Based on the reproducibility of basal VLDL-TG kinetics, our sample size would allow us to detect changes  $\geq 25\%$  in magnitude, which are considered physiologically relevant (35).

## RESULTS

**Training program, body composition and  $\dot{V}\text{O}_{2\text{peak}}$ .** Subjects in the training group exercised at an average heart rate of  $158 \pm 5$  beats/min, representing  $82 \pm 2\%$  of their age-predicted maximum heart rate. The total energy expenditure of the whole exercise training program was  $10,704 \pm 690$  kcal, i.e.,  $446 \pm 29$  kcal for each of the 24 sessions.

The training and control groups did not differ at baseline in body weight, body composition, and  $\dot{V}\text{O}_{2\text{peak}}$  (Table 1). Following the intervention,  $\dot{V}\text{O}_{2\text{peak}}$  ( $P$  for interaction = 0.001) increased significantly in the training group (by  $\sim 18\%$ ,  $P \leq 0.002$ ) and remained unchanged in the control group ( $P \geq$

0.35); body weight and body composition were not affected by the intervention (Table 1).

**Metabolic rate and substrate oxidation in the basal state.** There were no significant differences between groups, no significant effects of the intervention, and no significant interactions between time and group with respect to respiratory variables, RMR, and whole body substrate oxidation rates (all  $P > 0.05$ ) (Table 2).

**Plasma lipid concentrations in the fasting state.** Plasma FFA, total TG, and VLDL-TG concentrations did not differ between groups at baseline (Table 3). Following the intervention, plasma FFA and total TG concentrations were not altered in either group; however, there was a significant interaction between time and group for VLDL-TG concentration ( $P = 0.026$ ) that was reduced after the intervention in the training group (by  $\sim 28\%$ ,  $P = 0.042$ ) but not in the control group ( $P = 0.295$ ) (Table 3). As a result, VLDL-TG concentration was significantly lower in the training than in the control group postintervention ( $P = 0.031$ ).

**Basal VLDL-TG kinetics.** The FTR of VLDL-TG did not differ between groups at baseline (control:  $0.373 \pm 0.028$  pools/h, training:  $0.407 \pm 0.056$  pools/h;  $P = 0.571$ ) and was not affected by the intervention ( $P = 0.904$ ) in either group (control:  $0.377 \pm 0.032$  pools/h, training:  $0.396 \pm 0.043$  pools/h); there was no interaction between time and group ( $P = 0.829$ ).

A significant interaction was detected for hepatic VLDL-TG secretion, whether expressed as total secretion rate ( $P = 0.031$ ) or secretion rate per unit of plasma ( $P = 0.045$ ); this was reduced after the intervention in the training group (by  $\sim 35\%$ ,  $P \leq 0.05$ ) but not in the control group ( $P \geq 0.39$ ) (Fig. 2). Hence hepatic VLDL-TG secretion rate was significantly lower in the training than in the control group postintervention ( $P \leq 0.004$ ) but was not different at baseline ( $P \geq 0.55$ ) (Fig. 2).

VLDL-TG plasma clearance rate and the MRT of VLDL-TG in the circulation were not different between groups at baseline ( $P = 0.553$  and  $P = 0.892$ , respectively), nor were they affected by the intervention ( $P = 0.955$  and  $P = 0.734$ , respectively); no interactions between time and group were detected either ( $P = 0.755$  and  $P = 0.836$ , respectively) (Fig. 3). Values in both groups after the intervention were within 4% of respective baseline values.

## DISCUSSION

We examined basal VLDL-TG kinetics before and after short-term, high-intensity aerobic interval training in healthy, sedentary young men and observed that training, in the absence

Table 1. Body composition and  $\dot{V}\text{O}_{2\text{peak}}$  in the control and training groups, before and after the intervention

	Control (n = 8)		Training (n = 7)	
	Before	After	Before	After
Weight, kg	76.0 $\pm$ 2.0	76.2 $\pm$ 2.5	81.0 $\pm$ 5.6	79.3 $\pm$ 5.2
Body mass index, kg/m <sup>2</sup>	23.4 $\pm$ 0.6	23.4 $\pm$ 0.7	25.2 $\pm$ 1.2	24.6 $\pm$ 0.9
Body fat, %body wt	17.3 $\pm$ 1.4	16.9 $\pm$ 1.4	19.0 $\pm$ 1.8	19.2 $\pm$ 1.9
Fat mass, kg	13.1 $\pm$ 1.2	12.9 $\pm$ 1.3	15.2 $\pm$ 1.3	14.9 $\pm$ 1.4
Fat-free mass, kg	62.8 $\pm$ 2.0	63.3 $\pm$ 2.1	65.8 $\pm$ 5.4	64.4 $\pm$ 5.2
$\dot{V}\text{O}_{2\text{peak}}$ , l/min	2.98 $\pm$ 0.38	2.90 $\pm$ 0.34	3.03 $\pm$ 0.39	3.52 $\pm$ 0.39*
$\dot{V}\text{O}_{2\text{peak}}$ , ml $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup>	39.8 $\pm$ 5.6	38.4 $\pm$ 4.6	36.7 $\pm$ 2.7	43.9 $\pm$ 2.6*

Values are means  $\pm$  SE; n, no. of subjects.  $\dot{V}\text{O}_{2\text{peak}}$ , peak oxygen consumption. \*Significantly different from baseline value ( $P \leq 0.05$ ).

Table 2. Indirect calorimetry in the basal state in the control and training groups before and after the intervention

	Control (n = 8)		Training (n = 7)	
	Before	After	Before	After
Oxygen consumption, ml/min	225 ± 14	239 ± 5	244 ± 11	241 ± 10
Carbon dioxide production, ml/min	180 ± 11	193 ± 4	202 ± 11	189 ± 11
Respiratory exchange ratio	0.80 ± 0.01	0.81 ± 0.01	0.82 ± 0.02	0.78 ± 0.02
Resting metabolic rate, kcal/min	1.07 ± 0.07	1.14 ± 0.02	1.17 ± 0.05	1.14 ± 0.05
Carbohydrate oxidation, mg/min	62 (40, 96)	74 (53, 103)	58 (12, 289)	23 (2, 234)
Fat oxidation, mg/min	51 (37, 71)	57 (47, 69)	45 (26, 77)	64 (50, 83)

Values are means ± SE, except for substrate oxidation rates where means and 95% confidence intervals are provided.

of changes in body weight and body composition, reduces fasting plasma VLDL-TG concentration by suppressing hepatic VLDL-TG secretion rate, without affecting the plasma clearance rate and the MRT of VLDL-TG in the circulation, when measurements are made ~48 h from the last exercise session. This is consistent with results from available studies in exercise-trained animals (17, 41, 62) but directly contrasts results from our previous studies examining the effect of a single bout of prolonged endurance exercise on VLDL-TG kinetics the next morning (38, 57). We cannot attribute our present findings to exercise training per se, to the type of exercise performed, or, perhaps, to the greater time lapse after the last bout of exercise. Nevertheless, inclusion of a nonexercising control group in which we observed no changes in VLDL-TG concentration and kinetics strengthens our conclusion that, under these circumstances, exercise has the potential to suppress the rate of VLDL-TG secretion by the liver in humans in vivo.

Fasting plasma TG concentrations are reduced ~12–24 h after a single bout of prolonged endurance exercise and until 2–3 days later (2, 9, 55), and the same holds true after the last exercise bout in the trained state (22, 60). Our inability to observe a significant decrease in total plasma TG concentration 2 days after the last exercise training session is probably due to a type II statistical error because we measured total plasma TG concentration only in a single sample, before tracer injection, and this is associated with severalfold greater intraindividual variability compared with our VLDL-TG concentration measurement derived from serial samples (48); this, we believe, is responsible for the apparent discrepancy between VLDL-TG and total plasma TG concentrations. It is well established that exercise-induced reduction in total plasma TG concentration is predominantly due to decreased VLDL-TG concentration (2, 20, 39), and we did observe a significant reduction in VLDL-TG concentration after training.

We have previously demonstrated that fasting hypotriglyceridemia in response to a single bout of strenuous whole body

exercise, whether endurance or resistance, results from increased VLDL-TG plasma clearance rate (by ~25–40% compared with rest), which indicates enhanced efficiency of VLDL-TG removal from plasma ~12–24 h after exercise cessation (38, 56, 57). This is likely related to the secretion of fewer but TG-rich and therefore possibly also larger VLDL particles after exercise (38); in vivo studies in humans (36, 53) and animals (54, 61) indicate that the removal of TG from the core of TG-rich, large VLDL particles is more efficient than that from TG-poor, small VLDL, possibly because increasing TG content and size of lipoprotein particles enhances their susceptibility to hydrolysis by lipoprotein lipase (LPL) (13). In addition, LPL protein mass and activity in skeletal muscle, but not adipose tissue, increase transiently within 6–8 h after exercise and remain elevated for some 16–20 h postexercise (29, 49, 50); this could further facilitate VLDL-TG removal from the circulation the next day, at least across the previously exercised muscles (28, 39, 50).

In this study, we measured VLDL-TG kinetics ~48 h after the last bout of exercise and found no changes in the plasma clearance rate and the MRT of VLDL-TG, whereas fasting plasma VLDL-TG concentration and the rate of hepatic VLDL-TG secretion were ~30% lower compared with baseline, pretraining values. Other investigators, using the intravenous fat tolerance test, have shown that lower plasma TG concentrations 1 day after prolonged endurance exercise coincide with increased clearance rate of exogenous fat, but this is not the case 2 days after exercise, when plasma TG concentrations are still reduced compared with preexercise values but clearance of exogenous fat is not different (2). Furthermore, exercise-induced increases in skeletal muscle LPL mass and activity are not evident beyond 20–30 h after exercise cessation (29, 49, 50). These observations indicate that the greater time lapse from the last bout of exercise could be responsible for our divergent findings ~12–24 h after acute exercise, when low plasma VLDL-TG concentration is due to increased VLDL-TG plasma clearance rate (38, 56, 57) as opposed to

Table 3. Fasting plasma lipid concentrations in the basal state in the control and training groups, before and after the intervention

	Control (n = 8)		Training (n = 7)	
	Before	After	Before	After
Free fatty acids, mmol/l	0.41 ± 0.03	0.44 ± 0.05	0.52 ± 0.09	0.57 ± 0.07
Total plasma triglyceride, mmol/l	0.83 (0.68, 1.01)	0.87 (0.73, 1.05)	0.86 (0.55, 1.33)	0.82 (0.43, 1.54)
VLDL-triglyceride, mmol/l	0.46 ± 0.06	0.51 ± 0.05	0.40 ± 0.11	0.29 ± 0.08*†

Values are means ± SE, except for total plasma triglyceride, where means and 95% confidence intervals are provided. VLDL, very low-density lipoprotein.  $P \leq 0.05$ , significantly different from baseline value (\*) and significantly different from respective value in the control group (†).

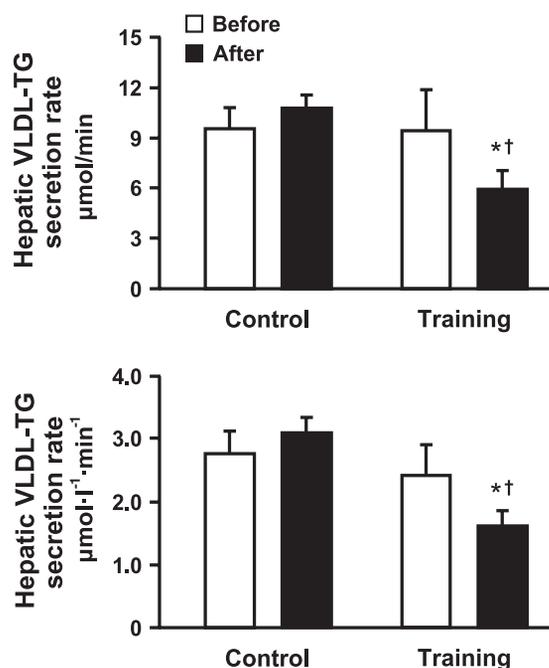


Fig. 2. Hepatic secretion rate of very low-density lipoprotein-triglyceride (VLDL-TG) in the basal state, expressed as total secretion rate (*top*) and secretion rate per unit of plasma (*bottom*), in the control and training groups before and after the intervention. Values are means  $\pm$  SE.  $P \leq 0.05$ , significantly different from baseline value (\*) and significantly different from respective value in the control group ( $\dagger$ ).

$\sim 48$  h postexercise in the trained state (present study), when low plasma VLDL-TG concentration is due to decreased hepatic VLDL-TG secretion rate. This possibility, if indeed true, implies that different mechanisms may be mediating exercise-induced hypotriglyceridemia over the first couple of days of recovery.

Studies in rodents suggest that the lower hepatic VLDL-TG secretion rate of exercise-trained compared with pair-fed sedentary animals *in vivo* is at least partly mediated by differences in substrate availability, the most prominent being the lower plasma FFA concentration in the trained state (41). In humans, FFA concentration and rate of appearance in plasma, *i.e.*, FFA availability to all tissues of the body (including the liver), are greatly augmented 12–16 h after a single bout of exercise by  $\sim 50\%$ , but hepatic VLDL-TG secretion rate does not increase (37, 38, 56), possibly because most of the additional FFA are utilized for energy production (38, 56) and restoration of skeletal muscle TG stores (29). This illustrates an uncoupling of hepatic VLDL-TG secretion from plasma FFA availability in the postexercise period. However, contrary to what is observed the day after a single bout of exercise (37, 38) or 1 day after the last exercise training session (46), several weeks of endurance training do not affect basal FFA rate of appearance and FFA concentration in plasma when measurements are made 36–72 h from the last exercise bout (15, 16, 25, 26, 51). These observations collectively suggest that exercise-induced augmentation of plasma FFA availability may persist for up to  $\sim 24$  h after exercise but not for longer (*i.e.*,  $\geq 36$  h), which is consistent with our finding that training did not affect fasting plasma FFA concentrations 2 days after the last exercise bout.

At the same time, *i.e.*,  $\sim 48$  h after the last training session, we observed that basal hepatic VLDL-TG secretion rate was

reduced significantly. The possibility cannot be excluded that exercise downregulates VLDL-TG secretion by the liver by some as yet unknown mechanism, but this effect is not readily evident the day after exercise ( $\leq 24$  h) because the higher plasma FFA availability induces a compensatory increase in VLDL-TG secretion (33), thereby preventing any change in hepatic VLDL-TG secretion rate from manifesting (37, 38, 56). At later time points ( $\geq 36$  h postexercise), however, plasma FFA availability is not increased, and this could unmask the exercise-induced lowering of VLDL-TG secretion by the liver, which was observed in this study. According to this hypothesis, the net balance of the metabolic interplay between the amount of FFA available after exercise and their use in pathways other than VLDL-TG synthesis and secretion in the liver assumes a key role. However, this is likely not the primary mechanism for the observed training-induced lowering of hepatic VLDL-TG secretion  $\sim 48$  h postexercise, because we found no changes in basal fat oxidation and plasma FFA concentration, and skeletal muscle TG stores should have been completely restored by that time (29).

Although there was considerable variability in our indirect calorimetry measurements between and within groups, previous studies also report no significant training-induced changes in basal energy and substrate metabolism when assessed  $\sim 24$ –72 h after the last bout of exercise (15, 16, 30, 51). It is also unlikely that training caused a selective increase in hepatic fatty acid oxidation and ketogenesis, thereby redirecting intrahepatic fatty acids away from VLDL-TG synthesis and secretion. Endurance training in animals does not alter malonyl-

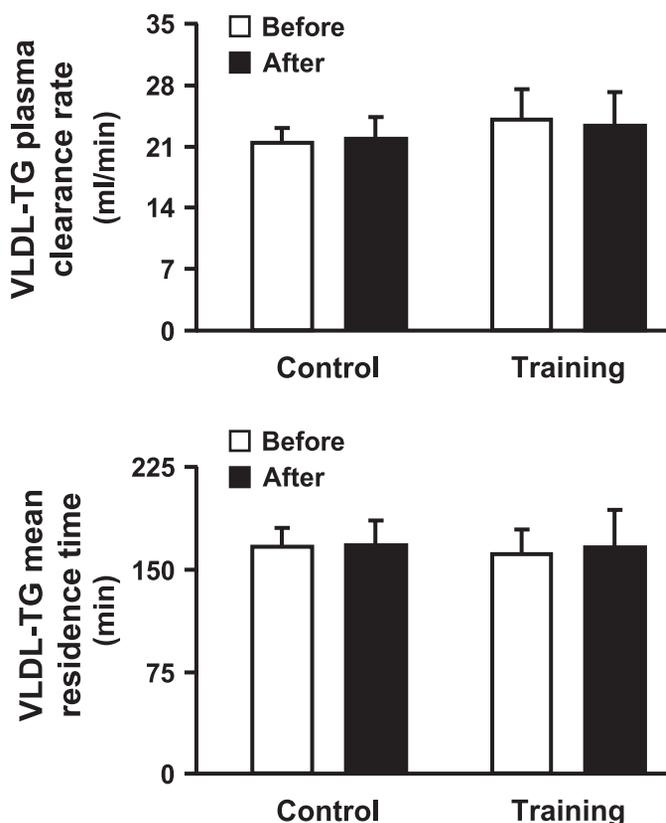


Fig. 3. Plasma clearance rate (*top*) and mean residence time (*bottom*) of VLDL-TG in the basal state, in the control and training groups before and after the intervention. Values are means  $\pm$  SE.

CoA content in the liver (3) and the activity of hepatic carnitine palmitoyltransferase-1 (the rate-limiting enzyme in mitochondrial fatty acid oxidation) and its sensitivity to inhibition by malonyl-CoA (21) in the basal state, ~12–24 h postexercise. Furthermore, although in vitro studies in animals indicate a reciprocal effect of exercise training on hepatic ketogenesis and VLDL-TG secretion (17), human studies indicate that the lower plasma TG concentration in the trained state, ~48 h after the last bout of exercise, does not coincide with any differences in  $\beta$ -hydroxybutyrate concentration (60), and increased  $\beta$ -hydroxybutyrate concentration one day after a single bout of exercise is not associated with any changes in hepatic VLDL-TG secretion (56). These observations argue against possible training-induced changes in hepatic fatty acid oxidation and ketogenesis being the primary factor mediating the reduction in basal VLDL-TG secretion rate 2 days after the last exercise bout. That being said, the effect of exercise on the key enzyme involved in hepatic VLDL assembly, i.e., microsomal TG transfer protein, is not known.<sup>1</sup>

We cannot ascertain whether aerobic training in itself is responsible for our present findings. There is little doubt that the majority of the hypotriglyceridemic effect associated with training is due to the last bout of exercise (24) and is acutely (within ~60 h) reversed by detraining (22, 23). However, the mechanisms behind these observations have never been investigated, and the possibility that training alters the VLDL-TG metabolism response to acute exercise cannot be excluded. Insulin sensitivity is increased for at least 48 h after acute and chronic exercise (40, 45), but whether or not and how this is related to the exercise-induced changes in VLDL-TG metabolism during the first couple of days of recovery is not clear. Enhanced insulin sensitivity would be consistent with reduced hepatic VLDL-TG secretion rate (19, 33, 34), but this is not observed 12–24 h after exercise (37, 38, 56, 57), perhaps due to the counteracting effect of increased postexercise FFA availability hypothesized above, since much of the suppressing effect of insulin on hepatic VLDL-TG secretion is mediated by the reduction in plasma FFA availability (34). However, a state of enhanced insulin sensitivity ~48 h after exercise (40, 45), which in addition would be more pronounced after training than after a single exercise session (45), and the absence of any compensatory effect from FFA at that time could lead to a lowering of hepatic VLDL-TG secretion rate, consistent with our observations. Another possible explanation could relate to the effect of training of liver fat. Observational studies in humans suggest that increased habitual physical activity is inversely associated with intrahepatic TG content (44), and endurance training in animals reduces liver fat accumulation

measured ~48 h after the last bout of exercise (18, 47). A possible training-induced reduction in intrahepatic TG content would be consistent with the lowering of hepatic VLDL-TG secretion in our study because intrahepatic TG content is strongly and positively associated with basal hepatic VLDL-TG secretion rate in humans, at least within the normal range of liver fatness (11). A single bout of exercise may also reduce intrahepatic TG content immediately postexercise (52), but the time course of liver TG repletion is not known.

It is interesting to refer to the type of exercise performed (i.e., high-intensity interval training) as a possible modulating factor of the VLDL-TG metabolism response to exercise. We observed a significant reduction in fasting plasma VLDL-TG concentration by ~30% compared with baseline, ~48 h after the last bout of training. The magnitude of this decrease is the same as that we observed 12–24 h after a single, prolonged bout of moderate-intensity endurance exercise (38, 57), but the estimated total energy expenditure of each training session in this study (~450 kcal) is much lower than that (900–1,200 kcal) of acute exercise studied previously (38, 57). In fact, a less prolonged bout of moderate-intensity endurance exercise, with an energy cost of ~600 kcal, still 33% higher than that in the present study, had no effect on basal VLDL-TG concentration and kinetics the next morning (37). It is well established that the hypotriglyceridemic effect of exercise (both its magnitude and duration) depends on the total exercise energy expenditure (2, 8, 58, 63), and exercise time and intensity are interchangeable when it comes to the magnitude of plasma TG lowering, provided that the exercise-induced total energy expenditure be held constant (7, 59). Whether the same mechanisms are responsible for hypotriglyceridemia under these conditions is not known, but these observations along with our findings indicate either that training lowers the energy expenditure threshold required for hypotriglyceridemia to manifest or that high-intensity interval exercise is much more effective than moderate-intensity continuous exercise in this respect. Available evidence suggests that high-intensity aerobic interval training is far more volume-effective than traditional training in increasing physical fitness and performance parameters (31), as also witnessed in the present study (~18% increase in  $\dot{V}O_{2\text{peak}}$  after 2 mo), and may in fact induce the same metabolic adaptations as traditional endurance training at only one-tenth of the total exercise energy expenditure (6). Therefore, it is possible that the relationship between hypotriglyceridemia and exercise-induced energy expenditure, and perhaps also the underlying mechanisms, may be different for different kinds of exercise.

In summary, we examined basal VLDL-TG metabolism before and after 2 mo of high-intensity aerobic interval training in healthy nonobese men, and in a nonexercising control group. Our data indicate that the trained state, in the absence of changes in body weight and body composition, is associated with lower fasting plasma VLDL-TG concentration secondary to reduced basal VLDL-TG secretion rate by the liver. It is unclear whether this represents an effect of aerobic training per se, an effect specific to the type of exercise performed, or simply an effect of the greater time lapse after the last bout of exercise.

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<sup>1</sup> In a recent study (Lira FS, Tavares FL, Yamashita AS, Koyama CH, Alves MJ, Caperuto EC, Batista ML Jr, Seelaender M. Effect of endurance training upon lipid metabolism in the liver of cachectic tumour-bearing rats. *Cell Biochem Funct* doi:10.1002/cbf.1495, 2008), it was shown that endurance training in control rats reduces basal hepatic VLDL-TG secretion rate in vivo and also suppresses the gene expression of apolipoprotein B and microsomal triglyceride transfer protein in the liver. These results are consistent with the effects of exercise training in humans, i.e., reduced hepatic VLDL-TG secretion rate (present study) and reduced hepatic VLDL-apolipoprotein B-100 secretion rate (Alam S, Stolinski M, Pentecost C, Boroujerdi MA, Jones RH, Sonksen PH, Umpleby AM. The effect of a six-month exercise program on very low-density lipoprotein apolipoprotein B secretion in type 2 diabetes. *J Clin Endocrinol Metab* 89: 688–694, 2004).

## GRANTS

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