Published in final edited form as:

Med Sci Sports Exerc. 2013 March ; 45(3): 455–461. doi:10.1249/MSS.0b013e318278183e.

Acute effects of exercise and calorie restriction on triglyceride metabolism in women

Elena Bellou¹, Aikaterina Siopi¹, Maria Galani¹, Maria Maraki¹, Yiannis E. Tsekouras¹, Demosthenes B. Panagiotakos¹, Stavros A. Kavouras¹, Faidon Magkos^{1,2}, and Labros S. Sidossis^{1,3}

¹Laboratory of Nutrition & Clinical Dietetics, Department of Nutrition and Dietetics, Harokopio University, Athens, Greece

²Department of Internal Medicine, Center for Human Nutrition and Atkins Center of Excellence in Obesity Medicine, Washington University School of Medicine, St. Louis, MO

³Department of Internal Medicine, Sealy Center on Aging, Institute for Translational Sciences and Shriners Hospital for Children, University of Texas Medical Branch at Galveston, TX

Abstract

The mechanisms by which exercise reduces fasting plasma triglyceride (TG) concentrations in women and the effect of negative energy balance independent of muscular contraction are not known.

Purpose—The aim of this study was to evaluate the effects of equivalent energy deficits induced by exercise or calorie restriction on basal very low-density lipoprotein (VLDL) TG metabolism in women.

Methods—Eleven healthy women (age: 23.5±2.7 years, BMI: 21.6±1.4 kg/m²) underwent a stable isotopically labeled tracer infusion study to determine basal VLDL-TG kinetics after performing, in random order, three experimental trials on the previous day: i) a single exercise bout (brisk walking at 60% of peak oxygen consumption for 123±18 min, with a net energy expenditure of 2.06±0.39 MJ (~500 kcal)), ii) dietary energy restriction of 2.10±0.41 MJ, and iii) a control day of isocaloric feeding and rest (zero energy balance).

Results—Fasting plasma VLDL-TG concentration was ~30% lower after the exercise trial compared to the control trial ($P<0.001$), whereas no significant change was detected after the calorie restriction trial ($P=0.297$ vs control). Relative to the control condition, exercise increased the plasma clearance rate of VLDL-TG by 22% ($P=0.001$) and reduced hepatic VLDL-TG secretion rate by ~17% ($P=0.042$), whereas hypocaloric diet had no effect on VLDL-TG kinetics ($P>0.2$).

Conclusion—(i) Exercise-induced hypotriglyceridemia in women manifests through a different mechanism (increased clearance and decreased secretion of VLDL-TG) than that previously described in men (increased clearance of VLDL-TG only), and (ii) exercise affects TG homeostasis by eliciting changes in VLDL-TG kinetics that cannot be reproduced by an equivalent

Copyright © 2012 American College of Sports Medicine

Address for correspondence and requests for reprints: Labros Sidossis, PhD. University of Texas Medical Branch at Galveston, 301 University Blvd, 6.128 Rebecca Sealy Hospital, Galveston, Texas 77555-0177, phone: 409-771-7894, fax: 409-772-1942, lasidoss@utmb.edu.

DISCLOSURES

The authors have no conflicts of interest.

The results of the present study do not constitute endorsement by ACSM.

diet-induced energy deficit, indicating that these changes are independent of the exercise-induced negative energy balance but instead are specific to muscular contraction.

Keywords

physical activity; hypocaloric diet; triacylglycerol; hypotriglyceridemia

INTRODUCTION

Elevated plasma triglyceride (TG) concentrations are associated with increased risk of coronary heart disease (CHD), particularly in women (16). Therefore, interventions that decrease or prevent an increase in plasma TG concentrations, such as exercise and diet, may help reduce CHD risk (1). It has long been known that exercise reduces total plasma TG concentration (17), almost exclusively due to reduced concentration of TG in very low density lipoproteins (VLDL) (3). This effect is not the result of repeated exercise sessions (i.e., training) (14), but instead is acute and short-lived, because plasma TG concentrations are decreased 12-18 h after a single bout of exercise and remain lower than pre-exercise values for 2-3 days (8, 39). Exercise-induced hypotriglyceridemia manifests above a certain threshold of exercise energy expenditure (7), independent of duration and intensity (41), and plateaus with progressively more exercise (9). In a series of studies in healthy non-obese men, we have shown that a single bout of aerobic exercise reduces fasting plasma TG concentrations the next day by increasing the clearance rate of VLDL-TG from the circulation, without affecting VLDL-TG secretion rate from the liver (23, 25, 26, 40). The exercise-induced increase in VLDL-TG plasma clearance rate also requires a certain energy expenditure threshold and plateaus thereafter (20). However, the mechanism by which a single bout of exercise reduces fasting plasma TG concentrations in women (12) remains elusive (24). Previous studies have revealed major sex differences in basal VLDL-TG kinetics (22, 31). For example, women have a much greater basal VLDL-TG secretion rate than men. It is thus possible that exercise-induced hypotriglyceridemia in women manifests via a different mechanism (e.g. reduced hepatic VLDL-TG secretion) than in men.

Recent data indicate that negative energy balance is a critical factor for exercise-induced TG lowering. Acutely increasing dietary energy intake to compensate for the energy expended during exercise abolishes the reduction in fasting total plasma TG concentration (6), whereas a single day of calorie restriction to induce a similar energy deficit as that caused by exercise decreases fasting plasma VLDL-TG concentration to the same extent (28). Still, although changes in energy balance appear to account for most of the exercise-induced TG-lowering, there is data to suggest that exercise is somewhat superior to calorie restriction in inducing hypotriglyceridemia (11, 29, 43). These observations suggest that the hypotriglyceridemic effect of exercise may be mediated by a mechanism other than, or in addition to, the negative energy balance and that dietary energy deficit may have an independent effect on the mechanisms regulating VLDL-TG homeostasis. Chronic dietary energy restriction leading to weight loss is accompanied by a reduction in hepatic VLDL-TG secretion rate (13, 32). However, the effects of acute dietary-induced negative energy balance on VLDL-TG kinetics are not known.

The overall aim of the present study was to assess the acute effects of exercise and calorie restriction, each tailored to induce the same negative energy balance, on VLDL-TG metabolism in women. The specific study purposes were to assess: 1) the mechanisms leading to exercise-induced hypotriglyceridemia in women, and 2) the independent hypotriglyceridemic effect of negative energy balance.

METHODS

Subjects

Eleven healthy, lean, sedentary women (age 23.5 ± 2.7 years; body mass index, BMI: 21.6 ± 1.4 kg/m²; peak oxygen consumption, $\text{VO}_{2\text{peak}}$: 1.6 ± 0.4 L/min) volunteered for the study. The selection of healthy lean volunteers was made in order to avoid influence of medication and obesity-related comorbidities. Exclusion criteria included contraindication to aerobic exercise, irregular menses, amenorrhea, polycystic ovary syndrome, pregnancy, acute or chronic illness, use of medications (including oral contraceptives) or dietary supplements, smoking, regular alcohol consumption (>1 drink per day), regular exercise participation (>1 time per week), and being on a special diet or having experienced weight fluctuations ≥ 2 kg at any time during the last 6 months. The Ethics Committee of Harokopio University approved the study protocol and all subjects gave written informed consent.

Preliminary testing

All preliminary tests were carried out during screening, approximately 1-2 weeks before the beginning of the experiment. Weight and height were measured and an overnight fasting blood sample was drawn for hematological and biochemical evaluations. Subjects were healthy on the basis of medical examination and routine laboratory tests; all were normoglycemic and normolipidemic. Total body fat mass and fat-free mass were determined with dual energy x-ray absorptiometry (model DPX-MD; Lunar, Madison, WI). Resting energy expenditure (REE) was measured by indirect calorimetry (Vmax229D; Sormedics, Yorba Linda, CA) in the morning, after subjects remained rested for at least 30 min (27). $\text{VO}_{2\text{peak}}$ was assessed with a submaximal incremental brisk walking exercise test based on the Balke treadmill protocol (2). Briefly, after a 5-min warm-up, subjects walked on a treadmill (Technogym Runrace, Gambettola, Italy) at constant speed and grade was increased by 2% every 3 min. Expiratory gases were collected (Vmax229D; Sormedics, Yorba Linda, CA) and heart rate was monitored continuously. The test was terminated at 80% of maximal heart rate, and $\text{VO}_{2\text{peak}}$ was predicted from the oxygen consumption - heart rate relationship (2).

Experimental protocol

We used a paired cross-over design, in which all subjects performed three trials, i.e., control, exercise-induced energy deficit, and dietary-induced energy deficit, in random order and at least one week apart, without considering the phase of the menstrual cycle, because we have previously shown that basal VLDL-TG kinetics are not affected by menstrual cycle phase (21). A stable isotopically labeled tracer infusion was performed on the day after each trial, following an overnight fast. Subjects were instructed to refrain from exercise for 2 days and avoid alcohol and caffeine consumption for 1 day before each trial. In addition, they were asked to record their diet during the day preceding the first trial and to replicate this diet on the day preceding the subsequent trials (i.e. purchase the same type and brand of food, use the same cooking methods and portions, etc.), in order to avoid pre-study differences in nutrient intake.

Control (isocaloric diet and rest)—Subjects were asked to abstain from exercise and carry out only the activities of daily living. During the afternoon of the day preceding the isotope infusion study, they remained rested at home. Subjects were instructed to follow a prescribed isocaloric diet (50% of energy from carbohydrate, 20% from protein and 30% from fat), which provided their estimated daily energy needs for weight maintenance, calculated by multiplying the measured REE with an activity factor of 1.4 representative of their very light to light physical activity habits (19). Subjects were thus assumed to be on zero energy balance during the control trial.

Exercise—Subjects were asked to abstain from exercise and carry out only the activities of daily living, with the exception of an exercise session at the laboratory in the afternoon. Each subject attended the laboratory midway between lunch and dinner and walked briskly on the treadmill (Technogym Runrace, Gambettola, Italy) at 60% of her VO_2 peak. Gas sampling was performed for 10 min every 20 min during exercise in order to estimate total exercise energy expenditure. The exercise bout was stopped when subjects reached an estimated exercise-induced net energy deficit of ~2 MJ, calculated by subtracting REE from the total energy expenditure of exercise. The duration of the bout was 123 ± 18 min (mean $\pm$ SD). Subjects were also instructed to follow a prescribed isocaloric diet which provided their estimated daily energy needs for weight maintenance without accounting for the exercise-induced energy deficit, i.e., the same diet as in the control trial.

Diet (hypocaloric diet and rest)—Subjects were asked to abstain from exercise and carry out only the activities of daily living. They were instructed to follow a prescribed hypocaloric diet (50% of energy from carbohydrate, 20% from protein and 30% from fat), which provided their estimated daily energy needs for weight maintenance minus 2 MJ (~500 kcal). The restriction of energy intake occurred at lunch, afternoon snack and dinner, so that the energy deficit occurred at similar times of day in the diet and exercise trials (11, 28).

For each trial (on the day before the infusion study), subjects recorded their dietary intake with a food diary (type of food, brand, portion, etc.) and a 24 hr food recall was obtained on the following day (during the infusion study). Subjects were allowed to make their own selections of brands, based on a prescribed diet for the first trial and were then instructed to consume the same type of foods (i.e. purchase the same brand, use the same cooking methods, etc.) with prescribed portions, for the remaining trials. Food records were analyzed by using Diet Analysis Plus 8 (Cengage Learning, Florence, KY).

Tracer infusion study

The morning following each of the three experimental trials (control, exercise, diet), subjects arrived at the laboratory at approximately 0800 h, after an overnight fast. A catheter was inserted in a forearm vein to administer stable isotopically labeled tracers and a second catheter was inserted in a contralateral hand vein for blood sampling, which was kept warm with a heating pad until the end of the metabolic study. Subjects were given 1 h to relax and familiarize with the catheters; during this time, a 24-hour diet recall was taken. At 0900 h, a baseline blood sample was obtained and immediately after a bolus of $[1,1,2,3,3\text{-}^2\text{H}_5]\text{glycerol}$ (75 $\mu\text{mol/kg}$ body weight; Goss Scientific Instruments, Essex, UK), dissolved in normal saline, was administered through the catheter in the forearm vein. Blood samples were taken every 15 min during the first hour after tracer administration and hourly thereafter for another 7 hours. Catheters were flushed with saline every 30 min to maintain patency. Subjects remained fasted (except for water) in a sitting position until the end of the metabolic study.

Sample collection, processing, and analysis

Blood samples were collected in precooled potassium-EDTA Monovettes (Sarstedt, Leicester, UK) and immediately placed on ice. Plasma was separated by centrifugation within 30 minutes of collection. A 3-ml aliquot of plasma was transferred into plastic culture tubes and kept in the refrigerator for immediate isolation of VLDL, and the remaining plasma samples were stored at -80°C until analyses. The VLDL fraction was prepared by density-gradient ultracentrifugation, VLDL-TG were isolated by thin-layer chromatography, hydrolyzed, and VLDL-TG-bound glycerol was derivatized with heptafluorobutyric anhydride, as previously described (25, 40). The tracer to tracee ratio (TTR) of glycerol in

VLDL-TG was measured 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 (25, 40).

Determination of plasma glucose, total plasma TG and VLDL-TG concentrations was performed by enzymatic colorimetric methods using commercially available kits (Alfa Wassermann Diagnostics, Woerden, The Netherlands) on an automated analyzer (ACE Schiapparelli Biosystems, Fairfield, NJ). Plasma free fatty acid (FFA) concentrations were measured by using a commercially available diagnostic kit (Waco Diagnostics, Richmond, VA). VLDL, intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) particle concentrations and HDL-cholesterol concentration were determined by using proton nuclear magnetic resonance spectroscopy (LipoScience; Raleigh, NC) (34). A separate blood sample was collected into non-heparinized serum tubes (Sarstedt, Leicester, UK), allowed to clot, spun in a centrifuge and then aliquoted and frozen immediately at -80°C , until measurement of insulin with a commercially available immunoenzymetric fluorescent method (ST AIA-PACK IRI, Tosoh Medics, San Francisco, CA) on an automated analyzer (Tosoh AIA 600II, Tosoh Medics, Inc., San Francisco, CA). All samples from each subject's trials were analyzed in the same batch.

Calculations

The gross energy expenditure of exercise was calculated by using the Weir equation (42) and non-protein respiratory quotient (36). Net energy expenditure was calculated by subtracting REE for an equivalent period of rest. The fractional turnover rate (FTR, $\text{pools}\cdot\text{h}^{-1}$) of VLDL-TG was determined by monoexponential analysis of VLDL-TG-glycerol TTR data (18, 35). The hepatic secretion rate of VLDL-TG ($\mu\text{mol}\cdot\text{min}^{-1}$) was calculated as $\text{FTR} \times \text{C} \times \text{PV} / 60$, where C is the concentration of VLDL-TG in plasma and PV is the plasma volume (55 ml per kg of fat-free mass (4)). It was assumed that VLDL-TG volume of distribution equals PV because VLDL particles are restricted to the plasma compartment (38). The plasma clearance rate of VLDL-TG ($\text{ml}\cdot\text{min}^{-1}$), 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 (which equals the secretion rate at steady state) by the plasma concentration of VLDL-TG.

Statistical Analysis

All datasets were tested for normality by using the Kolmogorov-Smirnov criterion. Normally distributed variables are presented as mean $\pm$ SD, whereas non-normally distributed variables were log-transformed for analyses and back-transformed for presentation as means with 95% confidence intervals. Generalized estimating equations (GEE) were fitted to evaluate differences among the three experimental trials (encoded as dummy variables). For all the dependent variables, the normal distribution was used for fitting GEE, with the identity as the link function. The unstructured formation of the correlation matrix was used after comparing various scenarios using the corresponding QIC (Quasi likelihood under the Independence criterion for model's goodness-of-fit). Post-hoc analysis for comparing mean values among trials was applied by using the Bonferroni correction rule to adjust for the inflation of type-I error due to multiple comparisons. All statistical analyses were carried out with SPSS 19 for Windows (IBM SPSS, Chicago, IL).

RESULTS

Dietary energy intake and exercise energy expenditure

The gross energy expenditure of exercise was 2.53 ± 0.08 MJ. Compared with the control condition (rest and isocaloric feeding; zero energy balance), subjects were in a negative energy balance of ~ 2 MJ during the exercise and hypocaloric diet trials (Table 1).

Metabolic profile

Fasting plasma glucose concentration was not different among trials, but fasting serum insulin concentration was significantly lower after dietary energy restriction than after the control trial ($p=0.005$) (Table 2). Plasma FFA concentration was not different after exercise or diet compared to the control condition, but was significantly greater after exercise compared to hypocaloric diet ($P=0.006$). Total plasma TG ($P=0.007$), VLDL-TG ($P<0.001$) and total and all VLDL subclass particle concentrations ($P<0.015$) were significantly lower after exercise compared to control (Tables 2 and 3). Dietary energy restriction did not significantly affect total plasma TG ($P=1.000$) and VLDL-TG ($P=0.297$) concentrations, but significantly reduced large ($P=0.019$) and small ($P=0.013$) VLDL particle concentrations relative to the control condition (Tables 2 and 3). Exercise did not affect total LDL particle concentration, but HDL-cholesterol concentration ($P=0.028$) (Table 2) and total HDL particle concentration ($P=0.017$) (Table 3) were greater after the exercise trial than after hypocaloric diet trial.

VLDL-TG kinetics

The fractional turnover rate of VLDL-TG was 0.60 ($0.54, 0.67$) $\text{pools} \cdot \text{h}^{-1}$ after the control trial (zero energy balance), and was significantly increased after the exercise-induced energy deficit (0.73 ($0.62, 0.86$) $\text{pools} \cdot \text{h}^{-1}$, $P=0.001$ vs. control) but did not change after the diet-induced energy deficit (0.66 ($0.60, 0.71$) $\text{pools} \cdot \text{h}^{-1}$, $P=0.213$ vs. control). Compared with the control trial, hepatic VLDL-TG secretion rate was reduced by $\sim 17\%$ ($P=0.042$) (Figure 1) and plasma clearance rate of VLDL-TG was increased by $\sim 22\%$ after exercise ($P=0.001$) (Figure 2). Hypocaloric diet had no effect on the hepatic secretion rate ($P=1.000$) or the plasma clearance rate ($P=0.227$) of VLDL-TG.

DISCUSSION

We evaluated the effects of aerobic exercise and dietary energy restriction of equivalent energy deficit (~ 2 MJ or 500 kcal) on basal VLDL-TG kinetics in healthy, lean, sedentary women. Compared with a control day of isocaloric feeding and rest, we found that exercise decreased fasting plasma VLDL-TG concentration by $\sim 30\%$, owing to a 17% reduction in hepatic VLDL-TG secretion rate and a 22% increase in the plasma clearance rate of VLDL-TG, whereas diet had no effect on VLDL-TG concentration and kinetics. The findings from our study indicate that (i) exercise-induced hypotriglyceridemia in women manifests through a different mechanism (increased clearance and decreased secretion of VLDL-TG) than that described previously in men (increased clearance of VLDL-TG only) (26, 40), and (ii) exercise affects TG homeostasis by eliciting changes in VLDL-TG kinetics that are independent of negative energy balance and specific to muscular contraction.

Studies measuring total plasma TG concentrations after single bouts of aerobic exercise indicate that exercise-induced hypotriglyceridemia in the basal state requires a certain amount of energy to be expended during exercise (7). The duration and intensity of exercise are interchangeable (increasing duration while decreasing intensity and vice versa) when it comes to eliciting hypotriglyceridemia, provided that the total energy expenditure is held constant (41). In previous studies we have shown that 60 min of exercise at 60% of

VO₂peak does not lead to hypotriglyceridemia in either men (23) or women (24). Results from several studies in healthy normolipidemic men indicate that the threshold of exercise required for hypotriglyceridemia to manifest lies near or around 500-600 kcal (20, 33). On the other hand, a single bout of prolonged moderate intensity endurance exercise (90-120 min at 60% of VO₂peak with an energy expenditure of 800-1200 kcal) reduced VLDL-TG concentrations by 25-30% in young healthy men (26, 40), consistent with the results from this study. Therefore, men and women are equally sensitive to a single bout of endurance exercise (12) despite the fact that the absolute energy expended during exercise is much lower for women than for men owing to their lower body weight and VO₂peak.

We have shown previously that basal hypotriglyceridemia after a single bout of prolonged moderate intensity endurance exercise (90-120 min at 60% of VO₂peak) results from increased plasma clearance rate of VLDL-TG in healthy non-obese men (26, 40). The present results demonstrate that, in women, exercise-induced hypotriglyceridemia manifests via both an increase in VLDL-TG plasma clearance rate and a decrease in the hepatic VLDL-TG secretion rate. This sexually dimorphic response of VLDL-TG metabolism in response to acute exercise is consistent with previous studies showing considerable sex differences in basal VLDL metabolism. Women have significantly greater VLDL-TG plasma clearance rates but also greater hepatic VLDL-TG secretion rates than men (22, 31). Given that the mechanisms of TG removal from the circulation are saturable (5) and that the stimulatory effect of exercise on VLDL-TG clearance appears to level off (20), it is possible that exercise has lesser of an effect on VLDL-TG plasma clearance rate in women (~20%, present study) than in men (~40%, (26, 40)) because women already exhibit very high rates of VLDL-TG removal from the circulation (22, 31). This hypothesis is consistent with data from studies showing that women exhibit considerably smaller exercise-induced increases in skeletal muscle LPL activity compared with men (37). Therefore, exercise in women lowers fasting plasma TG concentrations only partly by increasing VLDL-TG clearance. Our results demonstrate that approximately half of this effect is attributed to a reduction in VLDL-TG secretion from the liver, which is also considerably greater in women than in men at rest (22, 31). Unfortunately, the nature of our study cannot further describe the mechanisms responsible for this observation.

We found that part of the exercise-induced decrease in VLDL-TG concentration in women was due to reduced secretion of VLDL-TG from the liver. Although this contributes to a beneficial effect (i.e. reduction in plasma TG concentrations), it can be argued that it might also have negative consequences in view of the fact that VLDL-TG secretion buffers the excess amount of plasma FFA, which generally increases after exercise and would otherwise be cytotoxic (10). However, we did not observe an increase in plasma FFA concentration after exercise in women. Also, other studies have shown that, women exhibit lower exercise-induced increases in plasma FFA than men, and values after exercise return to baseline more readily in women than in men (15, 23, 24). This suggests that the increase in hepatic fatty acid availability after exercise might be lower in women, or that hepatic fatty acids are used more towards oxidation or tissue replenishment in women than in men. A previous study has shown that in response to another lipolytic stimuli (prolonged fasting), women have a greater ability to partition systemic fatty acids towards ketone body production rather than VLDL-TG synthesis, compared with young men, resulting in a more advantageous metabolic profile (30). It can be hypothesized that a similar difference might exist after exercise. Furthermore, the exercise induced-decrease in hepatic glycogen availability might have been higher for our subjects compared to other studies conducted in men (26, 40), thus resulting in a further increase in the use of hepatic fatty acids for oxidation and a limited use for TG synthesis and secretion.

Our results show that dietary energy restriction of a similar magnitude as the exercise-induced energy deficit does not reproduce the effects of exercise on VLDL-TG metabolism. However, we observed a great variability in the diet-induced changes (relative to the control trial) in VLDL-TG concentration among our subjects. VLDL-TG concentration decreased by 20-50% in 6 subjects, did not change in 3 subjects ($\pm 6\%$), and increased in 2 subjects by 40%. We have previously shown that a similar intervention was successful in decreasing fasting and postprandial triglyceridemia (29) and another study has shown that a greater diet induced-energy deficit (~ 900 kcal) for a longer period of time (5 days) reduced fasting plasma TG concentrations (43), although in both instances exercise appeared to cause a somewhat greater effect than diet (29, 43). Another study however found that a lower diet-induced calorie restriction (~ 1.4 MJ) had no effect on fasting plasma TG concentrations (11). It is thus possible that there is an energy deficit threshold for diet-induced TG lowering which lies around 2 MJ (500 kcal). Thus, acute dietary interventions near this threshold may (29) or may not (present study) induce a hypotriglyceridemic effect. Nevertheless, our results indicate that exercise has an effect on VLDL-TG concentration and kinetics which is specific to muscular contraction and independent of exercise-induced negative energy balance.

Our study has several limitations. First, we only studied healthy young normotriglyceridemic women; hence we cannot exclude the possibility that our results may be different in obese subjects or those with increased plasma TG concentrations. Secondly, we did not evaluate VLDL-apolipoprotein B-100 kinetics in this study, which is indicative of the metabolic behavior of the VLDL particle itself, as opposed to the metabolic behavior of core TG; this would have been helpful to better characterize the effects of diet and exercise on VLDL metabolism. Lastly, we did not perform a trial with less exercise, hence we cannot speculate whether less exercise, such as that recommended to the general public, has a similarly beneficial effect on TG homeostasis and VLDL metabolism.

In summary, we found that a single bout of moderate intensity aerobic exercise lowers fasting plasma VLDL-TG concentrations in women via a combination of reduced VLDL-TG secretion from the liver and increased VLDL-TG clearance from plasma. Calorie restriction tailored to induce the same energy deficit (~ 2 MJ / 500 kcal) had no effect on VLDL-TG concentration and kinetics. Therefore, exercise affects TG homeostasis in women by eliciting changes in VLDL-TG kinetics that are specific to muscular contraction and independent of the accompanying negative energy balance. Our study underpins the existence of sex differences in the regulation of postexercise TG metabolism, and the possibility of developing sex-specific interventions to improve plasma TG homeostasis.

Acknowledgments

This work was supported by the Graduate Program, Department of Nutrition and Dietetics of Harokopio University, the Hellenic Heart Foundation and the Greek Governmental Institute of Scholarships (E.B.). Support also came from the Institute for Translational Sciences at the University of Texas Medical Branch, supported in part by a Clinical and Translational Science Award (UL1TR000071) from the National Center for Advancing Translational Sciences, National Institutes of Health, the Shriners Hospital for Children (SSF 84090) and from the Sealy Center on Aging, University of Texas Medical Branch at Galveston. We are indebted to the subjects for their interest and participation in the study.

References

1. Abdel-Maksoud M, Sazonov V, Gutkin SW, Hokanson JE. Effects of modifying triglycerides and triglyceride-rich lipoproteins on cardiovascular outcomes. *J Cardiovasc Pharmacol.* 2008; 51(4): 331–51. [PubMed: 18427276]
2. American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription. Philadelphia: 2000. p. 57-85.

3. Annuzzi G, Jansson E, Kaijser L, Holmquist L, Carlson LA. Increased removal rate of exogenous triglycerides after prolonged exercise in man: time course and effect of exercise duration. *Metabolism*. 1987; 36(5):438–43. [PubMed: 3574133]
4. Boer P. Estimated lean body mass as an index for normalization of body fluid volumes in humans. *Am J Physiol*. 1984; 247(4 Pt 2):F632–6. [PubMed: 6496691]
5. Brunzell JD, Hazzard WR, Porte D Jr, Bierman EL. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest*. 1973; 52(7):1578–85. [PubMed: 4352459]
6. Burton FL, Malkova D, Caslake MJ, Gill JM. Energy replacement attenuates the effects of prior moderate exercise on postprandial metabolism in overweight/obese men. *Int J Obes (Lond)*. 2008; 32(3):481–9. [PubMed: 17998903]
7. Cullinane E, Siconolfi S, Saritelli A, Thompson PD. Acute decrease in serum triglycerides with exercise: is there a threshold for an exercise effect? *Metabolism*. 1982; 31(8):844–7. [PubMed: 7098853]
8. Dufaux B, Order U, Muller R, Hollmann W. Delayed effects of prolonged exercise on serum lipoproteins. *Metabolism*. 1986; 35(2):105–9. [PubMed: 3945185]
9. Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, Durstine JL. Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *J Appl Physiol*. 1998; 85(3):1169–74. [PubMed: 9729596]
10. Gibbons GF, Wiggins D, Brown AM, Hebbachi AM. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans*. 2004; 32(Pt 1):59–64. [PubMed: 14748713]
11. Gill JM, Hardman AE. Postprandial lipemia: effects of exercise and restriction of energy intake compared. *Am J Clin Nutr*. 2000; 71(2):465–71. [PubMed: 10648259]
12. Gill JM, Herd SL, Tsetsonis NV, Hardman AE. Are the reductions in triacylglycerol and insulin levels after exercise related? *Clin Sci (Lond)*. 2002; 102(2):223–31. [PubMed: 11834142]
13. Ginsberg HN, Le NA, Gibson JC. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. Effect of weight loss. *J Clin Invest*. 1985; 75(2):614–23. [PubMed: 3973021]
14. Hardman AE, Lawrence JE, Herd SL. Postprandial lipemia in endurance-trained people during a short interruption to training. *J Appl Physiol*. 1998; 84(6):1895–901. [PubMed: 9609782]
15. Henderson GC, Fattor JA, Horning MA, Faghihnia N, Johnson ML, Mau TL, Luke-Zeitoun M, Brooks GA. Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *J Physiol*. 2007; 584(Pt 3):963–81. [PubMed: 17855762]
16. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk*. 1996; 3(2):213–9. [PubMed: 8836866]
17. Holloszy JO, Skinner JS, Toro G, Cureton TK. Effects of a Six Month Program of Endurance Exercise on the Serum Lipids of Middle-Aged Man. *Am J Cardiol*. 1964; 14:753–60. [PubMed: 14232800]
18. Lemieux S, Patterson BW, Carpentier A, Lewis GF, Steiner G. A stable isotope method using a [(2)H(5)]glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans. *J Lipid Res*. 1999; 40(11):2111–7. [PubMed: 10553014]
19. Lin PH, Proschan MA, Bray GA, Fernandez CP, Hoben K, Most-Windhauser M, Karanja N, Obarzanek E. Estimation of energy requirements in a controlled feeding trial. *Am J Clin Nutr*. 2003; 77(3):639–45. [PubMed: 12600854]
20. Magkos F. Basal very low-density lipoprotein metabolism in response to exercise: mechanisms of hypotriacylglycerolemia. *Prog Lipid Res*. 2009; 48(3-4):171–90. [PubMed: 19275916]
21. Magkos F, Patterson BW, Mittendorfer B. No effect of menstrual cycle phase on basal very-low-density lipoprotein triglyceride and apolipoprotein B-100 kinetics. *Am J Physiol Endocrinol Metab*. 2006; 291(6):E1243–9. [PubMed: 16835398]
22. Magkos F, Patterson BW, Mohammed BS, Klein S, Mittendorfer B. Women produce fewer but triglyceride-rich very low-density lipoproteins than men. *J Clin Endocrinol Metab*. 2007; 92(4):1311–8. [PubMed: 17264179]

23. 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.* 2007; 292(6):E1568–74. [PubMed: 17264219]
24. Magkos F, Patterson BW, Mohammed BS, Mittendorfer B. Basal adipose tissue and hepatic lipid kinetics are not affected by a single exercise bout of moderate duration and intensity in sedentary women. *Clin Sci (Lond).* 2009; 116(4):327–34. [PubMed: 18752466]
25. Magkos F, Tsekouras YE, Prentzas KI, Basioukas KN, Matsama SG, Yanni AE, Kavouras SA, Sidossis LS. Acute exercise-induced changes in basal VLDL-triglyceride kinetics leading to hypotriglyceridemia manifest more readily after resistance than endurance exercise. *J Appl Physiol.* 2008; 105(4):1228–36. [PubMed: 18669933]
26. Magkos F, Wright DC, Patterson BW, Mohammed BS, Mittendorfer B. Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *Am J Physiol Endocrinol Metab.* 2006; 290(2):E355–62. [PubMed: 16219668]
27. Maraki M, Christodoulou N, Aggelopoulou N, Magkos F, Skenderi KP, Panagiotakos D, Kavouras SA, Sidossis LS. Exercise of low energy expenditure along with mild energy intake restriction acutely reduces fasting and postprandial triacylglycerolaemia in young women. *Br J Nutr.* 2009; 101(3):408–16. [PubMed: 18570693]
28. Maraki M, Magkos F, Christodoulou N, Aggelopoulou N, Skenderi KP, Panagiotakos D, Kavouras SA, Sidossis LS. One day of moderate energy deficit reduces fasting and postprandial triacylglycerolemia in women: The role of calorie restriction and exercise. *Clin Nutr.* 2010; 29(4):459–63. [PubMed: 19926367]
29. Maraki M, Sidossis LS. Effects of energy balance on postprandial triacylglycerol metabolism. *Curr Opin Clin Nutr Metab Care.* 2010; 13(6):608–17. [PubMed: 20829686]
30. Marinou K, Adiels M, Hodson L, Frayn KN, Karpe F, Fielding BA. Young women partition fatty acids towards ketone body production rather than VLDL-TAG synthesis, compared with young men. *Br J Nutr.* 2011; 105(6):857–65. [PubMed: 21251339]
31. Mittendorfer B, Patterson BW, Klein S. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. *Am J Clin Nutr.* 2003; 77(3):573–9. [PubMed: 12600845]
32. Mittendorfer B, Patterson BW, Klein S. Effect of weight loss on VLDL-triglyceride and apoB-100 kinetics in women with abdominal obesity. *Am J Physiol Endocrinol Metab.* 2003; 284(3):E549–56. [PubMed: 12475754]
33. Miyashita M, Burns SF, Stensel DJ. Exercise and postprandial lipemia: effect of continuous compared with intermittent activity patterns. *Am J Clin Nutr.* 2006; 83(1):24–9. [PubMed: 16400045]
34. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem.* 1992; 38(9):1632–8. [PubMed: 1326420]
35. Patterson BW, Mittendorfer B, Elias N, Satyanarayana R, Klein S. Use of stable isotopically labeled tracers to measure very low density lipoprotein-triglyceride turnover. *J Lipid Res.* 2002; 43(2):223–33. [PubMed: 11861664]
36. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci.* 1991; 16(1):23–9. [PubMed: 1645211]
37. Perreault L, Lavery JM, Kittelson JM, Horton TJ. Gender differences in lipoprotein lipase activity after acute exercise. *Obes Res.* 2004; 12(2):241–9. [PubMed: 14981216]
38. Reichl D. Lipoproteins of human peripheral lymph. *Eur Heart J.* 1990; 11(Suppl E):230–6. [PubMed: 2226530]
39. Thompson PD, Cullinane E, Henderson LO, Herbert PN. Acute effects of prolonged exercise on serum lipids. *Metabolism.* 1980; 29(7):662–5. [PubMed: 7382830]
40. Tsekouras YE, Yanni AE, Bougatsas D, Kavouras SA, Sidossis LS. A single bout of brisk walking increases basal very low-density lipoprotein triacylglycerol clearance in young men. *Metabolism.* 2007; 56(8):1037–43. [PubMed: 17618947]

41. Tsetsonis NV, Hardman AE. Reduction in postprandial lipemia after walking: influence of exercise intensity. *Med Sci Sports Exerc.* 1996; 28(10):1235–42. [PubMed: 8897379]
42. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol.* 1949; 109(1-2):1–9. [PubMed: 15394301]
43. Yamada T, Kurasawa S, Matsuzaki M, Tanaka A. Remnant lipoprotein metabolism is improved more when body weight is reduced by exercise than by dietary restriction. *Clin Chim Acta.* 2008; 388(1-2):28–32. [PubMed: 17980357]

Figure 1.

Acute effects of exercise and hypocaloric diet on the hepatic secretion rate of very low density lipoprotein triglyceride (VLDL-TG). Data are means $\pm$ SD ($n = 11$). *Significantly different from control, $P = 0.042$.

Abbreviations: TG, triacylglycerol; VLDL, very low density lipoprotein.

Figure 2.

Acute effects of exercise and hypocaloric diet on the plasma clearance rate of very low density lipoprotein triglyceride (VLDL-TG). Data are means $\pm$ SD (n = 11). *Significantly different from control, $P = 0.001$.

Abbreviations: TG, triacylglycerol; VLDL, very low density lipoprotein.

TABLE 1

Dietary energy intake, net energy expenditure of exercise, and energy balance for the three experimental trials

	Control	Exercise	Diet
Dietary energy intake (MJ)	7.01±1.21	7.04±1.38 [‡]	4.91±1.27 [*]
Net energy expenditure of exercise (MJ)	-	2.08±0.08	-
Energy balance (MJ)	0	-2.06±0.39 [*]	-2.10±0.41 [*]

Values are means ± SD (n = 11).

^{*} Significantly different from control, $P < 0.001$.[‡] Significantly different from diet, $P < 0.001$.

TABLE 2

Acute effects of exercise and hypocaloric diet on basal metabolic profile

	Control	Exercise	Diet
Glucose (mmol·L ⁻¹)	5.42 (5.23, 5.62)	5.24 (4.96, 5.53)	5.30 (5.09, 5.51)
Insulin (pmol·L ⁻¹)	5.84 ± 2.19	4.36 ± 1.81	4.37 ± 1.76 [*]
Free fatty acids (mmol·L ⁻¹)	0.72 ± 0.28	0.87 ± 0.26 [†]	0.68 ± 0.19
HDL-cholesterol (mmol·L ⁻¹)	1.32 ± 0.19	1.37 ± 0.18 [†]	1.29 ± 0.20
Total triglyceride (mmol·L ⁻¹)	0.59 ± 0.11	0.51 ± 0.06 [*]	0.57 ± 0.11
VLDL- triglyceride (mmol·L ⁻¹)	0.23 (0.18, 0.29)	0.16 (0.12, 0.20) [*]	0.19 (0.14, 0.27)

Values are means ± SD or means with 95% CI (n = 11).

^{*} Significantly different from control, $P < 0.01$.

[†] Significantly different from diet, $P < 0.05$.

Values for glucose, insulin, and free fatty acids are from time point 0h; values for HDL-cholesterol, total triglyceride and VLDL-triglyceride are averages of time points 0, 4, and 8 h.

Abbreviations: HDL, high-density lipoprotein; VLDL, very low density lipoprotein.

TABLE 3

Acute effects of exercise and hypocaloric diet on circulating lipoprotein particle concentrations

	Control	Exercise	Diet
VLDL particles, total (nmol·L ⁻¹)	29.5 ± 9.7	16.4 ± 7.3 *	24.5 ± 11.6
Large (nmol·L ⁻¹)	0.5 ± 0.3	0.3 ± 0.2 *	0.3 ± 0.2 *
Medium (nmol·L ⁻¹)	9.2 ± 4.4	5.2 ± 3.2 *	8.5 ± 6.4
Small (nmol·L ⁻¹)	19.8 ± 6.9	10.9 ± 5.9 *	15.7 ± 7.2 *
IDL particles (nmol·L ⁻¹)	14.3 ± 14.9	13.4 ± 8.9	18.1 ± 11.3
LDL particles, total (nmol·L ⁻¹)	704 ± 185	748 ± 229	743 ± 202
HDL particles, total (μmol·L ⁻¹)	26.2 ± 3.8	26.6 ± 3.9 †	25.2 ± 4.2

Values are means ± SD (n = 11).

* Significantly different from control, $P < 0.05$.† Significantly different from diet, $P < 0.05$.

Values for lipoprotein subclasses are averages of time points 0, 4, and 8 h.

Abbreviations: HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.