Intensity-dependent and sex-specific alterations in hepatic triglyceride metabolism in mice following acute exercise

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Tuazon MA, McConnell TR, Wilson GJ, Anthony TG, Henderson GC. Intensity-dependent and sex-specific alterations in hepatic triglyceride metabolism in mice following acute exercise. J Appl Physiol 118: 61–70, 2015. First published September 25, 2014; doi:10.1152/japplphysiol.00440.2014.—Precise regulation of hepatic lipid metabolism is linked with development of chronic disease. Elevated plasma triglyceride (TG) concentrations, circulating in the fasted state primarily as very low density lipoprotein-TG (VLDL-TG), and excess hepatic TG accumulation are associated with increased coronary heart disease risk (1) and insulin resistance (17, 29, 44), respectively. Both plasma TG (15) and hepatic TG content (47, 55) decrease with chronic endurance exercise. However, much of the effect of chronic exercise on TG metabolism is likely due to acute impacts of repeated single exercise bouts rather than training-induced adaptations per se (19, 25). There was some initial evidence in a human study that single bouts of moderate-intensity continuous exercise (CE) may reduce the rate of VLDL-TG secretion compared with preexercise baseline (45); however, this study lacked a sedentary control trial, so findings could not be conclusive. VLDL-TG kinetics and other aspects of hepatic TG metabolism likely vary with time since the prior meal, and so it is difficult to interpret postexercise data in the absence of time-matched sedentary controls. Because plasma TG levels as well as expression and activity of hepatic microsomal triglyceride transfer protein (MTP), which is involved in assembly and secretion of VLDL-TG, can vary within a 24-h period (39), it is possible that the apparent change in secretion after CE (45) could have been circadian in nature rather than a response to exercise, further emphasizing the necessity of comparing changes with exercise to time-matched sedentary controls as opposed to preexercise baseline. Although VLDL-TG secretion rate was not reduced by a recent bout of CE in men compared with a sedentary trial (34), for a study conducted in women performing an exercise session of similar duration and relative intensity vs. a sedentary trial, a significant reduction in VLDL-TG secretion was reported (3), revealing this aspect of lipid metabolism can be altered by acute exercise with possible sex differences. Additionally, it has been demonstrated that women exhibit a higher VLDL-TG secretion rate under basal sedentary conditions (33, 37), and an animal model of this phenomenon is needed to create opportunity to understand the molecular mechanisms responsible for the sexual dimorphism observed.

High-intensity interval exercise (HIIE) involves alternating between relatively easy and challenging intensities within a single exercise bout and may be a more effective means of improving hepatic lipid metabolism than the traditional endurance exercise approach of CE at a steady workload because of the potential metabolic effects of the short periods of high-intensity exertion. Chronic (50) but not acute (2) HIIE has been shown to lower VLDL-TG secretion rate in men. However, the effects of HIIE on VLDL-TG secretion in female participants have yet to be reported, and the effectiveness of HIIE vs. CE needs to be directly compared. As well, molecular mechanisms underlying changes in VLDL-TG secretion and other aspects of hepatic lipid metabolism by exercise are poorly understood.

It is plausible that one session of HIIE could lower VLDL-TG secretion rate to a greater degree than CE through modulation of hepatic AMPK activity. In rats, a single exercise session of CE was sufficient to increase activity of AMPK in the liver (9, 40), but it was not known if HIIE could have a more robust effect upon this pathway. This increase in AMPK activity could lead to reduced acetyl-CoA carboxylase (ACC) and increased malonyl-CoA decarboxylase activities, and as a result a decrease in malonyl-CoA content (40). In theory, the net effect would be a shift of fatty acids (FAs) toward oxidation...
and away from esterification into TG. Since hepatic AMPK and ACC are activated and deactivated, respectively, to greater extents by single bouts of high- compared with low-intensity exercise (9), HIIE is potentially more effective than CE for altering hepatic lipid metabolism because of the repeated bursts of highly intense activity.

In addition to potential effects on AMPK signaling, another route by which exercise might alter hepatic TG metabolism and secretion is through changes in lipid droplet (LD)-associated protein content. Perilipin 2 (PLIN2) is an LD-associated protein in the liver (48, 54) and other tissues (6), and is the primary perilipin isoform that is strictly associated with LDs in liver (54). PLIN2 may inhibit intracellular TG lipolysis by blocking access of LDs to cytosolic lipases (5), and thus changes in its expression can possibly alter intracellular TG abundance and FA trafficking (35). Indeed, loss of function of PLIN2 in mice results in 25–60% reductions in hepatic TG content (10, 11, 27), and involvement of PLIN2 in TG secretion has been demonstrated in PLIN2 transfected hepatocytes (35), revealing an important role of this protein in hepatic TG metabolism. There is some evidence in mice that acute CE transiently raises hepatic TG in the postexercise recovery period compared with time-matched sedentary controls (26); however, the role of PLIN2 and the influence of exercise intensity in this phenomenon is unknown.

The effects of single exercise bouts likely play a major role in the benefits of chronic exercise training (14, 22, 24) and could possibly be enhanced when exercise is performed at high intensities, thus highlighting the importance of assessing the acute impact of different exercise modalities. Therefore, the purpose of this study was to assess the influence of one session of HIIE vs. CE on hepatic TG metabolism and secretion as well as to determine molecular mechanisms underlying these physiological results. VLDL-TG secretion rates, hepatic TG content, and abundance and surrogate markers of activation for key hepatic proteins involved in TG metabolism and secretion were measured in mice that performed one session of either HIIE or CE, matched for work and bout duration. Because of the many known sex-based differences in exercise metabolism (49) as well as the known sex differences in basal VLDL kinetics in humans (33, 37), our purpose was also to determine sex differences in these responses. Furthermore, we included time-matched sedentary groups to control for potential diurnal variations in hepatic lipid metabolism as well as effects of the time-elapsed since food withdrawal. We hypothesized that VLDL-TG secretion rate would be reduced in association with lower hepatic TG concentration and enhanced AMPK signal- ing following HIIE, but to a lesser extent with CE, and that exercise effects would be more pronounced in female mice.

**METHODS**

**Animals.** This protocol was approved by the Rutgers University Institutional Animal Care and Use Committee. Male and female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were maintained on a 12-h light/dark photoperiod with all mice allowed ad libitum access to food and water. All mice consumed the LabDiet 5K52 diet (Purina Mills, Richmond, IN) for their lifetimes and were acclimated to the facility for at least 5 days prior to exercise. Body composition was assessed between 14–16 wk of age, prior to exercise, using an EchoMRI system (Echo Medical Systems, Houston, TX).

**Exercise protocols.** Mice were exercised between the ages of 14–16 wk on a treadmill (Exer-3/6, Columbus Instruments, Columbus, OH) with a shock grid set at a low intensity (on a scale of 0–10, set at 1). On the day before exercise, any mice were acclimated to the treadmill for 5 min at a speed of 5 m/min with no incline (0°). At 7:00 AM on the day of exercise food was withdrawn, and at 10:00 AM a drop (~15 μl) of whole blood was drawn from the tail for measurement of preexercise TG concentration using a handheld meter (Cariocheck, Polymer Technology Systems, Indianapolis, IN). Mice were then assigned to time-matched sedentary control (CON), CE, or HIIE groups matched for preexercise TG concentration followed by exercise beginning between 11:45 AM and 12:30 PM of the same day. CE and HIIE are described below, and CON was a time-of-day matched condition in which mice remained in their cages with water bottles withdrawn for the amount of time that CE and HIIE mice would spend away from their cages during a session (~35 min). Effects of CE and HIIE were compared only with CON within the same day to control for drift in sedentary baseline over time.

Preliminary development work was performed to establish an HIIE protocol that would be challenging but that could be consistently completed, and ultimately the protocol reported here was chosen. For HIIE, following a 5-min warmup at a slow walking speed (5 m/min), mice ran for 30-s intervals with 60-s walking rest periods (5 m/min) interspersed between intervals. The exercise session included 20 running intervals, the first at 15 m/min, next at 20 m/min, then at 25 m/min, followed by all remaining sprint intervals at a final speed of 30 m/min. Acceleration up to 15, 20, 25 and 30 m/min was performed within a 5-s ramping duration and deceleration back to 5 m/min within a 2-s duration. Both the warmup and the exercise session were performed at an incline of 25°. CE consisted of an incline-matched, duration-matched, and distance-matched continuous running session (13.8 m/min for 30 min) following the same 5-min warmup phase. To avoid repeated shocks and to maintain running speed, mice were gently prodded by hand when they closely approached the shock grid. As described below, measurements and tissue collections were made either on the day of exercise (day 1) or on the following day (day 2).

**Food intake.** Overnight food intake was measured in mice that were assigned to be killed on day 2. In these mice, at 7:00 PM on the day of exercise, a known amount of food was added back to cages, and the remaining food at 7:00 AM the following morning was weighed.

**Hepatic TG secretion.** Food was withdrawn at 7:00 AM on the day of experimentation. Blood TG rate of appearance (Ra), considered to represent the hepatic VLDL-TG secretion rate, was assessed at 3:00 PM following an 8-h fast on the day of exercise (day 1) and the day following exercise (day 2) in separate sets of mice, with a minimum of six mice per group. Blood TG was measured on samples (~15 μl) obtained from the tail after intraperitoneal injection of tyloxapol (500 mg/kg) (Sigma-Aldrich, St. Louis, MO) taken at 20-min intervals until TG exceeded 500 mg/dl, as described and discussed previously (21), with the same handheld meter used to measure preexercise TG concentration. Tyloxapol prevents circulating TG degradation such that blood TG concentration rises linearly, the slope of the linear regression indicating the rate of VLDL-TG secretion (21). A high degree of linearity ($R^2 = 0.99$) was consistently achieved. Hepatic VLDL-TG secretion rate was measured per unit of blood volume (mg·dl$^{-1}$·min$^{-1}$) and then converted to values normalized to body weight (mg/kg BW/min) based upon an assumption of blood volume (43).

**Tissue collection.** Tissue collection was performed in a separate set of mice than those used for measurement of VLDL-TG kinetics with a minimum of six mice per group. Again, food was withdrawn at 7:00 AM, and then tissues were collected at the same time of day as the hepatic TG secretion measurements described above. On the day of exercise (day 1) or the day after exercise (day 2), mice were euthanized by CO$_2$ inhalation followed by immediate collection of blood via cardiac puncture. Blood was collected in EDTA-coated tubes followed by isolation and storage of plasma at −80°C until analysis.
Liver tissues were quickly collected following blood draw, immediately frozen in liquid nitrogen, then stored at −80°C until analysis. Biochemical assays. Plasma glycerol (a marker of whole body lipolysis) and plasma TG concentrations were measured using a commercially available kit (Sigma-Aldrich). For measurement of hepatic TG concentration, lipids were extracted from ~8 mg of tissue, and TG content of the extract was determined using a commercially available kit (Sigma-Aldrich) (18, 23) and expressed as percentage of liver wet weight.

Western blotting. Western blotting was conducted as previously reported by our group (23) with minor modification. Briefly, ~40 mg of liver was homogenized followed by gel electrophoresis of 50 μg of protein and transferred onto membranes. Membranes were incubated with primary antibodies against total AMPKα (1:400; Cell Signaling Technology, Danvers, MA), phospho-AMPKαThr172 (1:800; Cell Signaling Technology), total ACC (1:8,000; Abcam, Cambridge, MA), phospho-ACCSer79 (1:1,000; Cell Signaling Technology), and β-actin (1:2,000; Cell Signaling Technology). Membranes were then incubated with IR Dye 680 (1:10,000; LI-COR Biosciences, Lincoln, NE) or IR Dye 800 (1:10,000; LI-COR Biosciences) secondary antibodies and bands quantified with Odyssey (Li-Cor Biosciences).

mRNA quantification. Approximately 20 mg of liver was pulverized under liquid nitrogen temperature and RNA was isolated (8) followed by treatment with RNaseAfree (Life Technologies, Grand Island, NY) and DNase I (Life Technologies). cDNA synthesis was performed using a commercially available kit (Life Technologies) and RT-PCR by using the following TaqMan Gene Expression Assays (Life Technologies): PLIN2 (Assay ID Mm00475794_m1), AMPKα1 (Assay ID Mm01204670_m1), ACC1 (Assay ID Mm01304257_m1), ACC2 (Assay ID Mm0120467_m1), MTP (Assay ID Mm00435015_m1), and 18s ribosomal RNA (cat. no. 4352930E) as the endogenous control as detailed previously (8); results are expressed as fold-difference relative to female sedentary control mice.

Statistical analysis. Data are presented as means ± SE. Responses to exercise (trial) were analyzed by 2-way ANOVA (sex-by-trial) within each day. As discussed above regarding the importance of controlling for drift in sedentary baseline over time, effects of exercise were only compared with CON of the same day. For determination of sex differences under sedentary conditions, ANCOVA examining the effect of sex across both days 1 and 2 (day as the covariate) was used in order to collectively analyze these data to test for general sex differences in the control condition. To directly probe differences between CE and HIIE, a priori planned comparisons by sex differences under sedentary conditions, ANCOVA examining the differences in the control condition. To directly probe differences in the control condition. To directly probe differences relative to female sedentary control mice.

RESULTS

Characteristics of sedentary female and male mice. Compared with males, females in the CON group exhibited a lower plasma [TG] (P < 0.05) and higher VLDL-TG secretion (P < 0.05), both as measured per unit blood volume and normalized to body weight with similar patterns on day 1 (5.54 ± 0.22 vs. 4.86 ± 0.41 mg·dl⁻¹·min⁻¹, 6.10 ± 0.24 vs. 5.34 ± 0.45 mg·kg·BW·min⁻¹) and day 2 (5.42 ± 0.35 vs. 4.30 ± 0.27 mg·dl⁻¹·min⁻¹, 5.96 ± 0.38 vs. 4.75 ± 0.30 mg·kg·BW·min⁻¹) (Table 1). Prior to exercise, bodyweight (P < 0.0001) and fat-free mass (P < 0.0001) were lower and percent body fat higher (P < 0.05) in females compared with males (Table 1).

Food intake. Overnight food intake is shown in Table 1. In response to CE and HIIE, food intakes were reduced compared with CON [main effect of trial, P < 0.01; CE: −11.5 ± 4.3% (P < 0.05); HIIE: −10.5 ± 2.9% (P < 0.05)]. There were no differences in the reductions in food intake between CE and HIIE and no sex-by-trial interactions.

TG kinetics. On day 1, VLDL-TG secretion rate was not altered by exercise (Fig. 1A). On day 2, there was a sex-by-trial interaction for VLDL-TG secretion (P < 0.05; Fig. 1B). VLDL-TG secretion was decreased by ~20% on day 2 with HIIE compared with CON in females (P < 0.05), with no effect of CE, and there were no significant exercise-related changes in males. Plasma [TG] was not altered by exercise on day 1 (females, CON: 38 ± 5 mg/dl; CE: 48 ± 5 mg/dl; HIIE: 51 ± 8 mg/dl; males, CON: 54 ± 5 mg/dl; CE: 62 ± 5 mg/dl; HIIE: 55 ± 4 mg/dl) or day 2 (females, CON: 41 ± 5 mg/dl; CE: 39 ± 5 mg/dl; HIIE: 39 ± 5 mg/dl; males, CON: 49 ± 7 mg/dl; CE: 35 ± 6 mg/dl; HIIE: 50 ± 7 mg/dl). Plasma [glycerol] was also not altered by exercise on day 1 (females, CON: 0.30 ± 0.03 mM; CE: 0.30 ± 0.03 mM; HIIE: 0.31 ± 0.03 mM; males, CON: 0.30 ± 0.02 mM; CE: 0.30 ± 0.02 mM; HIIE: 0.31 ± 0.02 mM) or day 2 (females, CON: 0.32 ± 0.02 mM; CE: 0.41 ± 0.04 mM; HIIE: 0.34 ± 0.04 mM; males, CON: 0.25 ± 0.02 mM; CE: 0.26 ± 0.02 mM; HIIE: 0.27 ± 0.03 mM).

Hepatic TG and PLIN2. Hepatic TG concentration on day 1 increased with exercise (main effect of trial, P < 0.0001; Fig. 2A) with post hoc testing indicating that both CE and HIIE were different from CON (P < 0.05). In females, the relative increase in hepatic TG concentration compared with CON was approximately twofold greater with HIIE than with CE (P < 0.05) with no significant difference between CE and HIIE in males. For PLIN2 protein on day 1, there was a main effect of trial (P < 0.0001) with post hoc testing indicating that both types of exercise increased abundance compared with CON with a greater increase with HIIE than CE (P < 0.05; Fig. 3A). mRNA expression followed a similar pattern as protein for PLIN2 (main effect of trial, P < 0.05; Fig. 3C) but with post hoc testing revealing that only HIIE and not CE resulted in significant increases in mRNA expression (P < 0.05). Regression analysis showed that group means for content of PLIN2

Table 1. Characteristics of sedentary female and male mice

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<thead>
<tr>
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<th>Females</th>
<th>Males</th>
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<tr>
<td>VLDL-TG secretion, mg/kg·BW·min⁻¹</td>
<td>6.10 ± 0.24</td>
<td>5.34 ± 0.45†</td>
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<tr>
<td>VLDL-TG secretion, mg·dl⁻¹·min⁻¹</td>
<td>5.54 ± 0.22</td>
<td>4.86 ± 0.41†</td>
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<tr>
<td>Plasma [TG], mg/dl</td>
<td>38 ± 5</td>
<td>54 ± 5†</td>
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<tr>
<td>Plasma [glycerol], mM</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>19.0 ± 0.2</td>
<td>25.4 ± 0.4*</td>
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<tr>
<td>Fat mass, g</td>
<td>2.0 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>FFM, g</td>
<td>17.1 ± 0.2</td>
<td>23.1 ± 0.3*</td>
</tr>
<tr>
<td>% Body fat</td>
<td>10.4 ± 0.4</td>
<td>8.9 ± 0.6†</td>
</tr>
<tr>
<td>Overnight food intake, g</td>
<td>3.51 ± 0.14</td>
<td>3.16 ± 0.14</td>
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</tbody>
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Values are means ± SE. Values shown for VLDL-TG secretion, plasma [TG], and plasma [glycerol] are from day 1. VLDL-TG, very low density lipoprotein–triglyceride; BW, body weight; FFM, fat-free mass. Different from females: *P < 0.0001, †P < 0.05.
mRNA and protein were highly correlated ($R^2 = 0.95$, $P = 0.001$). On day 2, hepatic TG concentration (main effect of sex, $P < 0.0001$; Fig. 2B) and PLIN2 protein content (main effect of sex, $P = 0.0001$; Fig. 3B) were higher in females than males. Compared with CON within day 2 in both sexes, TG and PLIN2 abundance in CE and HIIE were not different, indicating that the elevations found on day 1 (Figs. 2A and 3A) with both types of exercise in each sex had significantly subsided. However, there were divergent trends on day 2 between sexes in response to HIIE (Fig. 2B). Compared with CON, hepatic TG concentration tended to be elevated following HIIE in females ($P = 0.1$) and lowered following HIIE in males ($P = 0.1$). Regression analysis of group means showed that compared with CON, changes in VLDL-TG secretion rate and changes in hepatic TG concentration on day 2 were highly correlated inversely ($R^2 = 0.88$, $P < 0.01$; Fig. 2D) with no significant correlation on day 1 (Fig. 2C). There were no sex-by-trial interactions on day 1 or day 2 for hepatic TG or PLIN2 protein and mRNA.

**Intracellular signaling.** In sedentary mice, total ($P < 0.0001$) and phosphorylated ($P = 0.0001$) AMPK as well as total ($P = 0.0002$) and phosphorylated ($P = 0.0015$) ACC protein levels were greater in females than males. Similarly, on day 1 and day 2, protein levels of total and phosphorylated for both AMPK and ACC were also higher in females (main effects of sex, $P < 0.01$; Figs. 4 and 5). ACC1 ($P < 0.001$) and ACC2 ($P < 0.05$) mRNA were higher in females on day 2 (main effect of sex; Fig. 5). There were no significant main effects of trial or sex-by-trial interactions on day 1 or day 2 for total, phosphorylated, or the ratio of phosphorylated to total protein for AMPKα and ACC (Figs. 4 and 5) as well as for mRNA abundance of AMPKα1, ACC1, or ACC2 (Figs. 4 and 5). The ratio of phospho-CREB (a surrogate marker of PKA activity) was greater in sedentary males than females ($P < 0.01$, data not shown). There were no main effects of trial or sex-by-trial interactions on day 1 or day 2 for the ratio of phospho-CREB to total CREB (data not shown).

**Microsomal triglyceride transfer protein.** Protein expression for MTP was greater in females than males in sedentary control mice ($P < 0.05$). On day 1 and day 2, there were no main effects of trial and no sex-by-trial interactions for MTP protein and mRNA expression (Fig. 6).

Fig. 1. Relative differences in very low density lipoprotein-triglyceride (VLDL-TG) secretion compared with sedentary control (CON) of the same sex. Values are means ± SE. Males, black bars. Females, open bars. A: day 1. No main effects of sex or trial and no sex-by-trial interaction. B: day 2. Sex-by-trial interaction, $P < 0.05$. *High-intensity interval exercise (HIIE) was significantly different from continuous exercise (CE) and CON in females, $P < 0.05$. 

Fig. 2. Hepatic TG concentrations. Values are means ± SE. Males, black bars. Females, open bars. A: day 1. Main effect of trial, $P < 0.0001$. *CE and HIIE trials were significantly different from CON ($P < 0.05$) but not from each other. No main effect of sex or sex-by-trial interaction. **The relative increase compared with CON was greater with HIIE than with CE ($P < 0.05$). B: day 2. *Main effect of sex, $P < 0.0001$. No main effect of trial or sex-by-trial interaction. C: group changes in VLDL-TG secretion and changes in hepatic [TG] compared with CON of same sex on day 1. No significant correlation. D: group changes in VLDL-TG secretion and changes in hepatic [TG] compared with CON of same sex on day 2. Significant inverse correlation ($R^2 = 0.88$, $P = 0.0058$).
DISCUSSION

Accumulation of hepatic TG is associated with metabolic dysfunctions such as insulin resistance and elevated VLDL-TG secretion (17). Many of the benefits of habitual exercise are due to the most recent exercise bout rather than training-induced adaptations; however, little is known about the effects of acute exercise on hepatic TG metabolism and secretion and the influence of exercise intensity in males vs. females. In the present study, we examined the impact of single bouts of moderate-intensity CE vs. HIIE on hepatic TG metabolism and secretion in female and male mice, and our novel findings are as follows. Both CE and HIIE transiently increased hepatic TG concentration on day 1, and in females the increase was greater with HIIE. Hepatic protein abundance of the lipid droplet coating protein PLIN2 was also transiently increased by exercise on day 1 with a greater increase with HIIE in both sexes. Increases in PLIN2 protein with exercise were likely driven by increased gene transcription, and the elevations in hepatic TG as well as PLIN2 protein and mRNA with exercise compared with CON on day 1 were no longer present on day 2 in both sexes. And finally, the reduced VLDL-TG secretion rate on day 2 relative to CON which occurred only in females with HIIE was associated with a trend for increased hepatic TG content. Importantly, the differences between CE and HIIE occurred even despite bouts being identical in both total distance ran and bout duration and without differences between HIIE and CE for ad libitum food intake. These results reveal that both exercise intensity and sex alter hepatic TG metabolism and secretion following an acute bout of exercise. We also found greater basal VLDL-TG secretion rate in females, a sexual dimorphism present in humans that may be mediated by higher abundances of hepatic TG, AMPK, ACC, and MTP.

Our findings corroborate previous work with humans (16) and male mice (26) demonstrating increased hepatic TG after one bout of CE. In the present study, we extend these previous findings by demonstrating that the rise in hepatic TG with exercise can occur in both sexes and that the elevation largely subsides within ~28 h postexercise, as shown by greater hepatic TG in CE and HIIE compared with CON within day 1 and lack of differences between CE and HIIE vs. CON within day 2. Of importance, we found sexual dimorphism in this response in that in females HIIE elicits an approximately twofold greater TG elevation than CE, whereas in males both exercise modalities produce similar increases. To our knowledge, this is the first time it has been shown that sex differences in hepatic lipid metabolism in response to intense exercise exist. The transient elevation in hepatic TG appears not to be a result of increased de novo lipogenesis as no changes in ACC abundance or phosphorylation in response to exercise were observed. Enhanced adipose tissue lipolysis could result in increased delivery of FAs to the liver for esterification into TG. However, we did not detect any differences in plasma glycerol concentration, a marker of whole body lipolysis that closely tracks changes in plasma free fatty acid (FFA) concentration with exercise (21, 38, 46). It could be, however, that adipose lipolysis was elevated immediately after exercise but subsided prior to blood collection 3 h later (9, 26).

Rather than simply a mass action effect from increased de novo lipogenesis or whole body lipolysis, it appears that the increases in hepatic TG with exercise could likely be related to PLIN2 expression and the sequestration of available FAs into hepatic lipid droplets, as an association between enhanced PLIN2 protein expression and hepatic TG was found. PLIN2 promotes hepatic TG storage likely by inhibition of intracellular TG lipolysis (5), whereas increases in hepatic TG could possibly elevate PLIN2 protein abundance through prevention of protein degradation (36). Because of this interdependence of PLIN2 protein and TG level, determining the direction of causality between changes in PLIN2 protein and TG could be difficult in the absence of other measures. Thus we measured expression of PLIN2 mRNA as well, and we discovered a strong significant positive correlation ($R^2 = 0.95$, $P = 0.001$) between PLIN2 mRNA and protein expression levels. This finding suggests that in response to exercise, hepatic TG is
increased through transcriptional regulation of PLIN2 expression. We also found that PLIN2 transcription with exercise is intensity dependent, as evidenced by the statistically significant increase only with HIIE. The mechanism behind this is unknown, but it appears unrelated to hepatic AMPK signaling or PKA activation as measured by phosphorylation of AMPK and CREB, respectively. It could be related to exercise-induced PPARα activation or expression, as acute exercise stimulates its expression in the liver (28) and PPARα agonists stimulate hepatic PLIN2 expression (13), and this could be an important future area of investigation.

In addition to the relationship between PLIN2 and hepatic TG with exercise, abundance of TG and PLIN2 protein also tended to track each other from day 1 to day 2 in CON, suggesting a possible but unconfirmed role of PLIN2 in changes in hepatic TG metabolism over time. Although our experiments were not designed to examine this time-related change in CON, future work may elucidate relationships between PLIN2 and hepatic TG under various conditions other than exercise.

We originally hypothesized that hepatic TG of exercised mice would be lower than CON. Thus the transient increases in hepatic TG and PLIN2 did not support our original hypothesis. Reductions in hepatic TG with chronic exercise are thought to be related to improved insulin sensitivity; however, the role of hepatic TG in insulin sensitivity is not entirely clear. Temporary rises in hepatic TG after acute exercise might also be beneficial, reflecting a buffering of FAs away from synthesis of potentially lipotoxic FA metabolites. Acute bouts of CE prevent lipid-induced whole body insulin resistance by lowering concentrations of lipotoxic metabolites in skeletal muscle, partially by promoting storage of FAs as biologically inert TG (42). In addition, PLIN2 overexpression in muscle also increases insulin sensitivity in parallel with greater intramuscular TG (4). It could thus theoretically be possible that the transient
increase in hepatic TG with exercise that appears to be caused by augmented PLIN2 expression promotes insulin sensitivity in the liver after exercise, and this remains an interesting area for future research.

In agreement with a study in humans (3), exercise in the present study lowered VLDL-TG secretion in females. As well, the lack of changes in VLDL-TG secretion in males with CE and HIIE in our study is consistent with human studies showing that the effects of single bouts of prolonged or intense exercise on VLDL-TG kinetics in men are limited to only increased clearance (2, 34, 51, 52). Thus our animal and exercise model appears to be appropriate for inferring molecular underpinnings of human sex differences in VLDL-TG kinetics with exercise. Compared with CON on day 2, we found an ~20% decrease in VLDL-TG secretion rate with HIIE in females with no effect of CE and no impact of exercise on secretion on day 1. Although HIIE resulted in attenuation of VLDL-TG secretion, we did not detect a change in plasma TG concentration, potentially because of the twofold greater individual variability in plasma TG (38 vs. 20% between-animal coefficient of variation). As well, our kinetic approach involved sampling blood TG at several time points for confirmation of complete blockade of circulating TG degradation and accurate calculation of secretion rate for each individual mouse (21), and therefore it is a more robust measurement than determination of plasma TG concentration at a single time point.

Our original hypothesis was that reduced VLDL-TG secretion with HIIE would be mediated by AMPK activation, resulting in decreased hepatic TG. However, this potential mechanism was not supported by our findings. At first, our findings of unchanged AMPK phosphorylation by either exercise modality appear to be in disagreement with acute continuous exercise studies demonstrating increased hepatic AMPK activation with exercise and greater activation with high- vs. low-intensity exercise (9, 40). In these studies, however, livers were collected immediately after exercise as opposed to the present study in which they were sampled 3 h after the
mechanisms behind reduced VLDL-TG secretion in females with HIIE are unclear but appear to be related to partitioning of FAs away from VLDL assembly and toward retention in lipid droplets. In the formation of VLDL particles, cytosolic TG undergoes lipolysis and FAs are transported into the endoplasmic reticulum for reesterification into TG and incorporation into VLDL (32). Inhibition of this pathway theoretically would result in accumulation of TG in lipid droplets. Indeed, on day 2 the reduction in VLDL-TG secretion in females was associated with a trend for a \( \sim 50\% \) increase (\( P = 0.1 \)) in hepatic TG content. In addition, there was a significant inverse correlation between group changes in these two outcomes; correlations between group means rather than between individual data points should be interpreted with caution, but this finding may further demonstrate a biological relationship between VLDL-TG secretion and hepatic TG concentration. Interestingly, although hepatic TG content was increased by exercise on day 1 in both sexes, these changes were not associated with changes in VLDL-TG secretion at this time. Thus the relationship between hepatic TG content and secretion with exercise does not manifest relatively early in the postexercise period but rather later during the following day. Glucagon (53) and other hormones such as catecholamines that would enhance PKA activity (7) are expected to stimulate lipolysis and thus promote transfer of FAs from lipid droplets to the endoplasmic reticulum in the liver. Additionally, MTP is required for incorporation of these FAs into VLDL. However, we found no changes in CREB phosphorylation [a marker of PKA activity (30, 31, 56)] or MTP abundance with CE or HIIE. While the reduction in VLDL-TG secretion with chronic exercise training has been attributable to decreased MTP (12), based on our findings the lowering of TG secretion with acute exercise is due to a different mechanism.

In addition to sex differences in hepatic TG metabolism and secretion in response to exercise, we found that our work on a common laboratory animal recapitulated human sex differences in the basal state. Females exhibited higher VLDL-TG secretion, which has been previously shown in humans (33, 37), as well as higher hepatic TG content. The reason for more TG in livers of female mice could be attributed to the twofold greater ACC protein expression, as ACC catalyzes the formation of malonyl-CoA. Malonyl-CoA provides substrate for FA synthesis that can be incorporated into TG, and it also inhibits FA transport into the mitochondria. Thus higher ACC content can shift FAs away from oxidation and toward storage as TG. This can potentially lead to greater hepatic TG availability and provide higher FA supply via lipolysis into the endoplasmic reticulum, reesterification into TG, then through MTP incorporation into VLDL and secretion (32). As well, the higher hepatic AMPK content could play a role in this process by promoting TG lipolysis (20). Indeed, AMPK activation has been shown to stimulate hepatic TG secretion (41), and in the present study both AMPK and MTP were higher in sedentary females than males. Thus it appears that females relative to males exhibit a hepatic enzyme profile that supports the synthesis and secretion of VLDL-TG.

In conclusion, we have discovered a novel metabolic impact of exercise in which transient alterations in hepatic TG metabolism are exhibited after exercise. The changes in hepatic lipid trafficking appear to be modulated by exercise-induced alterations in PLIN2 expression, and this response may be important for achieving health benefits of exercise or for adaptation to the stresses of exercise participation. Second, we have discovered potential mechanisms for sex differences in VLDL-TG secretion in the basal state and in response to a recent bout of intense exercise, and these results shed light upon sex-specific regulation of energy metabolism and the integration of metabolism between the liver and other tissues. As many of the effects of chronic exercise are expected to be a result of acute effects of each individual exercise bout, we expect that these results would provide information about certain aspects of the chronic exercise training response as well. In future work the effects of chronic training with these exercise types (CE and HIIE) could be investigated to deter-

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Fig. 6. Hepatic microsomal TG transfer protein (MTP) expression. Values are means \( \pm \) SE. Females, black bars. Males, open bars. A: protein abundances on day 1. \( * \)Significantly greater in females than males in sedentary control mice (\( P < 0.05 \)). No main effect of trial or sex-by-trial interaction. B: protein abundances on day 2. \( * \)Significantly greater in females than males in sedentary control mice (\( P < 0.05 \)). C: mRNA abundances on day 1. No main effects of sex or trial and no sex-by-trial interaction. D: mRNA abundances on day 2. No main effects of sex or trial and no sex-by-trial interaction.
mine longer term effects on hepatic TG metabolism and ultimately the effect on metabolic health.

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AUTHOR CONTRIBUTIONS


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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