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Postprandial triglyceride and free fatty acid metabolism in obese women after either endurance or resistance exercise

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INADEQUATE PHYSICAL ACTIVITY level (PAL) is associated with the accumulation of excess body fat (32). Obesity has been linked with many disease risks, including cardiovascular disease (CVD) and type 2 diabetes (34, 62). Previously, it has been reported that obese individuals exhibit a lower capacity for lipid oxidation (Lox) compared with their lean counterparts (31, 38, 69). A reduced relative contribution of Lox is associated with increased risk of gaining weight over time (81), and so, substrate partitioning, along with effects of positive energy balance, could potentially contribute to the propensity for gain and retention of body fat. Fed-state plasma triglyceride (TG), referred to as postprandial lipemia, is elevated in obesity (13, 47) and is associated with increased CVD risk (4, 18, 46, 57, 67). Exercise can potentially address these metabolic alterations by impacting the resting metabolism for many hours after each session (26). People typically take a meal after exercise and are in the postprandial state for a majority of the day, and studies of the effects of exercise on postprandial metabolism are needed to improve understanding the integration of metabolic processes and for preclinical testing of exercise efficacy.

Benefits of chronic exercise represent the accumulation of the acute benefits from each individual exercise bout, and chronic exercise can reduce CVD risk (32). An individual exercise bout can increase subsequent resting Lox (21, 26, 28, 61, 71, 72) and reduce postprandial plasma TG concentration (1, 16, 19, 21, 22, 28, 35, 36, 50, 52, 56, 67, 71, 72–80). In addition to the effect of the energy expenditure (EE) of exercise (EEE) (22, 78), there has been some indication that exercise intensity could be important in the response of postprandial lipemia (35, 70) and postprandial Lox (70), although this intensity effect was not apparent in all cases (16, 71). If exercise intensity were a factor in determining the subsequent response of postprandial metabolism, one could expect that resistance exercise, because of its vigorous nature, could be useful in altering subsequent lipid metabolism. Attempts have also been made to compare the modalities alongside one another in a single study; in these studies, bouts of endurance exercise (E) and resistance exercise (R) were designed to match for EE. When doing so, the R bout was challenging (near the maximum intensity possible during the bout), whereas the E bout was quite easy, apparently, approximately one-half (or even less) of the intensity that an individual could tolerate well (49, 61). So, when matched for EEE, R appears to be superior to E in its metabolic impacts on plasma TG metabolism and Lox (49, 61). E inherently entails a high EEE, whereas R, although physiologically stressful in other ways, does not lead to high EEE when performed for a similar duration as E exercise. Matching EEE addresses an interesting biological question, but we sought to compare E and R in the present study in a manner that could be particularly translational and clinically relevant. We studied E and R bouts that were typical of those that may be attempted in an exercise program (matched for duration; each at a challenging intensity; each consistent with current exercise recommendations stating that higher intensities of exercise may carry additional benefits beyond those of lower intensities). In such an experimental design, applying a more challenging E bout could potentially clarify implications of previous results that had suggested inferiority of E vs. R.

Changes in postprandial lipid metabolism after exercise could entail an alteration in metabolism of the fat from the postexercise meal (exogenous fat), and alternatively, the changes could be for metabolism of fat that was already within the body before meal ingestion (endogenous fat). In identifying whether the benefits of exercise on postprandial lipid metabolism involve altered handling of the meal’s fat content, insight
is provided into the physiological impacts of exercise. In studies of postexercise metabolism, stable isotope tracers can be used to label meal fatty acid (FA) content to track dietary fat and differentiate between exogenous (meal-derived) and endogenous FA contribution to the plasma TG excursion, plasma free FA (FFA), and Lox (14, 24, 44, 45). In a small number of studies, FA tracers have been administered in postexercise meals (21, 74–76), and a subset of this analytical potential was realized as exogenous FA oxidation was measured by isotope labeling of carbon dioxide. However, in light of the potential to also follow these tracers through other fates and pathways, we expanded this methodological approach to study the relative labeling of circulating TG and FFA as well. As obese individuals exhibit exaggerated responses of plasma TG concentration to a meal (13, 47), we considered it important to study this population when attempting to manipulate metabolism of dietary fat with exercise. To our knowledge, this is the first study to use an oral-fed tracer to track alterations in Lox, as well as plasma TG and FFA concentrations and labeling after exercise.

Interventions that lead to a favorable postprandial FA metabolism may reduce CVD risk compared with a sedentary state and could also promote a lean phenotype if the achieved negative fat balances were maintained without compensation. Therefore, with the use of the methodology of stable isotope tracers, we studied the effects of a premeal exercise bout on postprandial TG and FA metabolism over the course of a postprandial period, following time-matched exercise bouts of different modalities, and compared the results with a sedentary control. We studied women, who are traditionally under-represented as study participants in the exercise literature, and obese individuals because of a similar under-representation, as well as a need to alter lipid metabolism in this group. We tested the hypothesis that FA oxidation in both exercise groups would increase during the postexercise period compared with the sedentary control. We also hypothesized that either exercise approach would decrease plasma TG concentrations during the postexercise period by a reduction in exogenous and endogenous fat content in circulation, whereas the concentration of plasma FFA from both sources would increase after exercise.

METHODS

Study participants. Twelve sedentary, premenopausal, obese (body mass index [BMI] > 30) women were recruited from Rutgers University (New Brunswick campus) and its surrounding community by posted notice. Participants were required to have partaken in <1 h/wk of physical activity at moderate or higher intensity during the prior 3 mo. Nine subjects were administered an isotope tracer, and three were provided unlabeled meals and solely included in indirect calorimetry (IDC) analysis. Due to a technical error in labeled meal preparation and analysis, four subjects were administered isotope tracers to study participants consumed a provided bagel at 7:30 AM (81.9% carbohydrate, 3.6% fat, 14.5% protein, 249 kcal) and arrived at the laboratory study participants consumed a provided bagel at 7:30 AM (81.9% carbohydrate, 3.6% fat, 14.5% protein, 249 kcal) and arrived at the laboratory 1 h/wk after each trial. Study participants were instructed to consume solely their standardized diet and water ad libitum and to abstain from structured physical exercise sessions but to continue typical activities of daily living and were fed for a PAL of 1.4, according to the current dietary reference intake guidelines of the Institutes of Medicine for daily estimated energy requirement (EER) (32). Each standardized diet was provided to the study participant 2 days before the upcoming trial and was consumed by the participant outside of the laboratory on the day before and morning of each trial at the instructed times. Dietary energy intake was individualized for each study participant (2,294.0 ± 81.2 kcal/day), and macronutrient composition was made similar among individuals for carbohydrate (CHO; 54.3 ± 0.7%), lipid (25.4 ± 0.5%), and protein (20.3 ± 0.4%). On the day of trials, study participants consumed a provided meal at 7:30 AM (81.9% CHO, 3.6% fat, 14.5% protein, 249 kcal) and arrived at the laboratory at 8:30 AM. Upon arrival at the laboratory, participants confirmed that they had consumed the standardized diets. We chose to feed our study participants 1.5 h before exercise to increase tolerance of the exercise protocol (pre-exercise meal described above).

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Fig. 1. Experimental design. Each study participant completed 3 different experimental trials in randomized order, involving endurance exercise (E), involving resistance exercise (R), and 1 with no exercise (C). Minutes = time clapsed since the study participant commenced meal consumption. E or R bouts were performed from 90 to 30 min or semisupine rest during this time period in the C trial.
Duration of E was exactly 60 min, and the R bout was designed to be ~60 min. The intensity for E was 60–65% \( V_O_{2\text{peak}} \), which in our experience, approximates the maximum intensity that can be completed over 1 h consistently by sedentary people. IDC was used during the E bout to achieve the desired intensity. The R trial was time-of-day matched and duration matched and consisted of eight whole-body exercises, with three sets and 10 repetitions of each exercise (bench press, lat pulldown, shoulder press, squat, leg curls, triceps pushdown, biceps curl, lunges) at 90% 10 RM. If 10 repetitions in a given set could not be completed, then the weight was reduced to complete 10 repetitions on the subsequent set. IDC was not used during the R session. Prior to exercise and at the same time of day in the sedentary control, a single-use needle was used for the first blood draw. After each exercise session (or again, same time in C), study participants sat in a chair where venous catheters were placed (described below). The test meal (also described below) was given 30 min postexercise, after which, participants remained seated for the remaining 400 min of the postprandial period, quietly reading or watching movies. On the day of each trial, at the same time of day, a catheter was placed in a hand vein to obtain arterialized blood using the heated hand-vein technique. In a previous study (29), blood was drawn simultaneously from the radial artery and from a heated hand vein, and there were no differences among blood-sampling sites for glucose, glycerol, or palmitate isotopic enrichment (IE) during tracer administration. The catheter was kept patent throughout the study day, using a 0.9% saline draw, which was 30 min after cessation of exercise. The test meal, which was heated to 85–90°C, using a hot plate with a 0.2 mol percent excess of carbon-13, was consumed within 20 min. The sides of the container and all subsequent voids were collected for the final analysis of urinary urea nitrogen (UUN).

To study the partitioning of dietary FA postexercise, [U-\(^{13}\)C]palmitate (Cambridge Isotope Laboratories, Andover, MA) was administered in a liquid test meal. Thirty minutes after the termination of exercise, the postexercise blood draw and first IDC measurements were taken. Blood samples for the analysis of TG and FA concentration and palmitate IE were collected in tubes containing EDTA. At each breath-sampling time point, pulmonary gas exchange was determined for assessment of metabolic rate and energy substrate partitioning, and an aliquot of expired breath was collected in evacuated Exetainer tubes for subsequent determination of \(^{13}\)CO\(_2\) IE by isotope ratio mass spectrometry (IRMS).

**Test meal.** The liquid test meal consisted of Boost Plus (Nestlé HealthCare Nutrition, Florham Park, NJ) and evaporated milk and was administered immediately following the postexercise breath draw, which was 30 min after cessation of exercise. The test meal provided 20 kcal/kg fat-free mass (FFM; 46.2 ± 1.1% of EER). The [U-\(^{13}\)C]palmitate isotopic tracer dose of 5 mg/kg FFM was added to the test meal, which was heated to 85–90°C, using a hot plate with constant stirring, using a magnetic stir bar. The temperature of the meal was monitored with a thermometer; this procedure ensures successful dissolution and delivery of the tracer, as reported previously (14). The IE of meal palmitate was 5.7 ± 0.2 mol percent excess (MPE), which we determined by liquid chromatography/mass spectrometry (LC/MS) of meal aliquots, as described below. The macro-nutrient composition of the drink was 47.8% CHO, 36.1% fat, and 16.1% protein (1076.4 ± 62.7 kcal), and the average volume was 591.5 ± 34.9 ml. To maintain consistency, participants were asked to consume the entire drink within 20 min. The sides of the container were scraped and that small remaining quantity also consumed.

**Urine collection.** Upon completion of exercise, subjects were wheeled to the restroom to void their bladder. This first urinary void was discarded, such that UUN would be from the postexercise period, and all subsequent voids were collected for the final analysis of nitrogen excretion and creatinine concentration. All urine collections from a trial over the 400-min postprandial period were pooled. At the end of the collection period, the urine volume was assessed and an aliquot saved for subsequent analysis.

**Calculation of energy substrate oxidation.** The last 5 min of each 10-min breath measurement were used from pulmonary gas exchange to calculate rates of EE, CHO oxidation (CHOox), and Lox (17). In the substrate oxidation calculations, we used nonprotein respiratory-quotient values for each time point that we derived from the respiratory exchange ratio (RER) and timed UUN excretion, assuming that the percentage of the resting metabolic rate fueled by protein was consistent across the entire postmeal period. We assumed that each gram of UUN represents oxidation of 6.25 g protein (17). EE, CHOox, and Lox were calculated as kcal/min or kcal/kg FFM/min, and results from statistical analyses were similar between these two manners of expressing the data. In addition to data expressed at each individual time point, postexercise averages (and those in the C trial at the corresponding time of day) were calculated as area under the curve, multiplied by duration (i.e., time-weighted average), and incremental concentrations were calculated as time-weighted average minus pre-exercise baseline values. Incremental concentration changes were calculated in the same manner for other variables, such as plasma lipids.

**Laboratory analyses.** Glucose concentration was analyzed enzymatically (Sigma-Aldrich, St. Louis, MO) in plasma. Urine was analyzed for UUN content using an enzymatic urea nitrogen kit (Stanbio Laboratory, Boerne, TX). Timed UUN output was calculated as (nitrogen content × urine volume)/time interval, reported as g/min, and was used to correct substrate oxidation for protein oxidation. Creatinine concentration in urine was analyzed using a colorimetric assay (Oxford Biomedical Research, Oxford, MI). With the assumption that creatinine excretion is relatively constant throughout the day, the UUN results were normalized to urinary creatinine concentration to derive a proxy index of protein oxidation.

**Isolation of lipids.** Following addition of known amounts of triheptadecanoin and heptadecanoic acid as internal standards, 0.5 ml plasma for TG and FFA analysis was extracted with 4 ml 30:70 heptane/isopropanol (v/v) and subsequently mixed with 2 ml 0.003 M sulfuric acid. The organic layer was removed and then dried under nitrogen gas, and the TG and FFA were isolated by TLC, as described previously (26, 73). Standard lanes in TLC contained oleic acid and trilinolein, and were visualized with iodine. The TG and FFA spots were scraped and extracted and dried under nitrogen gas. FA were resuspended in 90% acetoni-trile with 0.5 mM ammonium acetate: TGs underwent saponification using a 0.25 M KOH in 90% ethanol solution and heated for 45 min at 80°C. Next, to extract the released FA, we added 0.6 ml 1 M HCl and 4 ml hexane, mixed thoroughly, and then centrifuged to separate phases and subsequently transferred the top layer (hexane). The sample was then dried under nitrogen gas, and the remaining lipid was resuspended in 90% acetoni-trile with 0.5 mM ammonium acetate. Samples were then analyzed by LC/MS (Varian, Walnut Creek, CA). For analysis of the liquid test meal, 10 μl of the test-meal shake was combined with a known amount of heptadecanoic acid (internal standard). The mixture underwent saponification, as described above, and the organic phase of the subsequent hexane extraction was dried under nitrogen gas. Samples were then resuspended in 90% acetoni-trile with 0.5 mM ammonium acetate and analyzed by LC/MS by the same method as used for plasma samples.

**Mass spectrometry.** With minor modification, we used the LC/MS method of Persson et al. (60). With the use of an Agilent 1200 HPLC system, Ascentis C18 2.1 × 150 mm column (Sigma-Aldrich), and Varian 1200L quadrupole mass spectrometer with electrospray ionization, FA concentrations and palmitate IE were determined. Mobile phase A (mp-A) was 80% acetoni-trile with 0.5 mM ammonium acetate, and mp-B was 100% acetoni-trile with 0.5 mM ammonium acetate. The flow rate was 0.4 ml/min, and FA were eluted isocratically with 45% mp-A and 55% mp-B, followed by a column wash at higher organic strength. FA were identified by retention time and mass-to-charge ratio (m/z). The following ions were selectively mon-
Table 1. Characteristics of study participants

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Body fat, %</th>
<th>Fat mass, kg</th>
<th>VO₂peak, l/min</th>
<th>VO₂peak, ml·kg⁻¹·min⁻¹</th>
<th>VO₂peak, ml·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.8 ± 1.6</td>
<td>162.6 ± 2.3</td>
<td>98.9 ± 6.8</td>
<td>37.3 ± 2.3</td>
<td>45.4 ± 1.2</td>
<td>45.3 ± 4.0</td>
<td>2.4 ± 0.1</td>
<td>25.2 ± 1.4</td>
<td>46.0 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12. BMI, body mass index; FFM, fat-free mass; VO₂peak, peak oxygen consumption.

Table 2. Metabolic rate

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>E</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂, l/min</td>
<td>0.36 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.28 ± 0.01*</td>
</tr>
<tr>
<td>VCO₂, l/min</td>
<td>0.24 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>RER</td>
<td>0.91 ± 0.01</td>
<td>0.88 ± 0.01*</td>
<td>0.87 ± 0.01*</td>
</tr>
<tr>
<td>EE, kcal/min</td>
<td>1.37 ± 0.5</td>
<td>1.40 ± 0.5</td>
<td>1.43 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12. Postexercise metabolic rate, average of 400 min. C, no exercise (control) trial; E, endurance exercise trial; R, resistance exercise trial; VO₂, oxygen consumption; VCO₂, carbon dioxide production; RER, respiratory exchange ratio; EE, energy expenditure. *Significantly different from C trial in ANOVA, P < 0.05.

RESULTS

Characteristics of study participants and exercise bouts. Subject characteristics can be found in Table 1. All subjects were obese (BMI, 30.1–58.0). E was time matched with R and entailed 60 min of walking at an intensity of 62.5 ± 1.4% VO₂peak with a gross EEE of 430.8 ± 22.4 kcal. In the R trial, all three sets were completed by each study participant for every exercise at an intensity of 88.9 ± 2.3% of 10 RM, with an average number of 9.9 ± 0.03 completed repetitions/set. Habitual exercise participation was 0.5 ± 0.1 h/wk, indicating sedentary lifestyle status.

Metabolic rate and substrate partitioning. Average postmeal values are reported. A main effect of time was detected for all metabolic rate and substrate partitioning values (P < 0.05); however, there were no significant trial-by-time interactions. There was a significant main effect of trial for VO₂ (P < 0.05), with post hoc testing indicating the R trial VO₂ was elevated significantly above C but not statistically different from E (Table 2). There was no significant main effect of trial for VCO₂. In the postexercise recovery period, there was a main effect of trial (P < 0.05) for RER with post hoc testing indicating significantly decreased RER in E and R compared with C (Table 2 and Fig. 2A). RER value contributions toward energy substrate partition are addressed below. EE was not significantly different among trials when averaged throughout the postexercise time period. There was a significant main effect of trial (P < 0.05) for percentage of energy from fat and from CHO. E and R groups derived a significantly higher percentage of energy from fat compared with C (P < 0.05), with no significant difference between E and R compared with C (28.76 ± 3.0; E, 37.39 ± 3.0; R, 40.39 ± 3.0%; Fig. 2B). E and R groups derived a significantly lower percentage of energy from CHO compared with C (P < 0.05), and there was no significant difference between E and R (65.65 ± 2.1; E, 56.99 ± 2.1; R, 54.52 ± 2.1%; Fig. 2B). There were no significant main effects of trial for energy derived from protein based on total UUN (C, 1,241 ± 333; E, 1,304 ± 375; R, 1,187 ± 407 mg) or urea-to-creatinine ratio in urine (C, 19.2 ± 4.5; E, 22.5 ± 5.4; R, 17.3 ± 5.6). When considered across time during each trial, there was a significant main effect of trial for absolute Lox (P < 0.001); post hoc testing indicated significantly increased Lox in E and R compared with C, with no difference between E and R (C, 0.40 ± 0.05; E, 0.52 ± 0.05; R, 0.58 ± 0.05 kcal/min).

Metabolite concentrations. The pre-exercise values for all blood measurements were not statistically different among trials, and this time point was used for normalizing incremental concentration changes across trials. A main effect of time (P < 0.05) was observed for plasma glucose concentration but with no main effect of trial or trial-by-time interactions (average postexercise values: C, 105.7 ± 5.5; E, 106.4 ± 5.7; R, 102.6 ± 5.7 mg/dl; P > 0.05). The total plasma TG and

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incremental plasma TG concentration changes are shown in Fig. 3. A main effect of time was observed for total plasma TG concentration ($P < 0.05$). Both exercise conditions exhibited a significant decrease in total plasma TG as a main effect of trial with post hoc testing, indicating that E and R were significantly different (E, 23.4% reduction; R, 27.2% reduction) compared with C (C, 1.319 ± 64.9; E, 1.010.1 ± 64.9; R, 960.1 ± 64.9 μM). There was no significant difference between E and R and no significant trial-by-time interactions for total plasma TG concentration. Plasma TG incremental concentration changes for E and R were reduced substantially from the level in the C trial, and there was a significant main effect for trial, with post hoc testing indicating that E and R were significantly different from C but not different from one another (Fig. 3B). A main effect of time was observed for total plasma FFA concentration ($P < 0.05$). There was a main effect of trial ($P < 0.05$) for total FFA concentrations (C, 246.7 ± 21.6; E, 291.8 ± 21.6; R, 237.9 ± 21.6 μM), with post hoc testing indicating that total FFA concentrations were elevated significantly in the E group vs. R and C (23% and 18% elevations, respectively; Fig. 3C). There were no significant trial-by-time interactions for plasma total FFA concentrations, and there were no statistically significant differences for FFA incremental concentration changes as a function of trial, but the trends are shown in Fig. 3D.

FA sources in lipid pools. In our tracer methodology, the relative contributions of dietary fat to plasma TG and FFA were derived by comparing the IE of meal palmitate with that in plasma lipids. As the tracer was given in the test meal, any labeling of the body’s lipid pools would be considered to indicate contribution of the exogenous lipid source to those pools. By inference, the FA fraction not exhibiting labeling would be considered of endogenous origin. There was a main effect of trial ($P < 0.05$) and time ($P < 0.05$) and no significant interaction for endogenously derived plasma TG. Post hoc testing indicated that this circulating TG of endogenous origin (Fig. 4A) was significantly attenuated in the exercise trials (E, 24.0% reduction; R, 28.4% reduction) compared with C ($P < 0.05$), with no significant difference between E and R (C, 1.265.5 ± 68.2; E, 962.3 ± 68.2; R, 906.3 ± 68.2 μM). There was a significant main effect of trial ($P < 0.05$) and time ($P < 0.05$) and no significant interaction for endogenously derived plasma FFA concentration. Post hoc testing indicated that this endogenous FFA was elevated significantly ($P < 0.05$) in E compared with both C and R (C, 236.2 ± 20.3; E, 274.7 ± 20.3; R, 222.3 ± 20.3 μM; Fig. 4B). The abundance of exogenous FA in the plasma TG pool had a significant main effect of time ($P < 0.05$) but was not significant as a main effect of trial or trial-by-time interaction (Fig. 4C). For exogenously derived plasma FFA concentration, there was a significant main effect of trial ($P < 0.01$) and of time ($P < 0.05$), with no significant time-by-trial interaction. Post hoc testing indicated that these exogenous FFA were elevated significantly in both the E and R trials compared with C but not significantly different from each other (C, 10.5 ± 2.4; E, 17.1 ± 2.4; R, 15.6 ± 2.4 μM; Fig. 4D).

Breath $^{13}$CO$_2$ excretion continued to rise during the postprandial period, with a main effect of both time ($P < 0.05$) and trial ($P < 0.05$) and no significant time-by-trial interaction. $^{13}$CO$_2$ excretion is directly related to exogenous Lox, because tracer dose and test-meal size and composition were identical among trials. Post hoc testing indicated that both E and R
breath $^{13}$CO$_2$ excretion and corresponding rates of exogenous FA oxidation were elevated significantly above C (Fig. 5), with no significant differences between the exercise modalities. Exogenous FA oxidation rates (calculated from $^{13}$CO$_2$ excretion, tracer dose, and dietary fat dose) rose continuously throughout the trials to the following final values: C, 3.6 ± 0.5 kcal/h; E, 5.3 ± 0.7 kcal/h; R, 4.6 ± 0.5 kcal/h. In the three subjects who received unlabeled meals (no tracer), we confirmed that breath $^{13}$CO$_2$ excretion rates were negligible.

**DISCUSSION**

We investigated the effects of two different exercise modalities on postprandial TG and FFA metabolism in sedentary, obese women. In contrast to previous work matching bouts of endurance and resistance exercise for EEE, we used a challenging intensity for each and matched for duration of the exercise sessions. Our results indicate that a prior session of either E or R significantly attenuates the postprandial lipemic response, concomitantly increasing FA oxidation. We had hypothesized that FA oxidation would be increased and that both exogenous and endogenous TG would be reduced after either exercise bout. Our hypothesis for Lox was confirmed by both IDC and tracer methodology. However, contrary to the hypothesis, exercise was only effective at reducing the postprandial TG excursion from endogenous FA sources, with no effect of exercise on abundance of meal-derived FA in circulating TG. We had also hypothesized that both exogenously and endogenously derived plasma FFA would be elevated after exercise, although we observed that the impact of exercise on abundance of meal-derived FA in circulating TG was more pronounced than that of endogenous FFA. Below, we discuss these results in the context of modifying CVD risk, management of adiposity, and metabolic aspects of the partitioning and interaction between plasma lipid pools.

As noted previously, elevated postprandial lipemia is associated with increased risk for CVD (4, 18, 46, 57, 67). Thus it is worthwhile to compare different interventions for efficacy in...
lowering postprandial plasma TG excursion. A dose effect of exercise volume has been observed for attenuations in plasma TG following endurance exercise (22, 78), implying dependence on the EEE. Whereas we did not measure EE during the R bout, based on previous work, it would seem that the EEE of E exercise may have been approximately double that of R exercise (49, 61). From this, one may predict a greater impact of E in the present study, but as shown, the impacts on postprandial lipemia were similar between the two modalities. This can be understood in the context of previous research that suggested that exercise intensity is an important factor (35, 70) and that resistance exercise may be a more potent form of exercise to reduce plasma TG (22, 78), implying dependence on the EEE. Whereas we did not measure EE during the exercise bout affects subsequent postprandial lipemia, although a direct effect of substrate selection could not be established in a previous study (51). Due to the presumed difference in EEE between E and R in the present study, it can be inferred that R is more effective than E in lowering postprandial lipemia when normalized for EE, but an

Fig. 4. FA sources in lipid pools. Percent contribution of dietary fat (i.e., the exogenous FA source) to plasma TG and FFA was calculated as IEplasma/IEmeal × 100, where IEplasma represents isotopic enrichment (IE) of palmitate in either plasma TG or FFA, and IEmeal represents IE of meal palmitate. Absolute contents of exogenous (dietary) and endogenous TG and FFA were calculated from this derived percentage and the total concentration of TG and FFA in plasma. Values are means ± SE; n = 8. A: endogenous plasma TG; B: endogenous plasma FFA; C: exogenous FA in plasma TG; D: exogenous plasma FFA. *E and R trials significantly different from the C trial, P < 0.05. ^E trial significantly different from C and R trials, P < 0.05. Significant differences represent effects of trial in ANOVA. Main effects of time were also observed (P < 0.05) but no significant time-by-trial interactions.

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The reduction in postprandial TG is typically accompanied by other beneficial changes in lipid metabolism, such as an increase in Lox (21, 28, 61, 71, 72). The present results are consistent with previous studies that report that both endurance exercise (21, 26, 50, 72) and resistance exercise (49, 61) increase Lox after a previous bout of exercise. Factors determining postexercise Lox are not yet fully elucidated, but as discussed above for postprandial lipemia, the effects may be related to the relative proportion of the total body muscle mass that is recruited, as well as the degree of glycogen depletion. Exercise intensity seems to play little role in postexercise Lox (16, 26, 71), unless the intensity is quite high (70). Highly intense exercise or bouts of lower intensity but longer duration could cause a substantial net reduction in muscle glycogen content. Exercise-induced muscle glycogen depletion has been shown to activate glycogen synthase (GS) (10), and GS activation in muscle is associated with a higher relative contribution of Lox to EE (55). This may be through channeling of glucose toward storage so it competes less with FA as a substrate for oxidation. Under postabsorptive conditions, it appears that the negative energy balance, rather than the glycogen depletion, drives postexercise Lox (56), but it is possible that glycogen plays a bigger role under postprandial conditions when glycogen resynthesis is stimulated. The increased Lox after exercise may include intramuscular TG use (37), although by tracer methodology, we have shown that it also includes oxidation of fat from the postexercise meal. The ability to oxidize dietary FA is found to be negatively correlated with adiposity level in the postprandial state (75), and Lox is reduced in vitro in tissues from obese humans (31, 38) and in vivo in the postabsorptive state in human obesity (69), as well as in animal models in the postprandial state (7). Furthermore, an increased risk of weight gain is associated with an elevated RER (65, 81), indicating a potential detriment of low Lox. Our study confirms the ability of a prior bout of either E or R exercise to reduce postprandial RER and promote favorable changes in Lox. Given the health risks associated with obesity and obesity’s association with reduced Lox (31, 38, 62, 69), the results from this study suggest that obese individuals can use either E or R to increase total Lox. In addition to a clear role of negative energy balance in weight loss, we speculate that attaining a negative fat balance through increased Lox may be of additional benefit. However, since in the current study we did not track metabolism overnight following the exercise trials, we concede that the 24-h fat balance remains unknown. Previous work indicated that postexercise substrate selection compensates for that during exercise, such that the total impact on 24-h Lox is not different between different exercise intensities (64). In this work, participants were deliberately kept in energy balance by compensating for the EEE with increased energy intake (64). It may be that effects of exercise and differences between sessions with different intensities would be more readily apparent when participants remain in a negative energy balance or negative CHO balance. As discussed below, subsequent hunger is not appreciably altered after exercise (9, 30, 39, 40), so increasing energy intake to fully compensate for the EEE, although an interesting study maneuver, may not actually emulate the dietary response that would occur under ad libitum conditions. Therefore, we provided similar diets under each condition in the present study, and this approach likely facilitates postexercise Lox and possibly an effect of exercise on fat balance.

It has been suggested that the trafficking of dietary fat toward either storage or oxidation may play a role in the physiology of obesity (7). However, very limited data exist to date that describe the role of exercise on the subsequent partitioning of dietary fat (21, 74–76). The partitioning of dietary FA between oxidation and retention has been assessed, in which retention would simply include the sum total of all nonoxidative fates, such as incorporation into intramuscular, plasma, and adipose lipid pools. However, to our knowledge, relative contribution of dietary fat to plasma TG and FFA has not been investigated in the postexercise state. Previously, lean men performed endurance exercise the night before consuming a meal containing [1,1,1-13C]tripalmitin, and exercise (compared with a sedentary control) led to increased oxidation of dietary fat and reduced plasma TG concentration (21). Others have fed the isotopically labeled meal soon after exercise on the same day (74, 75). Exercise of various intensities was compared with a control condition, and significant enhancement of dietary and total Lox was observed after exercise, with only minimal effect of intensity (74) and no effect of the exact meal timing (76). These studies confirm the ability of E to significantly alter substrate oxidation in favor of dietary fat. Additionally, our current study was the first that we are aware of to compare E with R with a postprandial lipid tracer, and is the first, to our knowledge, to investigate an obese female population with this tracer approach after exercise. We report a significant increase in exogenously derived FA oxidation, irrespective of exercise modality compared with C. This exog-
enous FA oxidation after E confirms previous literature but expands the finding to a new population and adds recognition of R as an alternate means of deriving a similar outcome. Whereas other studies have not measured exogenous FA oxidation after R specifically, they have reported increased total Lox by IDC after R (49, 61), and our tracer findings show that exogenous fat contributes to this increase in Lox after exercise.

A prior exercise bout can reduce both VLDL and chylomicron TG concentrations (20, 21), but still, without assessing incorporation of a dietary FA tracer into the unfraccionted plasma TG pool, it is not yet definitively known if the reduction in postprandial lipemia was due to a reduction in both exogenously and endogenously derived FA in plasma TG. In the current study, we differentiated between exogenous and endogenous sources of plasma TG and FFA, and we discovered that the significant attenuation in total plasma TG came from endogenous TG only, not from meal-derived TG (Fig. 4). This could be a result of a reduction in hepatic VLDL-TG secretion, enhanced VLDL-TG clearance without enhanced chylomicron clearance, or increased relative contribution of recycled dietary FA from chylomicrons into VLDL. In support of this speculation, recently, it was reported that women exhibit reduced VLDL-TG secretion after a bout of endurance exercise in the postabsorptive state (6); it is possible that this physiological impact is also present in the postprandial state.

Rather than focus on specific lipoprotein particles per se, our questions were with regard to the role of the meal fat, and so we assessed whether the changes in plasma TG and FFA concentrations were because of altered abundance of the ingested fat in circulation. The inter-relationships between lipid pools dynamically change over time during meal absorption, so measuring lipid concentrations cannot sufficiently answer questions about the sources (dietary vs. endogenous). When investigators are interested in addressing contributions of exogenous and endogenous lipid to circulating TG, there may be a tendency to conceptualize the FA sources for TG content of lipid pools as solely exogenous for chylomicrons and solely endogenous for VLDL, but this is not entirely accurate. Furthermore, when plasma TG rises after a meal, it may be assumed by some scientists to be primarily the result of rising chylomicron levels, but this is also not accurate. During the course of absorbing an isotopically labeled meal, the IE of chylomicron TG changes over time (44, 63), possibly indicating impurity of the isolated fraction but also likely indicating changing relative contribution of meal fat and contribution of fat previously residing in intestinal cells. After a meal, plasma VLDL-TG can rise to a similar or even greater absolute extent than chylomicron-TG (20, 21), and FA from a meal rapidly label the VLDL pool, in addition the chylomicron pool (24, 44, 63), as chylomicron remnants, and dietary-derived plasma FFA can deliver FA to VLDL (5, 24, 25). As we were interested in the relative role of the actual meal fat in the TG and FFA excursions, we derived an approach and a simple calculation to compare the meal IE with IE of plasma TG and FFA, and this directly assessed the contribution of the meal to the plasma TG excursion and generated surprising and novel data about postprandial lipid metabolism after exercise.

Similar to previous research showing an elevation in total FFA concentration postexercise using E (21, 26, 50, 72), we have seen similar total FFA concentration increases after E but no change in total plasma FFA after R. Further analysis revealed a significant rise in exogenous FFA in both exercise groups but a significant rise in endogenous plasma FFA concentration after E only (Fig. 4). In the E trial, the significant rise in endogenous FFA concentration was likely a result of lipolysis and mobilization of FFA during the prior E bout, which is related to hormonal changes during exercise, such as increased catecholamines, growth hormone, and cortisol (26); removal of the most immediate postexercise time point eliminated the statistical significance for elevation of FFA concentration across the recovery period. The rise in plasma FFA of exogenous origin after either exercise bout indicates a potentially enhanced spillover of dietary FA, as exogenous FFA in plasma have been considered a surrogate marker for systemic dietary spillover (53, 54, 58). This process of spillover describes FA released by intravascular lipase activities (lipoprotein lipase and/or hepatic lipase) that were not channeled efficiently to direct uptake at the site of lipolysis and are thus released into venous circulation (53). In the process of spillover, FFA become systemically available and can ultimately be used by tissues after recirculation through the arterial vasculature. Although we consider the increased exogenously derived FFA concentration to be spillover, without arterial-venous sampling across tissues, this mechanism cannot be confirmed with absolute certainty; we note that an alternative explanation for this observation could be that dietary FA were briefly esterified intracellularly and then reliberated by lipolysis into the plasma FFA pool. Nonetheless, we observed the increased apparent postexercise spillover alongside increased oxidation of exogenous FA. It is possible that this process promoted oxidation by making labeled FA available to specific tissues that exhibit high rates of FA oxidation, such as the liver or others, and this increased FFA availability may also have increased nonoxidative disposal rates in specific tissues. Another major contributor to postexercise Lox could be partitioning of the endogenous lipid toward oxidation through changes in intracellular signaling related to glycogen concentration and cellular energy charge.

In each obese individual, positive fat balance has led to the storage of excess fat over time (23). Unless dietary compensation were to occur, enhancing the ability to oxidize lipid could help rectify this situation and could lead to a negative fat balance, which can ultimately lead to a reduction in body fat (23). Elevations in fat oxidation have been demonstrated in the current study after both E and R, with no significant differences between the exercise conditions. The increased Lox in conjunction with reductions in postprandial lipemia has implications for management of adiposity and of CVD risk. A critical question to ask is whether it would have been more appropriate to increase the size of the postexercise meals in the E and R trials compared with the C trial in the present study to prevent the negative energy balance resulting from exercise. Our goal was to mimic nonlaboratory conditions, and the common finding has been that an acute bout of exercise does not increase subsequent hunger or ad libitum energy intake throughout the rest of the day (8, 9, 11, 30, 39–43, 48). To describe this phenomenon, previously, the association between EE and energy intake has been described as being only a loose coupling (8). For this reason, we provided the same caloric content in the test meal for each condition rather than deliberately encouraging dietary compensation. As we focused on female subjects, we cannot comment on comparisons with an
obese male population, and such a comparison could be an important future direction to be explored. Our results provide obese women with more than one means of increasing Lox and attenuating the postprandial lipemic response.

In conclusion, we have demonstrated that a prior bout of either 60 min of E or R attenuated postprandial lipemia by reducing the circulating abundance of endogenously derived plasma TG and that prior exercise also increased both total FA and exogenous FA oxidation in obese women. Our findings indicate that when matched for duration, R provides an at least equivalent effect on postprandial lipemia and fat oxidation as E in obese women. These results may be distinct from those that would have been obtained if bouts were matched for EE. The results exemplify the complex relationship between lipid pools and the ability of lifestyle changes to alter these relationships. Additional preclinical studies comparing potential exercise prescriptions on postprandial lipemia and the trafficking of dietary fat toward oxidation are needed in various populations with both acute and chronic application of the exercise interventions.

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DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS


REFERENCES


