Published in final edited form as:

Med Sci Sports Exerc. 2013 October ; 45(10): 1897–1905. doi:10.1249/MSS.0b013e318294b225.

Prior exercise and postprandial incretin responses in lean and obese individuals

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Abstract

Purpose—The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) help regulate postprandial triacylglycerol (TAG) and insulin concentrations, but the effects of acute aerobic exercise on GLP-1 or GIP responses are unclear. The purpose of this study was to determine if reductions in postprandial TAG and insulin with exercise are associated with GLP-1 and GIP responses.

Methods—Thirteen normal-weight (NW) and 13 Obese (Ob) individuals participated in two, 4-d trials in random order including an exercise (EX) and a no exercise (NoEX) trial. Diet was controlled during both trials. The EX trial consisted of 1 h of treadmill walking (55–60% of $\text{VO}_{2\text{peak}}$) during the evening of day 3 of the trial, 12 h prior to a 4 h mixed meal test on day 4, during which frequent blood samples were taken to assess postprandial lipemia, glycemia, insulin, c-peptide, GIP, and GLP-1 responses. Insulin secretion was estimated using the Insulinogenic Index and insulin clearance was estimated using the ratio of insulin to c-peptide.

Results—Postprandial TAG's were 29% lower after EX in Ob individuals ($P<0.05$) but were not significantly altered in NW individuals ($P>0.05$). The drop in postprandial high-density lipoprotein cholesterol was attenuated with EX in Ob individuals ($P<0.05$). Insulin responses were 14% lower after EX in Ob individuals ($P<0.05$), and this was associated with reduced insulin secretion ($P<0.05$), with no change in insulin clearance ($P>0.05$). Glucose, c-peptide, GIP, and GLP-1 were not different between trials.

Conclusion—A 1 h bout of moderate intensity aerobic exercise the night prior to a mixed meal attenuates TAG and insulin responses in Ob, but not NW, individuals, an effect not associated with altered GLP-1 or GIP responses.

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CONFLICT OF INTEREST

We have no conflicts of interest to report.

We have no conflicts of interest to declare.

Keywords

Glucagon like peptide 1; glucose-dependent insulinitropic polypeptide; insulin; physical activity; obesity; lipemia

INTRODUCTION

Abnormally elevated postprandial triacylglycerol (TAG) (21) and insulin (16) concentrations increase the risk of cardiovascular disease as both are associated with endothelial dysfunction (23, 33), an early event in the atherosclerotic process. In addition, elevated postprandial TAG concentrations contribute indirectly to CVD by reducing anti-atherogenic high density lipoprotein cholesterol (HDL-C) concentrations (6). Hyperinsulinemia is indicative of reduced insulin sensitivity as insulin secretion from pancreatic β -cells increases to compensate for reduced insulin action in cells. Over time, this compensatory hyperinsulinemia as a result of reduced insulin sensitivity may lead to reduced β -cell function and ultimately type 2 diabetes. Therefore, therapies that reduce postprandial TAG and improve insulin sensitivity, with a subsequent lowering of insulin concentrations, are likely to aid in the prevention of type 2 diabetes and cardiovascular disease.

Aerobic exercise has beneficial effects on postprandial lipemia and insulinemia in normal-weight (NW) and obese (Ob) individuals. For instance, acute moderate intensity aerobic exercise (30–90 min) performed 12–18 h prior to an oral fat tolerance test (38, 40) or mixed meal test (3, 8, 34) reduces postprandial TAG and insulin concentrations, and this response is largely dependent upon the aerobic exercise-induced energy deficit as the response is abolished when the calories expended during exercise are replaced (3, 18). However, the mechanisms by which the acute aerobic exercise-induced energy deficit reduces postprandial TAG or insulin concentrations are not entirely understood.

Postprandial lipemia and glycemia are regulated by numerous tissues that interact with each other by secreting hormones into circulation. Insulin stimulates glucose uptake into skeletal muscle and adipose tissue (28), reduces hepatic glucose secretion (31), and increases TAG hydrolysis by increasing adipose tissue lipoprotein lipase activity (29). Insulin secretion is not only potentiated by blood glucose concentrations but also by several gut-derived peptides or incretins including glucose dependent insulinitropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1), and this phenomenon is known as the incretin effect. GIP is secreted from endocrine K-cells of the duodenum and jejunum of the small intestine in response to macronutrient ingestion, and this hormone potentiates pancreatic β -cell insulin secretion in a glucose dependent manner, inhibits glucagon secretion, and stimulates fatty acid synthesis and re-esterification in adipose tissue (4). Furthermore, postprandial GIP is elevated in some obese individuals (26) and has been shown to increase lipoprotein lipase activity in adipocytes (13), while GIP administration reduces postprandial TAG concentrations in a canine model (37). GLP-1 is secreted predominately from endocrine L-cells located in the ileum and colon of the intestine in a biphasic manner in response to nutrient ingestion, and its functions include inhibiting acid secretion, gastric emptying, and stimulates insulin release in a glucose-dependent fashion (14). Postprandial GLP-1 responses are attenuated in obese individuals (26), while intravenous administration of GLP-1 completely abolishes the rise in postprandial TAG and reduces postprandial glycemia by inhibiting gastric emptying and potentiating insulin release (17). The physiological roles of these incretin hormones have been established. Given that both GIP and GLP-1 play an important role in regulating postprandial TAG and insulin concentrations, it is plausible that

reductions in postprandial TAG and insulin by an acute aerobic exercise induced energy deficit are mediated by alterations in GIP and GLP-1 responses.

The response of postprandial GIP or GLP-1 after acute aerobic exercise has not been studied extensively. Two studies have examined the impact of acute exercise on the immediate post-exercise incretin response to glucose ingestion. In young trained athletes, the insulin and GIP response, but not the GLP-1 response, was attenuated in response to glucose ingestion after 2 h of aerobic exercise (2), while the GIP, but not insulin, response was attenuated in response to glucose ingestion after exhaustive aerobic exercise in young, untrained men (22). Only one study has examined the impact of aerobic exercise on the incretin effect in response to fat ingestion. In hypertriacylglycerolemic Ob men, 1 h of aerobic exercise performed 16 h prior to an oral fat tolerance test reduced postprandial TAG, but did not alter insulin, GLP-1, or GIP responses to an oral fat tolerance test (7). Taken together, it is unknown how a single bout of prior aerobic exercise (12–18 h before a meal) modulates postprandial incretin concentrations in response to a mixed meal containing protein, fat, and carbohydrate in NW or Ob individuals as prior studies have used a single nutrient in their test meals (either glucose or fat) and a single population (either NW or Ob). A mixed meal test allows for the assessment of the effect of prior aerobic exercise on incretin hormone responses under physiologically relevant situations and how these peptides associate with postprandial TAG and insulin concentrations. This test is advantageous versus an oral fat tolerance test as it is more similar to what most people in Western cultures typically eat.

The purpose of this study was to examine postprandial lipemia, glycemia, insulin, c-peptide, GIP, and GLP-1 in response to a mixed meal test 12 h after 60 min of moderate intensity (55–60% of $\text{VO}_{2\text{peak}}$) walking and physical inactivity in NW and Ob individuals to determine if alterations in incretin hormones are associated with changes in postprandial lipemia and insulinemia. We hypothesized prior aerobic exercise would reduce postprandial TAG and insulin concentrations in both NW and Ob individuals and that these alterations would be mediated, in part, via alterations in postprandial incretin hormone responses.

METHODS

Participants

The University of Missouri Health Sciences Institutional Review Board approved this study protocol and informed consent, and all participants provided written informed consent before participating and were screened to determine eligibility. The screening included assessment of height, weight, body composition via the BOD POD®, completion of a physical activity questionnaire, diet history questionnaire, medical history questionnaire, and VO_2 peak during a stress test. Inclusion criteria included individuals between 21–40 y of age, a body mass index (BMI) between 18.5–24.9 kg/m^2 (normal-weight) or 30–46 kg/m^2 (obese), weight stable over prior 3 months (< 2 kg weight gain or loss), not regular exercisers (< 2 d/wk of 30 min or more of planned exercise), no abnormalities found during stress test (determined by cardiologist), no prior history of heart, lung, kidney, endocrine, or gastrointestinal disease, and not taking any medications known to alter glucose or lipid metabolism. Women were not pregnant or lactating and were premenopausal.

Study Design

Each participant underwent a stress test and maximal oxygen consumption test as part of the screening. Qualified individuals performed two trials (each were 4 d in length) in random order including an exercise trial (EX) and a no exercise trial (NoEX). The trials were at least 7 d apart, and for women both trials occurred during the first week of the follicular phase of the menstrual cycle. During days 1–3 of both trials, the participants kept detailed dietary

records and consumed the same self selected foods and drinks and did not consume alcohol. During the EX trial the participants performed 1 h of treadmill walking between 1900–2100 h of the evening of day 3, 12 h prior to the mixed meal test in the morning (0700–0900 h) of day 4. During the NoEX trial the participants remained sedentary on the evening of day 3. During both trials, the participants fasted (water was allowed *ad libitum*) for 13 h prior to the mixed meal test on day 4, and no food was ingested after the exercise bout. During day 3 of both trials, the participants wore a pedometer to assess physical activity levels.

Body Composition

The manufacturer's guidelines for the BOD PODR were followed (COSMED USA, Concord, CA). Before body fat testing, all participants fasted for 3 h. All participants wore skintight clothing, a swim cap, and removed all jewelry and eyeglasses before testing. Lung volume was measured and body fat was estimated using the Siri equation from the computer program.

Exercise Testing and Free-living Physical Activity

The stress test was performed on a Quinton Q-Stress TM55 (Cardiac Science, Bothell, WA) motor driven treadmill. Simultaneously, this test was used to measure aerobic fitness. A twelve lead electrocardiograph (ECG) was used to assess the electrical activity of the heart before, during, and after exercise. Indirect calorimetry was used to measure oxygen consumption (ParvoMedics' TrueOne 2400 metabolic cart, Salt Lake City, Utah). The progressive exercise protocol started with a treadmill velocity of 2.5 mph at 0% grade. Every 2 min thereafter the speed was increased by 0.5 mph until 3.5 mph was reached, and then the grade was increased 2% every 2 min thereafter until volitional fatigue. The highest oxygen consumption value recorded was used as the participant's peak oxygen consumption (VO_2 peak).

The participants wore a pedometer (Walk4life, Inc, Oswego, IL) on their hip during day 3 of both trials. The pedometer was worn throughout the day until bed, including the acute exercise bout. For the acute exercise bout, the participants walked on a treadmill at an exercise intensity between 55–60% of their VO_2 peak. Oxygen consumption and carbon dioxide production was measured, via indirect calorimetry, every 15 s during the entire 1 h exercise bout. The speed and grade of the treadmill was adjusted throughout the exercise bout so that the oxygen consumption was within the target range.

Mixed Meal Test

The mixed meal was administered between 0700–0900 h following a 13 h overnight fast. The participants were given 15 min to eat the test meal, which was 600 calories and consisted of common breakfast foods including scrambled eggs, English muffin with margarine, and 10 fl oz of an artificially flavored fructose and glucose sweetened drink (fructose to glucose ratio 55 to 45 to mimic the common sweetener high fructose corn syrup-55). The percent of energy from each macronutrient in the meal was 45% carbohydrate, 40% fat, and 15% protein. We selected this meal composition instead of an oral fat tolerance test as it is more similar to what most people typically eat, thus more physiologically relevant.

Blood Collection and Analysis

Upon arrival for the mixed meal test a venous catheter was inserted into the antecubital vein of the forearm and venous blood samples were taken at baseline and 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, and 240 min after ingestion of the meal. Blood samples at each time point (~3 mL) were collected by syringes, and half of the blood (~1.5 ml) was

transferred immediately into serum separator tubes and was allowed to clot at room temperature while the other half was put into chilled EDTA tubes pretreated with dipeptidyl peptidase-4 inhibitor (DPP-IV, Millipore, Billerica, MA) to inhibit the inactivation and breakdown of GLP-1 and GIP (12), and immediately put into an ice bath. We added 15 µl of DPP-IV inhibitor to the 1.5 ml of blood put into DPP-IV treated tubes, as suggested by the manufacturer. At the time points 60, 120, 180, and 240 min, an additional 3 ml of blood was taken and added into EDTA tubes without dipeptidyl peptidase-4 inhibitor and immediately put into an ice bath. Within 30 min from taking the sample, the blood was separated by centrifugation at 3000 RPM for 15 min at 4°C and frozen at -80° C until analysis. Plasma hormone concentrations of c-peptide, GIP (total), GLP-1 (active), and insulin were determined from the same sample using a MILLIPLEX magnetic bead-based quantitative multiplex immunoassay with the MAGPIX instrumentation (Millipore, Billerica, MA). The intra-assay coefficients of variability (CV) were 5.6%, 5.1%, 5.8%, and 4.7% for c-peptide, GIP, GLP-1, and insulin, respectively. The inter-assay CV was 14.7%, 8.7%, 12.1%, and 12.8% for c-peptide, GIP, GLP-1, and insulin, respectively. To eliminate inter-assay variation, plasma glucose, TAG, HDL-C, and serum FFA were analyzed in the same run. Plasma glucose was determined using the glucose oxidase method (Sigma-Aldrich, St Louis, MO) and the intra-assay CV was 1.8%. Plasma TAG and total HDL-C concentrations were determined using colorimetric assays (Fischer Scientific, Houston, TX) and the intra-assay CV's were 1.5% and 1%, respectively. Serum non-esterified fatty acids were determined using a colorimetric assay (Wako Chemicals USA, Inc. Richmond, VA, USA) and the intra-assay CV was 2.5%. Only the baseline and 1, 2, 3 and 4 h samples were analyzed for TAG, HDL-C, and FFA concentrations. The quantitative insulin sensitivity check index (QUICKI) was used as a surrogate measure of insulin sensitivity (11).

Dietary Records

Participants kept detailed written dietary records with the exact timing of all meals, snacks, and beverages consumed during days 1–3 of each trial. To control for diet, copies of these records were given to the participant's and they were asked to precisely replicate these records during the subsequent trial. The macronutrient composition of each meal was determined by the Nutrition Data System for Research (NDSR, University of Minnesota, Minneapolis, MN).

Calculations

Postprandial TAG, HDL-C, FFA, glucose, and hormone responses between conditions were quantified and compared using the total and incremental area under the curve (AUC and *i*AUC, respectively), which were calculated using the trapezoidal method (25). In addition, for the glucose and hormone responses the peak, 0–30 min (calculated as 30 min – 0 min), the early phase AUC and *i*AUC (0–30 min), and the late phase AUC and *i*AUC (30–240 min) were determined. A modified insulinogenic index (total AUC_{c-peptide} / total AUC_{glucose}) was used to estimate glucose stimulated insulin secretion (30). For this calculation we used the total AUC instead of the *i*AUC because we had several negative numbers when we used the *i*AUC in the equation. In addition, we used c-peptide instead of insulin in the equation, as c-peptide is not affected by first-pass metabolism through the liver. Insulin clearance was estimated using the molar ratio of insulin to c-peptide and this was calculated as a percent ($[1 - (iAUC_{\text{insulin}}/iAUC_{\text{c-peptide}})] * 100$) (24).

Statistical Analysis

Statistical analyses were performed using the IBM SPSS statistical software version 20 (IBM Corporation, Armonk, NY, USA). Baseline characteristics and the metabolic assessments during exercise between NW and Ob groups were compared using an independent samples t-test. Daily caloric intake and step counts between each condition,

within each group, were compared using a paired samples t-test. A 2 (condition) by 2 (group) mixed model repeated measures ANOVA with follow up Bonferroni adjusted t-tests were used to test for statistically significant differences between fasting, peak, 0–30 min, AUC and *i*AUC, insulinogenic index, and insulin clearance values between conditions and groups. To determine if the meal significantly altered postprandial concentrations of TAG, glucose, insulin, GLP-1, GIP, FFA, and HDL-C, a 2 (group) $\times$ 2 (condition) $\times$ time ANOVA with repeated measures was employed. Statistical significance was set at $P = 0.05$.

RESULTS

Participants

Thirteen normal-weight (BMI = 18.5–24.9 kg/m², 7 male and 6 female) and thirteen obese (BMI = > 30 kg/m², 7 female, 6 male) individuals completed this study. The baseline characteristics of all participants are listed in Table 1. Daily caloric intake was not significantly different between days 1–3 of each trial, so the daily caloric intake for days 1–3 of each trial were pooled together and the mean caloric intake from all three days were used in the analysis. The mean daily caloric intake in the NW (EX: 1,828 $\pm$ 184 calories, NoEX: 1,912 $\pm$ 116 calories) and Ob (EX: 1,910 $\pm$ 144 calories, NoEX: 1,859 $\pm$ 99 calories) groups were not statistically different ($P > 0.05$) between conditions. Physical activity levels (step counts) were significantly greater on day 3 during the EX trial compared to the NoEX trial for both NW (EX: 12,269 $\pm$ 822 steps, NoEX: 6,538 $\pm$ 840 steps, $P < 0.05$) and Ob (EX: 13,256 $\pm$ 569 steps, NoEX: 4,971 $\pm$ 613 steps, $P < 0.05$).

Exercise

The mean speed and grade of the acute exercise bout was 3.47 $\pm$ 0.08 mph and 5.27 $\pm$ 0.47% grade in the NW group and 3.20 $\pm$ 0.07 mph and 3.69 $\pm$ 0.43% grade in the Ob group. The gross energy expenditure (calories), mean VO₂ (ml/kg/min), intensity (% of VO₂ peak), and mean heart rate during the 1 h exercise bout are listed in Table 2. The mean VO₂ (ml/kg/min) was significantly lower ($P < 0.05$) in Ob individuals compared to NW individuals, while all other variables were not significantly different between groups during exercise ($P > 0.05$).

Fasting and postprandial lipemia and glycemia

Figure 1 and Table 3 illustrate the fasting and postprandial lipemia responses. Fasting TAG concentrations were not different between trials or groups ($P > 0.05$). The meal significantly increased postprandial TAG concentrations (peak was 36 – 51% above baseline) within each group, within each condition ($P < 0.05$). The mean peak TAG concentrations were ~10% lower after EX in both groups, but this was not significant ($P > 0.05$). There was a trend ($P = 0.07$) for the total AUC for TAG to be greater in the Ob group, compared to the NW group, during the NoEX condition. The *i*AUC for TAG was 21% and 29% lower in NW and Ob individuals, respectively, after EX compared to NoEX, but this decline was only significant in Ob ($P < 0.05$).

Fasting HDL-C concentrations were not significantly different between conditions within each group ($P > 0.05$). Fasting HDL-C concentrations were significantly lower in the Ob group compared to the NW group, regardless of trial ($P = 0.04$). The meal significantly reduced postprandial HDL-C concentrations within each group, within each condition ($P < 0.05$). The total AUC did not significantly differ ($P > 0.05$) between conditions within each group or between groups. There were no significant differences ($P > 0.05$) between trials in the NW group but in the Ob group, the HDL-C *i*AUC declined less ($P < 0.01$) in the EX trial compared to NoEX.

The meal significantly reduced postprandial FFA concentrations ($P < 0.05$). Fasting or postprandial FFA concentrations were not significantly different ($P > 0.05$) between conditions within each group or between groups.

Figure 2 and Table 3 illustrates the glycemic responses. The meal significantly increased postprandial glucose concentrations within each group, within each condition ($P < 0.05$). Fasting, peak, 0–30 min, and total, early phase, or late phase AUC or *i*AUC glucose values were not different between trials or groups ($P > 0.05$).

Fasting and postprandial pancreatic hormone responses

Figure 2 and Table 3 illustrate the fasting and pancreatic hormone responses. Fasting c-peptide concentrations were significantly greater ($P < 0.01$) in Ob compared to NW, regardless of trial. The meal significantly increased postprandial c-peptide concentrations within each group, within each condition ($P < 0.05$). The c-peptide peak, 0–30 min, and total, early phase, or late phase AUC or *i*AUC were not statistically different ($P > 0.05$) between trials or groups, but there was a trend ($P = 0.07$) for the total AUC to be lower in the EX trial compared to the NoEX trial in the Ob group.

The meal significantly increased postprandial insulin concentrations within each group, within each condition ($P < 0.05$). Fasting, peak insulin, and 0–30 min were significantly greater ($P = 0.03$) in Ob compared to NW during the NoEX, but not during the EX trial ($P > 0.05$). The insulin AUC and *i*AUC were 14% lower in the EX trial compared to NoEX in Ob ($P < 0.05$), whereas insulin was not different between trials in the NW group ($P > 0.05$). In addition, the 0–30 min was 30% lower in the EX trial compared to NoEX in Ob individuals ($P < 0.05$). During the NoEX trial, the insulin AUC and *i*AUC were significantly greater ($P < 0.05$) in Ob individuals compared to NW individuals, while there was no significant difference ($P > 0.05$) between the groups during the EX trial.

Fasting and postprandial incretin hormone responses

Figure 3 and Table 3 illustrate the incretin hormone responses. Fasting GIP was significantly greater ($P = 0.03$) during the NoEX trial in the Ob group compared to the NoEX trial in the NW group. The meal significantly increased postprandial GIP concentrations within each group, within each condition ($P < 0.05$). Peak GIP concentrations were not different between groups or conditions. The 0–30 min and GIP total, early phase, and late phase AUC and *i*AUC were not significantly different between trials or groups ($P > 0.05$).

The meal significantly increased postprandial GLP-1 concentrations within each group, within each condition ($P < 0.05$). Fasting or peak GLP-1 responses were not significantly different between trials or groups. In addition, the GLP-1 0–30 min, total, early phase, and late phase AUC or *i*AUC were not different between trials or groups ($P > 0.05$).

Insulin Secretion and Clearance

The Insulinogenic Index was not different between trials in the NW group (EX: 205 ± 15 pmol/mmol, NoEX: 217 ± 16 pmol/mmol, $P > 0.05$). However, in the Ob group the insulinogenic index was significantly lower ($P < 0.05$) in the EX trial (234 ± 8 pmol/mmol) compared to the NoEX trial (255 ± 13 pmol/mmol), suggesting that EX decreased postprandial insulin secretion. Insulin clearance was not different between trials within either group ($P > 0.05$). Insulin clearance was significantly greater in NW compared to Ob during the EX trial ($P < 0.05$, NW: $80 \pm 1\%$, Ob: $73 \pm 3\%$) and NoEX trial ($P < 0.05$, NW: $79 \pm 2\%$, Ob: $70 \pm 3\%$).

Insulin Sensitivity

The QUICKI was not significantly different between trials in the NW group, but was significantly higher ($P < 0.05$) with EX in the Ob group (EX: 0.329 ± 0.005 , NoEX: 0.318 ± 0.006), indicating an improvement in insulin sensitivity. In addition, QUICKI was significantly ($P < 0.02$) lower in Ob compared to NW during the EX (NW: 0.350 ± 0.006 , Ob: 0.329 ± 0.005) and NoEX (NW: 0.339 ± 0.005 , Ob: 0.318 ± 0.006) trials.

DISCUSSION

Prior aerobic exercise, without energy replacement, reduces postprandial TAG and insulin concentrations, but the mechanisms are not entirely understood. The incretin hormones GIP and GLP-1 inhibit gut motility and gastric emptying in addition to potentiating postprandial pancreatic insulin secretion, thus these peptides play an important role in the regulation of postprandial lipemia and insulinemia. We hypothesized that in response to a mixed meal (600 calories) reductions in postprandial lipemia and insulinemia with prior exercise would be associated with alterations in postprandial GIP and GLP-1 responses. Contrary to our hypothesis, we have shown for the first time that reductions in postprandial TAG and insulin concentrations are not associated with altered GIP or GLP-1 concentrations in Ob individuals. These data suggest that reductions in postprandial TAG and insulin concentrations in Ob individuals may not be mediated by the incretin response to a moderate size meal of mixed composition.

In the Ob group, the postprandial TAG and insulin concentrations declined 29% and 14%, respectively, with the exercise intervention compared to no exercise. This is in agreement with other studies that report prior exercise reduces postprandial TAG and insulin responses in Ob individuals (3, 8, 38). However, the lack of a significant effect of exercise on postprandial TAG and insulin in the NW group does not match previous reports in which prior exercise significantly reduced postprandial TAG and insulin responses in lean individuals (34, 40). It is not completely clear why we did not find a significant effect of exercise on postprandial TAG or insulin in the lean group, but we speculate this finding may be due to differences in the test meals given and differences in the acute exercise bout. In the present study, the lean participants expended ~450 calories during exercise and consumed 600 calories as 26.6 g of fat, 67.5 g carbohydrate, and 22.5 g of protein in the test meal. In contrast, Zhang et al. (40) reported that brisk walking for 1 h at 60% $\text{VO}_{2\text{max}}$ (energy expenditure not reported) 12 h prior to a 980 calorie test meal (~92% fat) with 100 g fat, 17 g carbohydrate, and 3 g of protein significantly reduced the postprandial TAG response. Similarly, Tsetsonis and Hardman (34) reported that 1.5 h of moderate intensity treadmill walking (~63% $\text{VO}_{2\text{max}}$) resulting in an energy expenditure of ~1022 calories the day prior to a high fat meal (~67% fat, ~1,268 calories, ~94 g fat, ~92 g carbohydrate, and ~13 g protein) significantly reduced postprandial TAG and insulin responses in lean individuals. Thus, in the present study the smaller meal provided (with a lower amount of fat) caused a smaller increase in postprandial TAG and insulin response as reported in these earlier studies with an oral fat tolerance test. Thus, it is possible that prior exercise would have less of an impact in reducing postprandial TAG and insulin, which was evident in our lean, metabolically healthy subjects.

The concentration of TAG in circulation is ultimately determined by the balance between secretion into circulation and clearance out of circulation. In the present study, the reduced postprandial TAG concentrations with EX in the Ob group were likely mediated by increased clearance. Prior exercise increases whole body fat oxidation, which is mediated by increased skeletal muscle and potentially liver fatty acid oxidation, an effect that may facilitate an increase in glycogen resynthesis to replace diminished glycogen stores (8). This increase in fatty acid oxidation by an exercise-induced energy deficit may stimulate skeletal

muscle lipoprotein lipase (LPL) activity (10, 39), a water soluble enzyme located on the luminal surface of endothelial cells in skeletal muscle capillaries that functions to hydrolyze TAG in chylomicrons and very low-density lipoproteins (VLDL) into nonesterified fatty acids, which are subsequently taken up by the tissue (36). Further, prior exercise has been shown to increase blood flow to the exercised muscle and to the liver (9). Increased skeletal muscle blood flow is important for postprandial TAG clearance as this may increase skeletal muscle and liver capillary recruitment in previously non-flowing capillaries, thereby increasing capillary blood volume and allowing an opportunity for more widespread hydrolysis of circulating TAG (9). In addition, there is evidence that prior exercise increases VLDL₁-TAG particle size, an effect that may increase the affinity of these particles for LPL hydrolysis and thus clearance from the circulation (1). Much less work has examined the effect of exercise on postprandial TAG secretion. During the postprandial period plasma TAG's are secreted from the liver as VLDL-TAG and from the intestine as chylomicron TAG. One study, using an intralipid infusion, showed no change in hepatic VLDL-TAG secretion after acute moderate intensity aerobic exercise (1). To our knowledge, no study has directly examined if exercise reduces intestinal chylomicron TAG secretion. Research has shown that prior exercise does not alter gastric emptying (5), and data from the present study shows no change in GLP-1 concentrations, a hormone that alters gut motility and gastric emptying which could alter intestinal chylomicron TAG secretion.

In line with other studies, fasting HDL-C concentrations were lower in the Ob compared to the NW group (20). In the present study, acute aerobic exercise attenuated the decline from baseline (assessed via *i*AUC) in HDL-C concentrations in Ob, but not NW, individuals. Exercise may indirectly improve HDL-C by reducing TAG concentrations. Elevated TAG increase the exchange of cholesterol ester and TAG via cholesterol ester transfer protein between TAG and HDL-C (27), and TAG enriched HDL-C increases the clearance of HDL-C apolipoprotein AI, via hepatic lipase, from the circulation (15).

In the present study, the Ob group experienced improved insulin sensitivity with EX (assessed via QUICKI) and as a result, postprandial insulin concentrations were lower as less insulin was needed to maintain euglycemia. The improvement in insulin sensitivity most likely occurred in the skeletal muscles that were involved in the exercise bout and the liver as a result of reduced glycogen stores, and the increased insulin sensitivity serves to restore diminished glycogen stores in these tissues (19). Further, the decreased insulin concentrations may enhance lipid utilization by skeletal muscle and perhaps liver, thus sparing glucose. Our data suggests that exercise may reduce postprandial insulin concentrations via reduced pancreatic β cell insulin secretion (assessed using the Insulinogenic Index). Interestingly, the reduction in insulin secretion in the exercise trial occurred in the face of similar plasma glucose, FFA, GIP, and GLP-1 concentrations as compared to the no exercise trial. As insulin secretion is complex and not only modulated by blood glucose, FFA, and incretin hormones, but is also influenced by other hormones and the nervous system, it is possible that the insulin potentiating effects of glucose, GIP, and GLP-1 are masked by alterations in these other variables such that the β cell is less sensitive to the insulinotropic effects of glucose and the incretins, and thus secrete less insulin after exercise without modulation of glucose or incretin concentrations. In addition, these data show that insulin clearance was not altered with exercise, suggesting that reductions in postprandial insulin concentrations are primarily mediated by reduced insulin secretion. The lack of an effect of exercise on insulin clearance in the present study counters another study that demonstrated increased insulin clearance with exercise, assessed via a hyperinsulinemic clamp (35). One reason for the discrepancy in findings may be the dose of exercise used and methodology. We used a 1 h bout of moderate intensity walking and used the molar ratio of insulin to c-peptide to estimate insulin clearance, whereas Tuominen et al. (35) directly tested insulin clearance using a hyperinsulinemic clamp after a marathon run or 2 h of

treadmill exercise. A high dose of acute aerobic exercise or exercise training might be required to induce significant changes in insulin clearance.

For the first time, the present study demonstrates that 60 min of moderate intensity aerobic exercise performed the night prior to a mixed meal does not alter the postprandial responses of the incretin hormones GIP and GLP-1. Similarly, Dekker et al. (7) reported that 60 min of treadmill walking performed ~16 h prior to an oral fat tolerance test did not alter the postprandial responses of GIP or GLP-1. It may be that a greater exercise intensity, energy deficit, higher carbohydrate meal, and/or weight loss with exercise are required for changes in postprandial incretin responses to occur. For instance, 2 h of moderate intensity treadmill running in trained men (22) or treadmill exercise until exhaustion in untrained men (2) reduced the GIP response to glucose ingestion in the immediate post-exercise recovery period. In another study, a low-glycemic index diet and exercise intervention with weight loss suppressed the postprandial GIP response to an oral glucose tolerance test (32).

This study has some limitations that should be considered when interpreting the results. We utilized young, metabolically healthy NW and Ob individuals. Therefore, extrapolation of these findings to other populations with hypertriglyceridemia, metabolic syndrome, or type 2 diabetes is limited. Another limitation is that we found prior exercise in the Ob group resulted in rather small, albeit statistically significant, beneficial changes in postprandial TAG and HDL-C concentrations. This could be a type I statistical error. However, we utilized Bonferroni adjusted *post hoc* tests to control for type I statistical error. Although the changes in TAG and HDL-C concentrations are small, it is likely that small, beneficial changes can aid in the prevention of chronic disease.

In conclusion, 1 h of prior aerobic exercise performed the night prior to a mixed meal (~600 cal) improved postprandial TAG and insulin responses in Ob, but not NW, individuals. These responses were not associated with changes in the incretin hormones GIP or GLP-1. Mechanisms other than modulation of the incretin hormones may mediate reduced postprandial TAG and insulin with prior aerobic exercise in Ob individuals.

Acknowledgments

This study was funded by a University of Missouri Research Council Grant (JAK) and University of Missouri Institute for Clinical and Translational Sciences Pilot Grant (TDH).

This study was funded by a MU Research Council Grant (J.A.K.) and a MU Institute for Clinical and Translational Science Pilot Grant (T.D.H.). The results of the present study do not constitute endorsement by ACSM.

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Figure 1.

Fasting and postprandial lipemia

TAG = Triacylglycerol

HDL-C = High-density lipoprotein cholesterol

EX = Exercise

NoEX = No Exercise

^aSignificantly different compared to NoEX trial within obese group.Values are means $\pm$ standard error of the mean.

Figure 2.

Fasting and postprandial glycemia and pancreatic hormone responses

EX = Exercise

NoEX = No Exercise

^aSignificantly greater compared to EX trial within obese group.

^bSignificantly greater compared to NoEX trial in normal-weight group.

Values are means $\pm$ standard error of the mean.

Figure 3.
Fasting and postprandial incretin hormone responses
EX = Exercise
NoEX = No Exercise
Values are means $\pm$ standard error of the mean.

Table 1

Participant Characteristics

	Normal-weight (n = 13)	Obese (n = 13)	P value
Age (y)	26.0 ± 2	25.4 ± 1	0.79
Height (m)	1.8 ± 3	1.7 ± 4	0.65
Weight (kg)	71.4 ± 3	104.9 ± 7	< 0.0001
Body mass index (kg/m ²)	23.0 ± 0.5	34.6 ± 1	< 0.0001
Body fat %	24.6 ± 3	40.0 ± 2	< 0.0001
Systolic (mm Hg)	107 ± 2	119 ± 4	0.01
Diastolic (mm Hg)	75 ± 4	80 ± 3	0.35
Waist (cm)	81.8 ± 3	104.2 ± 5	0.0014
Hip (cm)	97 ± 1	118.3 ± 2	< 0.0001

Values are means ± standard error of the mean

Normal-weight group: 7 men / 6 women; Obese group: 6 men / 7 women.

Table 2

Energy expenditure, oxygen consumption, and heart rate during acute exercise bout

	Normal-weight (n = 13)	Obese (n = 13)	P value
Calories Expended	448 ± 28	534 ± 40	0.09
VO ₂ (ml/kg/min)	21.1 ± 1	16.9 ± 0.54	0.0004
Intensity (VO ₂ as %Peak)	56 ± 2	57 ± 1	0.62
HR (bpm)	142 ± 5	143 ± 4	0.86

Values are means ± standard error of the mean

Table 3

Total area under the curve responses for all variables

	Normal-weight (n = 13)		Obese (n = 13)	
	EX	NoEX	EX	NoEX
Lipids and Glucose (mmol/L per 4 h)				
TAG	4.1 ± 0.3	4.4 ± 0.3	5.5 ± 0.7	6.0 ± 0.8
HDL-C	5.5 ± 0.2	5.5 ± 0.2	4.7 ± 0.2	4.7 ± 0.8
FFA	1.01 ± 0.07	1.00 ± 0.09	1.03 ± 0.09	0.93 ± 0.06
Glucose	1236 ± 30	1200 ± 26	1250 ± 21	1273 ± 34
Pancreatic Hormones (pmol/L per 4 h)				
Insulin	43,867 ± 3,832	46,344 ± 4200	58,734 ± 6,360 ^a	68,278 ± 5,804 ^b
c-Peptide	253,090 ± 19,103	262,293 ± 21,558	292,440 ± 12,985	325,035 ± 20,277
Incretin Hormones (pg/ml per 4 h)				
GLP-1	10,621 ± 1,078	10,237 ± 1,125	11,595 ± 1,291	10,499 ± 1,264
GIP	21,085 ± 1,792	21,637 ± 1,497	23,910 ± 3,032	25,130 ± 3,016

Values are means ± standard error of the mean

^a = P < 0.05 compared to NoEX trial within group.^b = P < 0.05 compared to NoEX trial in normal-weight group.