ABSTRACT

The Effect of Exercise Intensity on Postprandial Blood Lipids

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The purpose of the present investigation was to determine the effects of exercise intensity on the blood lipid response to a high-fat test meal in sedentary, overweight men, and to determine the contribution of excess post-exercise oxygen consumption (EPOC) to changes in postprandial lipemia. Seven men (Age = 43 $\pm$ 10 years; BMI = 31.8 $\pm$ 4.5 kg/m^2; Waist = 107.2 $\pm$ 14.9 cm; and VO_2peak = 31.7 $\pm$ 7.5 ml/kg/min) participated in 4 experimental conditions: control, low-intensity (LI = 40-50% of VO_2 reserve), high-intensity (HI = 70-80% of VO_2 reserve), and HI exercise plus EPOC re-feeding (HI + EERM) where the difference in EPOC following LI and HI was re-fed in the form of a meal bar (Peanut Butter PowerBar ®). Exercise sessions were isocaloric (500 calories) and completed in the morning after a 12-hour overnight fast. Blood samples were taken before and after exercise, immediately before, and 2, 4, and 6 hours after a high-fat test meal (1010 calories, 100 g fat, 99 g saturated fat, 17 g carbohydrate, 3 g protein). Serum samples were measured for triglycerides (TG), lipoprotein cholesterol, non-esterified fatty acids (NEFA), glucose and insulin and were analyzed using 2-way repeated-

measures ANOVAs. Repeated measures ANOVAs were used to examine triglyceride and insulin total (AUC_T) and incremental (AUC_I) areas under the curve. Comparisonwise significance was set at $p < 0.05$. Compared to control, TG were lower at 4 hours after both exercise intensities and remained lower at 6 hours after LI only. LI and HI significantly reduced postprandial triglyceride AUC_I by 31 and 27%. AUC_I and AUC_T were similar between exercise intensities and changes in other dependent variables were of similar magnitude and direction in all conditions. The lower postprandial TG observed after exercise was not diminished in the HI+EERM trial. The results of this study indicate that similar significant reductions in postprandial triglycerides occur with LI and HI exercise with and without replacement of EPOC calories. In sedentary overweight men, EPOC does not contribute substantially to energy expenditure or reductions in postprandial lipemia.

The Effect of Exercise Intensity on Postprandial Blood Lipids

by

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A Dissertation

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CHAPTER ONE

Introduction

Postprandial Lipemia and Metabolic Syndrome

Postprandial lipemia is an exaggerated elevation of non-fasting triglycerides (1). Since multiple meals are ingested in the course of a 24 hour period, those who exhibit postprandial lipemia are exposed to elevated triglycerides over large portion of the day (2). Postprandial hypertriglyceridemia has been established as an independent risk factor for cardiovascular disease (CVD), and is a stronger predictor of disease risk than fasting triglycerides (3-4). In a large prospective study, elevated non-fasting triglycerides were predictive of myocardial infarction, ischemic heart disease, and death in the general population (5). Postprandial lipemia has been observed in metabolic syndrome (MetS), coronary heart disease (CHD) and hypertension, and is associated with obesity and visceral fat accumulation (6-9).

The increased risk for CVD observed in association with postprandial lipemia largely results from prolonged exposure to triglyceride-rich lipoprotein remnants that are capable of infiltrating the arterial wall (10). In addition, blood triglycerides are directly and inversely related to high-density lipoprotein cholesterol (HDLC), and are a surrogate measure of non-HDLC (NHDLC) (1). Elevated plasma triglycerides are associated with atherogenic lipid abnormalities including an elevated number of small, dense LDLC particles, increased oxidized LDLC, and a reduced level of HDLC (11-12). Because of the atherogenic potential of high triglycerides, and due to the fact that the majority of the

day is spent in the postprandial state, it has been suggested that measuring non-fasting triglycerides would be a more clinically useful tool than the traditionally measured fasting values (1).

MetS currently affects 27% of adults in the United States and is defined as a clustering of atherogenic risk factors (13-14). These may include abdominal obesity, hypertriglyceridemia, low HDLC, elevated blood pressure, and elevated fasting glucose (15). Central to the development of MetS are abdominal obesity and insulin resistance (16). It is commonly recognized that these 2 features may induce the secondary dyslipidemia observed in MetS. Increased adipose tissue lipolysis, observed with abdominal obesity and in insulin resistant states, results in elevated plasma non-esterified fatty acid (NEFA) concentrations (17-18). Chronically elevated plasma NEFA concentrations favor fatty acid flux into muscle, and due to the absence of a concurrent increase in fatty acid oxidation, encourage the accumulation of intramuscular triglycerides (IMTG) (19). This promotes the accumulation of diacylglycerols (DAG) and ceramides, byproducts of incomplete fatty acid metabolism, which have been shown to inhibit insulin signaling and are increased in obese insulin resistant subjects (20-21). Insulin resistance contributes to liver production of VLDL, increasing the number of circulating atherogenic lipoproteins (22). Men with MetS exhibit significantly greater elevations in blood triglycerides following a high-fat meal when compared to healthy men without MetS (7). Strategies that reduce insulin resistance and increase substrate oxidation may transiently ameliorate metabolic dysfunction in those with MetS.

Aerobic Exercise and Postprandial Lipemia

The role of aerobic exercise in attenuating postprandial lipemia is well established (23-25). Aerobic exercise performed 1 to 16 hours before the ingestion of a high-fat meal has been shown to lower postprandial triglycerides significantly, with reductions of 18 to 51% (26-29).

Gill et al (30) was the first to suggest that the attenuation in postprandial triglycerides following acute exercise is directly related to the energy expenditure of the prior session. Support for this hypothesis arises from studies demonstrating that exercise of greater caloric expenditure, either by increased intensity or duration, enhances the reduction in postprandial triglycerides. Gill et al (31) have shown that the postprandial triglyceride and insulin reduction following exercise at 50% of VO_2max for 2 hours is greater than that observed after exercise at the same intensity performed for 1 hour. Ninety minutes of treadmill walking at 61% of maximal capacity results in a significantly lower postprandial triglyceride response when compared to 90 minutes at 31% (32). Tsetsonis and Hardman (33) have demonstrated that isocaloric exercise at intensities of 32 and 63% of VO_2max significantly reduce postprandial triglycerides to a similar extent when compared to a non-exercise control. In men with MetS, isocaloric low-intensity and moderate-intensity exercise (e.g. 35-45% of VO_2peak and 60-70% of VO_2peak) performed on the evening before a high-fat meal results in a significantly reduced triglyceride response when compared to a non-exercise control and accumulated moderate-intensity exercise, with no difference between the 2 conditions (34). In a similar group of subjects, exercise at 60% of VO_2peak for 45 and 60 minutes reduced postprandial lipemia to a significantly greater extent than did exercise for 30 minutes

(35). Therefore, exercise of sufficient energy expenditure is effective at reducing postprandial lipemia, despite differences in intensity and duration.

Replacing the energy that was expended during exercise lessens the positive effect of exercise on postprandial triglyceride concentration, but does not completely account for the reductions (36-37). In obese men moderate-intensity exercise at 50% of VO_2max without energy replacement results in significantly lower postprandial triglyceride and insulin responses, and significantly higher postprandial fat oxidation when compared to both a non-exercise control and an exercise session where the energy expended during exercise was replaced (36). When compared to non-exercise control conditions, maximal exercise has also been shown to significantly decrease triglycerides postprandially if the energy expended during exercise is not replaced following the sessions (37-38). The significant reduction in postprandial triglycerides following exercise without energy replacement ranges from 10 to 12% when compared to exercise sessions where the energy that was expended is replaced, and may be as great as 40% when compared to non-exercise control (36-38). Thus triglycerides are consistently reduced following exercise when the energy expended during the session is not replaced.

Further evidence for the effect of exercise energy expenditure on postprandial lipemia is provided from studies that have compared exercise energy expenditure and dietary energy restriction. When dietary energy restriction is compared to exercise of equal energy expenditure, exercise is effective at significantly lowering postprandial triglycerides below both control and dietary energy restriction (39). Dietary restriction, dietary restriction combined with exercise energy expenditure, and exercise energy expenditure that are equal in caloric requirement lower fasting and postprandial

triglycerides significantly below control (40). However, energy expenditure achieved through exercise produces significantly lower postprandial triglycerides than dietary restriction and exercise combined (40). These results indicate that the reduction in postprandial triglycerides is specifically related to the energy expenditure of exercise, and cannot be induced to the same extent by dietary restriction.

The underlying physiological mechanisms responsible for the consistent reduction in postprandial triglycerides following aerobic exercise have not been fully described, however are partially attributable to increased triglyceride clearance facilitated by enhanced lipoprotein lipase (LPL) activity (41-42). Increases in skeletal muscle LPL activity have been seen in as little as 4 and up to 24 and 48 hours following exercise at 70-75% of maximum capacity (43-44). Altered metabolism in skeletal muscle and liver due to substrate deficits created by exercise, decreased hepatic VLDL output, and/or increased skeletal muscle blood flow are likely to contribute to decrements in postprandial triglycerides (37,45-48).

Moderate-intensity exercise is effective at reducing insulin concentration in the postprandial period significantly below non-exercise control conditions. Exercise at 60% of VO_2max 12 hours before an oral fat tolerance test results in significantly lower postprandial insulin concentration when compared to non-exercise control (24,39). LPL activity in skeletal muscle is decreased under conditions of elevated insulin concentration, and therefore lower insulin levels following exercise may allow for enhanced LPL activity and subsequent triglyceride hydrolysis (43).

Insulin action is improved for 1 to 3 days following exercise and acute exercise increases glucose uptake in the absence of insulin (49). Evidence suggests that

contraction and stretch mediated mechanisms that involve changes in intracellular calcium, AMPK, and p38 MAPK contribute to increased GLUT 4 translocation or protein turnover (50-54). Increased hepatic glucose delivery in the presence of impaired insulin action results in enhanced VLDL assembly by the liver, and subsequently may elevate circulating blood triglycerides (55). Acutely reducing glucose levels through exercise in men with MetS who are likely insulin resistant may attenuate liver VLDL production, and, as a result, lessen postprandial lipemia.

Post-exercise Oxygen Consumption and High-intensity Exercise

The benefits of exercise that incorporate a mix of high-intensity (e.g. 90% of maximum capacity) and low-intensity intervals versus continuous moderate-intensity exercise include improvements in insulin sensitivity, flow-mediated-dilation, blood pressure, and aerobic capacity (56-59). The efficacy of using high-intensity aerobic interval training in persons with cardiometabolic disease has been demonstrated. VO_2peak is improved to a greater extent than that observed with continuous exercise training in patients with coronary artery disease (CAD), heart failure (HF) and MetS (56-57,60). Additionally, in patients with MetS aerobic interval training at 90% of the highest measured heart rate has been shown to have superior effects on endothelial function and insulin sensitivity when compared to isovolumetric continuous exercise (56). Katsanos et al (61) have shown that exercise at 65% of maximal capacity performed one hour before a high-fat meal significantly reduces postprandial triglycerides and insulin when compared to low-intensity exercise at 25% of maximal capacity. This work supports the contention that high-intensity exercise may confer additional beneficial effects toward lowering postprandial lipemia.

During high-intensity exercise the utilization of IMTG is significantly greater when compared to low-intensity exercise. Acute exercise at 65 and 85% of VO_2max utilizes IMTG stores to a greater extent than does exercise at 25% of VO_2max, and exercise prior to a lipid infusion creates a metabolic environment that favors IMTG storage with concomitant improvements in insulin sensitivity (62-63). In MetS the accumulation of IMTG and lipid byproducts is pathological largely due to an imbalance between IMTG content and oxidative capacity (19). Therefore, the increased oxidation of IMTG during exercise may be beneficial for men with MetS. Furthermore, the contribution of carbohydrate to energy production increases with exercise intensity and glycogen utilization is higher at high-intensity exercise (62,64-65). The effects of glycogen depletion on insulin action are favorable, with increases in insulin sensitivity persisting under conditions of glycogen depletion (66-67). The greater contribution of glycogen to energy metabolism during high-intensity exercise favors improvements in insulin action on the day following an exercise session, and thus may be beneficial for men with MetS. Despite potential differences in substrate utilization between high- and low-intensity exercise, the factor that most greatly influences postprandial lipemia appears to be energy expenditure (31-34).

The metabolic rate remains elevated following aerobic exercise and is measured as the excess post-exercise oxygen consumption (EPOC) (68). Causes for increased EPOC following exercise include the energy costs of clearing lactate, restoring heart rate, respiratory muscles, stabilizing active musculature to a resting state, replenishing intracellular substrates, repairing tissue damage, and mobilizing fatty acids (68-70). Elevation in oxygen consumption, and thus caloric expenditure, is greater following high-

intensity exercise when compared to moderate- or low-intensity exercise (68-69). Exercise for 80 minutes at 29, 50, or 75% of VO_2max results in increased O_2 consumption for 0.3, 3.3, and 10.1 hours following exercise (71). Measuring EPOC after high-intensity exercise provides an index of the additional substrate deficit created by exercise when compared to exercise sessions of similar caloric expenditure but low-intensity. High-intensity exercise may have a greater impact on reducing postprandial triglycerides than low-intensity exercise due to increased post-exercise energy expenditure.

Purpose

The purpose of this investigation was to determine the effects of low-intensity and high-intensity exercise of equal caloric expenditure on the postprandial blood lipid response to a high-fat test meal in men with MetS. In addition, we aimed to determine if differences in the postprandial lipemic response could be explained by a greater post-exercise energy expenditure following high-intensity exercise.

Hypotheses

It was hypothesized that the postprandial triglyceride response following high-intensity exercise would be decreased to a significantly greater extent when compared to low-intensity exercise of equal energy expenditure. It was hypothesized that the greater reduction in postprandial lipemia following high- vs. low-intensity exercise would be negated by replacing the difference in post-exercise energy expenditure between these two conditions.

Questions

1. What are the effects of low- and high-intensity exercise on the postprandial blood lipid response to a high-fat meal in men with MetS?
2. If a significant difference in postprandial blood lipids exists following low- and high-intensity exercise, can this be explained by a greater energy expenditure following high-intensity exercise?

Rationale

Exercise of sufficient volume, completed over a wide range of intensities, is effective at reducing postprandial triglycerides. The magnitude of triglyceride reduction is partially dependent on the energy expenditure of the exercise session. To our knowledge no studies have directly compared isocaloric sessions of low- and high-intensity exercise in order to assess differences in postprandial lipemia in men with MetS. Because high-intensity exercise is linked to increased post-exercise energy expenditure, this is a potential explanation for the proposed additional benefits of high-intensity exercise on postprandial lipemia. In studies of postprandial lipemia where the energy deficit created by exercise has been replaced, authors have administered either mixed or carbohydrate meals that are equal to or slightly above the caloric expenditure of the session, but have not measured EPOC to determine the exact contribution to energy expenditure or its relationship to blood lipid concentration in the postprandial period (36-37).

Assumptions, Limitations & Delimitations

Assumptions

1. Young and middle-aged men with MetS exhibiting secondary dyslipidemia, recruited from the Waco area, displayed the population response to low- and high-intensity exercise and to an oral fat tolerance test.
2. Study participants complied with the research protocol.

Limitations

1. Participants were recruited from Waco, Texas and surrounding areas only.
2. Self-report measures were used for the quantification of dietary and physical activity habits, factors that are known to influence blood lipids.
3. Women were not included in the study sample due to potential differences between genders in the response to exercise and postprandial lipid metabolism.
4. Indirect calorimetry was used to estimate exercise and resting energy expenditure.
5. Volunteers were lactose tolerant.

Delimitations

1. Participants were apparently healthy, with no known cardiometabolic or pulmonary diseases. They were not taking glucose or lipid altering medications, and had no physical conditions that would preclude them from exercise.
2. Only men with MetS between the ages of 25 and 55 were recruited for this study.
3. Physically inactive men with a BMI above 25 kg/m^2 were examined.

4. Aerobic exercise at 40-50 and 70-80% of HRR was used to expend 500 calories.

5. The period of measurement for EPOC was confined to 2 hours.

6. The energy replacement meal contained protein, fat, and carbohydrate, and was given immediately after exercise.

7. The test meal was high-fat and consist of primarily saturated fatty acids from milk (29,34,72).

Significance of the Study

Men with MetS possess an elevated risk for cardiovascular disease and are frequently exposed to postprandial lipemia. Exercise of differing intensities can be undertaken to lower postprandial triglycerides and to reduce CVD risk. High-intensity exercise is known to improve exercise tolerance and cardiorespiratory fitness beyond continuous moderate-intensity exercise, lowering CVD mortality risk (59). The acute benefits of performing high-intensity exercise include improved blood pressure and flow mediated dilation. High-intensity exercise in those who are not physically fit and exhibit heightened cardiometabolic risk is gaining interest among clinicians and exercise professionals; however with respect to its effects on dyslipidemia, has remained largely unexplored.

The energy expenditure of the exercise session has been linked to the reduction in postprandial triglycerides, and due to the observation that high-intensity exercise produces greater post-exercise energy expenditure than low-intensity exercise, this study will be undertaken to determine the effects of high-intensity exercise with and without energy replacement on postprandial lipemia. Exercise is a therapeutic and inexpensive

means for persons at high risk for cardiovascular disease to reduce exaggerated postprandial triglyceride responses. Gaining further understanding in the specific metabolic benefits of high-intensity exercise will allow for optimal exercise interventions for at-risk individuals.

CHAPTER TWO

Review of Literature

Introduction

In 2008, CVD accounted for 33% of deaths in America, and remained the leading cause of mortality among men and women (73). In middle-aged overweight adults, following adjustment for traditional risk factors, non-fasting triglycerides remain a significant predictor of CVD, while fasting triglycerides do not (3-4). Exposure of the vascular endothelium to triglyceride-rich lipoprotein (TRL) particles and their remnants promotes atherosclerosis and is fundamental in the etiology of CVD (10,74-75). For certain individuals, including those with MetS, hypertension, CVD and type 2 diabetes, non-fasting triglycerides remain elevated above healthy control values for 6 to 8 hours following a meal, increasing exposure of the vasculature to atherogenic particles (7-8,76). A recent expert panel statement indicated that a desirable postprandial triglyceride response to a high-fat meal is no higher than 220 mg/dl (2). Based on these guidelines, even otherwise-healthy overweight control subjects may display elevated postprandial lipemia in response to a high-fat meal (4). Because non-fasting triglycerides are not typically measured in the general population, CVD risk for many individuals who are exposed to postprandial lipemia may be underestimated. Aerobic exercise serves as a non-pharmacologic therapeutic intervention that lessens postprandial lipemia, and may substantially reduce cardiovascular disease risk.

Metabolic Syndrome

The combination of increased dietary intake, sub-optimal physical activity, and increased overweight and obesity has placed Americans at an elevated risk for the development of metabolic diseases. Approximately 27% of the population met the criteria for MetS in 2000, this percentage significantly increased from 23% in 1994 (14). Men with MetS display increased postprandial lipemia when compared to healthy controls and are at elevated risk for the development of CVD, Type 2 Diabetes and Stroke (7,77). Multiple organizations provide differing criteria for the identification of MetS (16,78). Despite these differences, abdominal obesity and insulin resistance are key features thought to underlie MetS, and subsequently CVD risk (16). Diagnosis of the condition is useful for identifying those with multiple risk factors for CVD, but does not identify all subjects who are insulin resistant. Salazar, et., al. (79) have shown that 60% of men not meeting the criteria for MetS were insulin resistant, defined as those in the top 25% of fasting plasma insulin concentration. In the same group of subjects, insulin resistant participants who did not have diagnosable MetS had a significantly higher BMI, e.g. 27 vs. 24.9 kg/m^2 than non-insulin resistant subjects (79). Other reports have shown that even normal weight individuals may be insulin resistant (80). Thus, overweight and normal weight subjects without diagnosable MetS may be insulin resistant and, as a result, be prone to secondary dyslipidemia.

Dyslipidemia

Dyslipidemia is a common feature of atherosclerotic diseases including MetS and can be characterized by elevated triglycerides, an increased number of small, dense LDLC particles, and low HDLC (11-12). Elevations in triglyceride observed with insulin

resistance and MetS are associated with increases in VLDL particle size and number (22,81-83). The large, triglyceride-rich VLDL produced under conditions of insulin resistance serve as precursors to atherogenic small, dense LDLC particles that are slowly degraded (84). The combined effects of elevated plasma triglycerides in conjunction with increased activities of hepatic triglyceride lipase (HTGL) and cholesterol ester transfer protein (CETP) result in small, dense HDLC particles that are rapidly cleared from the circulation, and atherogenic small, dense LDLC (84-87).

A large body of evidence confirms that elevated triglycerides and reduced HDLC are significantly associated with CVD risk (15,88-89) Meta-analysis has shown that, in men, the relative risk (RR) for CVD associated with a 1 mmol/l increase in triglycerides is significant at 32% (88). Upon accounting for HDLC and other CVD risk factors, the RR was reduced to 14%, yet remained statistically significant (88). The anti-atherogenic properties of HDLC have primarily been associated with reverse cholesterol transport, and antioxidant and anti-inflammatory functions (90). In men, the CHD risk reduction associated with a 1 mg/dl increase in HDLC is approximately 2% (89).

It has been proposed that the independent effect of elevated triglycerides in promoting CVD may be attributable to the presence of triglyceride-rich lipoprotein remnants (TRL) (10,74). Lipoprotein remnants of VLDL and chylomicrons are formed as a result of the activity of LPL and increased triglyceride levels (10,86). These remnants are rich in cholesterol ester and can be deposited in the arterial wall. Triglyceride level and remnant lipoproteins are strongly and significantly associated, and are predictive of future CVD development (91). Remnant-like cholesterol is strongly and significantly

associated with carotid intima-meida thickness in healthy middle-aged men, this independent of triglyceride level (92).

Postprandial Lipemia

A recent panel statement clarified the usefulness of non-fasting triglycerides in the prediction of CVD by reviewing large-scale trials that included postprandial measurements (2). The results from this review indicate that the peak triglyceride response can be observed 4 hours postprandially and that this response should be less than 220 mg/dl (2). TRL, including chylomicrons and VLDL, are increased acutely following a meal (93). Chylomicrons contain predominantly apo B 48, and are secreted from the intestine postprandially, while apo B 100 containing VLDL particles are hepatically derived (94).

In those with normotriglyceridemia and hypertriglyceridemia (e.g. mean triglyceride values of 93 and 244 mg/dl, respectively) the postprandial increase in both chylomicrons and large VLDL is statistically significant (95). In the same groups, chylomicrons were increased significantly following a high-fat meal, and to a significantly greater extent in those with elevated fasting triglycerides (95). In healthy controls and in CAD patients with normal and elevated fasting triglycerides, there is a statistically significant increase in large chylomicrons and VLDL 3 hours following a high-fat meal (96). When compared to a control group, CAD patients with hypertriglyceridemia have significantly increased large chylomicrons and VLDL at 3 and 6 hours postprandially (96). Sedentary middle-aged men with a mean BMI of 25.7 kg/m^{-2}, and with fasting triglycerides below 221 mg/dl, display a significantly higher triglyceride AUC_I when compared to younger sedentary men with a mean BMI of 23

kg/m^{-2} (97). In apparently healthy control subjects with a BMI above 25.0, postprandial lipid excursions have been shown to exceed the recommended cut-point of 220 mg/dl (4). Patsch, et.al., (4) have shown that healthy control participants with normal fasting triglycerides have elevated postprandial triglyceride responses of 263 and 225 mg/dl, respectively, at 4 and 6 hours. Although these values were significantly lower than a group with CVD, they are above the recommended cut-point suggested in the latest panel statement. It can be concluded that significant postprandial increases in lipoproteins are observed even in healthy weight subjects with normal triglycerides, and these increases are more pronounced in the middle aged, and in those with hypertriglyceridemia and heart disease.

Factors Influencing Metabolic Dyslipidemia

Altered activities cholesterol ester transfer protein (CETP), hepatic triglyceride lipase (HTGL), and LPL may contribute to secondary dyslipidemia by altering the composition of lipoproteins and/or affecting their clearance rate (86-87). In obese subjects and in men with Met S, the mass of CETP is increased above healthy controls, and CETP activity has been shown to be increased following a meal (85,98-99). In men, BMI is strongly and significantly associated with increased HTGL activity, and LPL activity is significantly reduced in obese subjects (100).

Cholesterol Ester Transfer Protein

CETP is responsible for the transfer of cholesterol ester and triglyceride between HDLC and lipids containing apoprotein B (apoB) particles including VLDL, LDLC, chylomicrons, and intermediate density lipoprotein (IDL) (85-86). The results of

increased CETP activity and hypertriglyceridemia are HDLC enriched with triglyceride and apo B particles that are enriched with cholesterol ester (101). Among Apo B containing particles, LDLC is responsible for accepting the preponderance of cholesterol ester (101). Due, in part, to the action of CETP, the resulting triglyceride rich HDLC are cleared more rapidly from the circulation, resulting in low HDLC levels (85,87,102-103). Among remnant lipoproteins, VLDL particles appear to accept a greater amount of cholesterol ester than do chylomicron remnants, which may lead to an abundance of VLDL saturated with cholesterol ester (22,101).

Small, Dense LDLC

HTGL plays a role in the conversion of VLDL to small, dense LDLC particles and its activity is significantly and negatively correlated with LDLC size and buoyancy (104). Small, dense LDLC has been shown to have a lower affinity for the LDLC receptor due to conformational changes in apo B 100, and therefore may be present in circulation for an extended period of time (105). The atherogenicity of small, dense LDLC particles arises from their increased susceptibility to oxidation when compared to larger LDLC particles (106).

Oxidized LDLC particles contribute to atherosclerosis and inflammation in multiple ways, being recognized most commonly for their ability to be taken up by macrophages through scavenger receptors, leading to the development of foam cells (107-108). In patients with CVD, the number of small, dense LDLC particles is significantly increased when compared to healthy controls, despite similar LDLC levels (109). Gazi, et. al, have shown that small, dense LDLC particles are increased in MetS when compared to healthy controls, and that triglyceride concentration is a significant

predictor of particle number (102). Additionally, these authors showed that, in a small sub-set of patients with MetS who had triglyceride values below 150 mg/dl, no difference was evident in mean LDLC particle size compared to healthy controls (102). These results confirm the close association between serum triglyceride concentration and increased small, dense LDLC (11,102).

Lipoprotein Lipase

LPL is responsible for the hydrolysis of triglyceride contained in LDLC, VLDL, and chylomicrons and its activity is partially modulated by insulin (110-111). In adipose tissue, LPL activity is greater with increased insulin concentration, while in skeletal muscle, the activity of LPL is reduced under similar conditions (43,112-113). In subjects with MetS, the pre-heparin mass of LPL is significantly and positively correlated with HDLC, and significantly and negatively correlated with triglyceride, blood glucose, and body weight (110,114). Post-heparin LPL activity is significantly reduced in obese compared to lean subjects, and insulin resistant subjects have lower LPL mRNA and protein content in skeletal muscle than do non-insulin resistant controls (98,115). Sedentary, overweight, middle-aged men have significantly lower post-heparin LPL activity in both the fasted state and 9 hours following a mixed meal when compared to younger normal weight men (97). Thus, reductions in LPL activity have been observed in obese, insulin resistant, and overweight sedentary individuals, and likely contribute to reduced TRL clearance.

TRL are cleared in a manner that is dependent on LPL mediated hydrolysis of chylomicrons and VLDL (110). The ability of LPL to hydrolyze TRL may become overwhelmed in the presence of elevated lipids (116-117). In healthy men, the

administration of a chylomicron-like lipid emulsion results in substantial increases in plasma triglycerides, and linear increases in large VLDL particles (116). Following the lipid emulsion, Bjorkegren, et.al.,(116) observed that the catabolic rate of large VLDL particles was reduced substantially when compared to a saline infusion control condition, and that the rate of conversion of large VLDL to small VLDL was decreased (116). Thus, it appears that increased chylomicrons in the plasma impede clearance of VLDL particles, and reduce the conversion of large VLDL to small VLDL. Postprandial increases in chylomicrons would lead to increases in circulating TRL and large VLDL particles.

Insulin Resistance

Insulin resistance and consequent hyperinsulinemia exacerbate hypertriglyceridemia (118). In conditions of metabolic dysfunction, skeletal muscle, with adipose and hepatic tissues, may become resistant to the effects of insulin (119). As a result, glucose uptake into skeletal muscle is decreased and insulin's ability to suppress hepatic glucose secretion may be compromised (120). Plasma NEFA concentrations may be elevated due to insulin resistant adipose tissue (22). The elevations in plasma NEFA, in conjunction with increased glucose flux resulting from hyperglycemia, provide ample substrate for hepatic VLDL overproduction (22,55). In middle-aged men and women, the estimation of insulin resistance using the homeostatic model assessment (HOMA) score has been shown to be significantly correlated with $VLDL_1$ rate of production (121). Furthermore, when compared to lean counterparts, obese subjects have a higher VLDL secretion rate of apoB (122). It can be concluded that obesity and, specifically, insulin resistance, are associated with increased hepatic output of triglyceride-rich VLDL.

Lifestyle

Overweight and obesity, along with dietary composition and physical inactivity are associated with blood lipid abnormalities (80,123-125). While insulin resistance is observed in normal weight subjects, a significant increase in BMI has been observed with increasing measures of insulin resistance, as defined as the top 25% of plasma insulin concentration (79-80). In obesity, the effect of insulin to suppress hepatic lipid assembly is compromised (80). In addition, the activities of CETP and HL have been shown to be increased in obesity, while LPL activity is negatively correlated with bodyweight (85,87,98). A prospective 6.5 follow up study has shown that, with weight gain of 5% of initial body weight and final BMI of less than 30 kg/m^2, the number of large VLDL particles increases significantly, by approximately a third (126). Thus even those who experience modest gains in body weight may develop secondary dyslipidemia. Elevations in postprandial lipemia are known to occur as a result of a high-fat meal, and individuals consuming a high-fat diets are exposed to postprandial lipemia as a result (95,127). The chronic effects of overweight and obesity on dyslipidemia are coupled with the acute detrimental blood lipid alterations that occur as a result of a high fat meal.

Summary

It can be concluded that postprandial increases in triglyceride-rich VLDL and chylomicrons promote atherogenic dyslipidemia. Increases in TRL promote low HDLC and increased small, dense LDLC. Increased activity of CETP and HL contribute to this effect by creating apo B particles laden with cholesterol ester and triglyceride-rich HDLC, meanwhile facilitating the conversion of VLDL to dense LDLC (85,98,104). Reductions in LPL activity lead to compromised ability to clear TRL (97,104).

Additionally, the effects of insulin resistance exacerbate postprandial lipemia and processes that contribute to atherogenic dyslipidemia. These alterations in enzyme activity and metabolic function that favor dyslipidemia have been described in MetS and obesity (85,97-98).

Exercise and Postprandial Lipemia

Aerobic exercise, performed 1 to 16 hours before a high-fat meal, significantly reduces postprandial triglyceride levels between 18 and 51% below non-exercise control values (25-26,29,46). Studies that support these effects have used treadmill or cycling exercise of low-, moderate- , and maximal-intensity ranging from 25 to 100% of VO_2max (30,34,38,46,61). Gill, et.al., (46), have shown that 90 minutes of exercise at 50% of VO_2peak performed on the day before a high-fat meal significantly reduces postprandial chylomicrons, VLDL, and remnant lipoproteins by 29, 34, and 35% when compared to non-exercise control in middle-aged overweight men. Thus, aerobic exercise is capable of reducing the postprandial increase in TRL, and substantially reducing CVD risk.

The reduction in postprandial lipemia has been shown to be related to the energy that was expended during exercise (31-33,36). When low- and moderate-intensity exercise is compared, regardless of intensity or duration, a threshold energy expenditure appears to exist below which alterations in postprandial lipemia are not statistically significant (30). Zhang, et.al., (35), have shown that the postprandial reduction in triglycerides is significant following exercise at 60% of maximal capacity only after sessions where 450 calories or greater were expended, and not following a session with a 300-calorie energy expenditure. At similar exercise intensity, the postprandial triglyceride reduction is significant following caloric expenditure exceeding 800 calories,

but not 400 calories (32). Mestek et.al., (34) has shown that a 500-calorie energy expenditure at 35-45 or 60-70% of maximal capacity lowers postprandial triglycerides similarly and significantly below non-exercise control. Likewise, exercise intensities of 32 and 63% of maximal capacity and equal caloric expenditure of approximately 1,000 calories lower postprandial triglycerides, with no differences between the two conditions (33). Thus, following low- and moderate- intensity exercise a caloric expenditure of 450-500 calories is sufficient to favorably alter postprandial triglycerides. It is clear that following low- and moderate- exercise, energy expenditure, instead of intensity or duration, appears to determine reductions in postprandial lipemia.

While intensity, per se, does not seem to be the primary determinant of changes in postprandial lipemia, the only studies that have directly compared exercise of differing intensities have used a narrow range of 31 to 60-70% of maximal capacity (32,34). Furthermore, no studies have directly compared low- and high-intensity exercise. In contrast to the previously mentioned investigations, the postprandial responses to isocaloric exercise sessions at 25 and 65% of maximal capacity performed 1 hour before a high-fat meal suggest that the alterations in postprandial triglycerides following low- and moderate- intensity exercise may indeed differ (61). Despite similar energy expenditures of the exercise sessions, postprandial triglycerides following exercise at 65% of VO_2peak were significantly attenuated when compared to non-exercise control, while the postprandial response following exercise at 25% of VO_2peak was similar to non-exercise control. One investigation, reporting the effects of maximal-intensity exercise on postprandial lipemia, has shown that 4 30-second all-out sprints separated by 4 minutes of active recovery significantly lower postprandial triglycerides below non-

exercise control (38). The approximate caloric expenditure of this session was 287 calories, substantially below the apparent threshold of 450-500 mentioned for low- and moderate- intensity exercise. This finding may indicate that the threshold energy expenditure required for favorable alterations in postprandial lipemia is lower following maximal- when compared to moderate- or low-intensity exercise. Comparisons between low- and high- intensity exercise should be made to examine whether isocaloric exercise sessions affect postprandial lipemia differently.

Support for the hypothesis that exercise energy expenditure determines reductions in postprandial lipemia comes from studies where the energy that was expended during exercise is replaced. Three studies have reported the effects of exercise with and without energy replacement on postprandial lipemia (36-38). The caloric expenditures for these studies are approximately 287, 670, and 1500 calories. The corresponding significant postprandial triglyceride reductions were 21, 14, and 40% following exercise without energy replacement when compared to non-exercise control (36-38).

Burton, et.al, (36) has shown that exercise at 50% of maximal capacity with a caloric expenditure of 670 calories without energy replacement significantly lowers postprandial triglycerides 14% below non-exercise control, and 10% below exercise with energy replacement in obese and overweight men (mean BMI 31.1 kg/m^{-2}). The energy expended during exercise was re-fed in the form of a mixed meal. No difference in postprandial triglycerides was observed between the exercise with energy replacement trial and non-exercise control (36). When compared to non-exercise control, the postprandial insulin concentration was significantly reduced by 18 and 10% following exercise with energy deficit and exercise with energy replacement (36). There was also a

10% statistically significantly lower postprandial insulin response following exercise with energy deficit when compared to exercise with energy replacement. Of the 3 studies examining the effects of energy replacement on postprandial lipemia, this is the only experiment where investigators report a significantly different insulin response following exercise trials with and without energy replacement. This is also the only investigation to examine exercise energy replacement and postprandial lipemia in obese and overweight subjects, a population is known to be prone to insulin resistance and glucose intolerance (80).

Harrison, et.al., (37) provided similar results using recreationally active men with a mean BMI of 26 kg/m^2. In the energy-replacement trial, the participants were fed glucose in an amount equal to the carbohydrate utilized during exercise. Higher-intensity exercise combining continuous exercise at 70% of maximal capacity and maximal-effort bouts, and producing a 1500 calorie energy expenditure, yielded a statistically significant 40% difference in postprandial triglycerides between exercise with energy deficit and non-exercise control. A smaller, but still significant (e.g. approximately 20%) difference between exercise with energy deficit and exercise with energy replacement was also found (37). In agreement with Burton, et.al. (36), no differences in postprandial triglycerides were observed between the exercise with energy replacement and non-exercise control condition. In contrast to the findings of Burton, et.al., (36) insulin levels did not differ across conditions. When compared to the work of Burton (36) the caloric expenditure achieved in this study is substantially greater, and the relative reduction in postprandial triglycerides much higher (e.g. 40% compared to 14%), supporting the role of energy expenditure in reducing postprandial triglycerides.

Freese et. al., (38) completed a similar study using maximal intensity exercise of short duration. The caloric expenditure of the exercise session was approximately 287 calories, and was re-fed in the form of a mixed meal. Participants completed 4 maximal 30 second cycling sprints interspersed with 4 minutes of active recovery (38). The significant differences between trials for postprandial triglycerides were equal to 21% between exercise with energy deficit and non-exercise control, 12% between exercise with energy deficit and exercise with energy replacement, and 10% between exercise with energy replacement and control (38). Of the 3 studies where the energy expended during exercise has been replaced, this is the only work documenting a significantly lower postprandial triglyceride response following exercise with energy replacement when compared to non-exercise control. This may indicate a specific benefit of higher-intensity exercise on lowering postprandial triglycerides, even when the energy expended during exercise is replaced. This is one of the few studies reporting the effects of only maximal intensity exercise, and it is possible that a lower caloric expenditure is required following this type of exercise to produce significant reductions in postprandial triglycerides.

Excess Post-Exercise Oxygen Consumption

There is evidence for the effect of exercise intensity in increasing post-exercise energy expenditure (68-69). Following isocaloric cycling exercise of 500 calorie energy expenditure, EPOC is significantly greater after a session at 75% of VO_2max when compared to one at 50% of VO_2max (e.g. 4.8 L vs. 9 L) (128). Even high intensity exercise of low caloric expenditure and short duration produce greater EPOC than lower-intensity exercise of longer duration (129). When measured for 14 hours post-exercise,

80 minutes of cycling at 75% of maximal capacity results in a 30.1 L EPOC, compared to only 5.7 and 1.3 L following exercise for 80 minutes at 50 and 29% (71). Gore and Withers (130), examined the differences in EPOC following treadmill exercise at a variety of intensities and durations. Subjects performed exercise bouts at 30, 50, and 70% of VO_2max for 20, 50 and 80 minutes (130). These authors found no statistically significant difference across time for the bouts at 30% of maximal capacity. Fifty minutes of exercise at 50% and 70% of VO_2max produced EPOC values of 5.19 and 10.04 L, and 80 minutes at the corresponding intensities yielded 6.10 and 14.59L (130). It was concluded that intensity is the primary factor determining increases in EPOC (130). It is evident that exercise at or above 70% of maximal capacity produces greater EPOC than exercise at or below 50% of maximal capacity. No studies have measured EPOC in order to determine its contribution to changes in postprandial lipemia. Because most studies examining postprandial lipemia have not explored higher-intensity exercise, favorable effects attributable to EPOC energy expenditure have likely been overlooked.

Potential Mechanisms

While energy expenditure has been indicated as a primary explanation for the decrements in postprandial lipemia following exercise, the precise physiological mechanisms are elusive. Increased clearance of VLDL and chylomicrons due to greater LPL activity offers one explanation for the consistent reduction in postprandial triglycerides following aerobic exercise (42-43). Increases in skeletal muscle LPL activity have been observed 24 hours following running exercise at 75% VO_2max, and at as little as 4 hours after 60 minutes of knee extensor exercise at 75% of maximum

capacity (43). LPL protein content in the vastus lateralis is increased significantly 22 hours post exercise following 60 minutes of cycling exercise at 65% VO_2max (42).

A recent study by Al-Shayji, Caslake, and Gill (131) supports the hypothesis that in middle-aged overweight men, the clearance of VLDL, particularly in the larger fraction, is increased on the day following exercise at 50% VO_2max. Men with a mean BMI of 31.1 kg/m^2 underwent Intralipid infusion designed to block catabolism of large VLDL particles. Following exercise, VLDL triglyceride was significantly lower, and the catabolic rates of VLDL triglyceride and apo B were significantly greater when compared to non-exercise control (131). VLDL production was not changed following the exercise or non-exercise control conditions. The composition of the VLDL particle was changed following exercise when compared to non-exercise control, with each VLDL particle containing a greater amount of triglyceride (131). The authors conclude that alterations in the composition of the VLDL particle itself may in fact lend the particle to being cleared more rapidly (131). This work strongly suggests that increased clearance of triglyceride-rich particles following exercise may explain improvements in postprandial lipemia.

Hepatic production of VLDL may decrease following a bout of exercise. A recent study using healthy normal weight women showed that, on the morning following exercise at 60% of VO_2peak where 500 calories are expended, fasting VLDL is significantly reduced when compared to non-exercise control (132). When compared to non-exercise control, the exercise trial significantly increased VLDL clearance and significantly reduced hepatic secretion (132). Up to 79% of the postprandial triglyceride reduction on the day following exercise is attributable to hepatically derived large VLDL

(46,133). Together, results from Bellou, et.al., (132) and Al-Shayji, et.al., (131) indicate that the reduction in triglycerides in the hours following exercise is due to decreases in VLDL production and increases in VLDL clearance.

The ability of exercise to increase glucose uptake and improve insulin sensitivity may play a role in reducing hepatic VLDL production (49). During exercise glucose uptake is increased in the absence of insulin (134). Exercise reduces insulin concentration in the postprandial period: exercise at 60% of VO_2max 12 hours before an oral fat tolerance test lowers postprandial insulin concentration significantly when compared to non-exercise control and exercise 24 hours before a meal (24,39). In obese men, high-intensity exercise, e.g. 85% of VO_2max to exhaustion, performed 12 hours before a euglycemic hyperinsulinemic clamp significantly increases glucose disposal (135). LPL activity in skeletal muscle is decreased under conditions of elevated insulin concentration and therefore the lower insulin concentrations observed following exercise may allow for enhanced LPL activity and subsequent triglyceride hydrolysis (43). In middle-aged overweight subjects, insulin resistance, estimated by the Homeostatic Model Assessment (HOMA-IR), is significantly correlated with large VLDL production rate (121). The effect of exercise in increasing insulin sensitivity and glucose disposal may permit improvements in hyperglycemia in subjects with metabolic disease. Reduced glucose delivery to the liver would lessen substrate availability for VLDL assembly, and subsequently could reduce hepatic VLDL production (55).

Hurren, Balanos, and Blannin (48), have shown that, on the day following 90 minutes of exercise at 60% of maximal capacity, total blood flow through the femoral artery and hepatic portal vein is significantly increased during the postprandial period

when compared to a non-exercise control condition. In addition to increased blood flow, a 22% lower postprandial triglyceride response was observed following exercise when compared to non-exercise control (48). This study, conducted in sedentary overweight men, provides evidence that alterations in blood flow on the day following moderate-intensity exercise may indeed affect postprandial substrate delivery to the tissues responsible for metabolizing fatty acids.

Potential differences in postprandial lipemia following low- and high- intensity exercise may be observed due to increased EPOC energy expenditure following high-intensity exercise. Increased energy expenditure would require greater mobilization of energy stores from hepatic tissue and skeletal muscle. IMTG has been shown to be relied upon more heavily during exercise at 65 and 85% when compared to 25% of maximal capacity (62). Although carbohydrate energy stores make a greater contribution to energy demands with increases in exercise intensity, lipid oxidation may be increased in the hours following exercise (62,136). During the 3 hours following moderate intensity exercise at 45 and 65% of VO_2peak, lipid oxidation is increased when compared to non-exercise control and pre-exercise values (126). Enhanced lipid oxidation in the post-exercise period coupled with increased EPOC following high-intensity exercise, may contribute to an increased substrate deficit. The increased utilization of IMTG during exercise and lipid oxidation following exercise may be partially responsible for alterations in postprandial lipemia.

In summary, multiple factors likely contribute to improvements in postprandial lipemia following aerobic exercise. Following moderate- and high-intensity exercise skeletal muscle LPL activity is likely increased, resulting in greater ability to clear TRL

(42-43). In addition, hepatic VLDL output may be reduced in the hours following moderate-intensity exercise (46,131). Reductions in postprandial insulin concentration have been observed following moderate exercise, and glucose disposal has been shown to be increased following higher-intensity exercise (24,135). These factors, in combination with increased blood flow to skeletal muscle and hepatic tissue, may contribute to the beneficial effects of aerobic exercise on postprandial blood lipids (48).

Conclusions

Atherogenic disease is the leading cause of death in United States, with the prevalence of obesity and MetS increasing (73,137). Blood lipid changes that occur in the postprandial state promote CVD, with triglyceride-rich particles elevated following meals (10,95). Low-, moderate-, and high- intensity aerobic exercise is known to favorably alter postprandial lipids, and is a useful modality for reducing CVD risk (32,38). The energy expenditure of exercise appears to dictate alterations in postprandial lipemia (30). While multiple studies have compared low- and moderate- intensity exercise, none have directly compared low- and high- intensity exercise. Higher-intensity exercise is known to produce greater post-exercise energy expenditure (68). The contribution of EPOC to changes in postprandial lipemia has not been determined. Because changes in postprandial lipemia have been linked to exercise energy expenditure, it is possible that increased EPOC following high-intensity exercise explains the favorable effects of exercise of greater intensity on postprandial lipemia.

CHAPTER THREE

Materials and Methods

Subjects and Methods

Modifications to reduce cardiovascular disease risk are generally of concern to sedentary, overweight individuals. Therefore, our target population was young to middle-aged, overweight and unfit men. Nine male volunteers were recruited from Baylor University, Waco, and surrounding communities by word of mouth and flyers posted at approved locations (APPENDIX A). Advertisements were also placed in newspapers and organizational newsletters. Volunteers who met the following criteria were admitted to the study: 1) healthy males between 25 and 55 years of age; 2) overweight or obese – defined as having a body mass index (BMI) above 25 kg/m^2; 3) physically-inactive – defined as engaging in no regular leisure-time or work-related physical activity, or completing less than 2.5 hours of low-moderate physical activity per week, over the last 6 months; 4) non-smokers; 5) not currently taking medications known to influence lipids and lipoproteins, or glucose metabolism; 6) able to ingest dairy products (e.g., volunteers are lactose tolerant), and; 7) free from orthopedic problems that would preclude walking/jogging on a treadmill.

Screening

Phone, e-mail, or in-person interview were used initially to screen volunteers to determine eligibility (APPENDIX B). Volunteers who met entry criteria were invited to the Baylor Laboratories for Exercise Science and Technology (BLEST, MMG 127).

During this initial visit, all of the experimental procedures were explained and reviewed. Volunteers were given a copy of a consent document, approved by the Baylor University Institutional Review Board, which was reviewed and signed (APPENDIX C). As part of this meeting, the volunteers completed a Health History Questionnaire (HHQ) and height and weight were measured to determine BMI criteria (APPENDIX D). The participant's height was measured to the nearest 0.25 inch using a stadiometer and weight was recorded to the nearest 0.25 pounds using a digital scale (SECA, Hamburg, Germany). Body mass index was calculated as mass (kg) / height (m^2). Waist circumference was measured in triplicate at the level of the umbilicus. Values were recorded to the nearest 0.5 cm.

Between the first and second visit to our lab, the volunteer's Health History Questionnaire responses were reviewed by the physician co-investigators affiliated with our lab, Dr. Ron Wilson, M.D., Jackson Griggs, M.D., and Nick Schwedock, M.D., for possible contraindications to exercise. Based on our physician's recommendation volunteers were asked to: 1) return to the lab to undergo additional physiological measurements related to the objectives of this study described below, or; 2) required to obtain medical clearance from their personal doctor before scheduling a second visit to the lab, or; 3) determined to be ineligible to participate in this study.

Physiological Assessment

Participants who met all entry criteria reported to the BLEST, MMG 127 after an 8- to 10-hour fast (limited to water ingestion only). All of the experimental procedures were reviewed again with each participant and any questions they had concerning this study were answered. Next, a small blood sample (17 ml) was obtained by venipuncture

from an antecubital vein for the determination of baseline blood glucose and lipids (Becton Dickinson (BD) Vacutainer, Franklin Lakes, NJ, SST 16 x 100 mm, 7.5 mg). Body composition was determined using dual-energy x-ray absorptiometry (DXA) (Hologic, Bedford, MA).

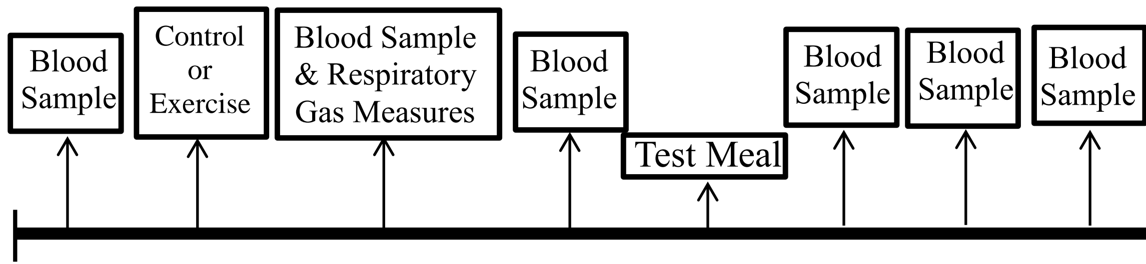
Next, participants performed a standardized maximal graded exercise test on a treadmill using the modified Bruce protocol to determine their cardiovascular fitness (138). The cardiovascular response to exercise was determined using continuous 12- lead electrocardiography (Cardio Control, Welch Allyn, Skaneateles, NY). Blood pressure and rating of perceived exertion (RPE) were obtained during the last minute of each 3-minute stage via a mercury sphygmomanometer and a 20-point Borg scale. Oxygen consumption and carbon dioxide production were measured throughout the test and averaged over a 15-second period by an automated analyzer (ParvoMedics, Sandy, UT). VO_2peak was defined as the highest VO_2 maintained for one minute and was corroborated by validating two of three criteria: 1) heart rate within 10 beats of age predicted maximum; 2) rating of perceived exertion ≥ 18, or; 3) respiratory exchange ratio (RER) ≥ 1.15. The maximum heart rate and VO_2peak obtained from the participant's graded exercise test was used to determine exercise intensities that are equal to 40-50% and 70-80% of heart rate reserve (HRR) and VO_2 reserve (VO_2R) (139). Participants who met all inclusion criteria and were cleared to exercise based on a normal cardiovascular response to exercise as reviewed by our physician co-investigator were asked to continue to take part in the study.

Overview

Volunteers who met all eligibility criteria above, provided their consent to participate, and completed all preliminary measurements were asked to visit the lab on 4 different occasions in order to address the objectives of this study. Participants completed 4 experimental conditions: control; low-intensity exercise (LI), and; high-intensity exercise (HI), and high-intensity exercise + EPOC energy replacement (HI + EERM). Each condition was separated by at least 5 days and no more than 14 days. Except for the fourth condition, all conditions were completed in random order. On the morning of each trial, the participants completed a non-exercise control or one of the exercise sessions. After each exercise session, 2 hours of resting post-exercise oxygen consumption was measured. Immediately after the final HI session, participants consumed a meal with a caloric content equal to the difference in calories spent in the hours after the LI and HI sessions. Two hours following the control or exercise session, participants consumed a high-fat test meal. Blood samples were obtained prior to ingesting the high-fat test meal, and again at 2, 4, and 6 hours after the test meal. All experimental conditions were completed at the same time of day. A study schematic is provided in figure 1.

Dietary Analysis

All participants were instructed on keeping daily records of their diet and physical activity (APPENDICES E & F). Participants completed daily records for 3 days prior to each of the conditions. We requested that all participants follow the diet and activity recorded prior to the first testing session as closely as possible before all subsequent trials. This was done to ensure that alterations in energy or nutrient intake and physical activity did not influence the postprandial lipid response to the test meal



Study Schematic

FIGURE 1.. Study schematic. Volunteers who met all criteria underwent 4 experimental conditions to determine the effects of exercise intensity on the postprandial lipid response to a high-fat test meal. Each trial required the participant to either rest or exercise followed by 2 hours of respiratory gas measurement. Exercise consisted of 500 calorie energy expenditure at either 40-50 or 70-80% of VO_2R. Two hours after control or exercise, blood samples were be taken prior to the ingestion of a high-fat test meal. Blood was sampled again at 2, 4, and 6 hours postprandially. Following the final HI exercise session, the difference in post-exercise energy expenditure between LI and HI exercise was re-fed in the form of a meal bar.

Dietary intake and macronutrient composition were estimated using nutritional analysis software (Food Processor SLQ, Version 10.7, ESHA Research, Salem, OR). Following the initial visit, the food consumed 3 days prior to the test meal, including type and quantity was e-mailed or hand delivered to the participant before the next testing session to encourage compliance with the protocol. Physical activity logs were used to quantify the type and amount of activity engaged in before the experimental conditions. Participants reported the time they spent in a variety of activities and were asked to repeat these habits in the 3 days leading up to the test meal. Records were used to verify sedentary behavior and remind participants to engage in similar behavior in the days leading up to the experiment.

Non-Exercise Control

Participants reported to the lab after a 12-hr fast limited to water intake only. After obtaining diet and activity records from the participant, each was measured for height and weight, and fitted with a heart rate monitor (Polar, Lake Success, NY). Heart rate and blood pressure were measured after 5 minutes of seated rest. Participants sat

upright and respiratory gasses were measured using a portable respiratory gas analysis system for 45 minutes (VO_{2000}, Medgraphics, St. Paul, MN). The final 10 minutes of each interval were averaged for the determination of resting oxygen consumption. This measurement allowed for the estimation of caloric expenditure under fasting and non-exercised conditions.

Exercise Interventions

Participants reported to the lab in the morning after a 12-hr fast limited to water intake only. After obtaining diet and activity records from the participant, each was measured for height and weight, and fitted with a heart rate monitor. Heart rate and blood pressure were measured after 5 minutes of seated rest. Participants sat upright and respiratory gasses were measured for 15 minutes. The final 10 minutes of oxygen consumption were averaged and used for the calculation of resting caloric expenditure. Next, participants were asked to walk or jog on a treadmill in order to expend 500 calories of energy. Warm-up consisted of walking for three minutes at 2.5 miles per hour and a 2% grade.

The approximate time needed for each session and the rate of caloric expenditure was estimated before each session using the oxygen consumption data obtained from the participant's graded exercise test and a 5 calorie*L^{-1} of O_2 equivalent. During the HI session participants were asked to exercise at 70-80% of VO_2R for approximately 45-60 minutes. During the LI session participants were asked to exercise continuously at 40-50% VO_2R for approximately 70-90 minutes. Respiratory gasses were measured regularly to verify oxygen consumption and to determine that a 500 calorie energy

expenditure had been achieved. During both HI and LI heart rate was measured continuously.

EPOC Measurement and Post-Exercise Energy Expenditure

Immediately following the exercise sessions, post-exercise energy expenditure was determined from respiratory gasses measured while the participant sat quietly for 2 hours. During this time participants were allowed to drink water only, and were asked to remain seated in the lab. When an exercise energy expenditure of 500 calories was reached, EPOC was measured until the oxygen consumption, averaged over a 10-minute period, returned to the rate of oxygen consumption measured in rest during the pre-exercise period. Oxygen consumption was averaged over 1-minute intervals and was used to calculate caloric expenditure. The difference in post-exercise energy expenditure between the HI and LI sessions was used to determine the caloric content of the meal ingested immediately after exercise in the fourth experimental condition.

Following the final HI session, participants were fed a small meal that had a caloric content equal to the difference in calories spent during EPOC after the LI vs. HI sessions that they previously completed. This meal was a portioned amount of a commercially-available sports nutrition bar (Peanut Butter Power Bar®: 240 calories; 4 g fat; 44 g carbohydrate; 9 g protein).

High-fat Test Meals and Experimental Blood Sampling

Two hours after control, LI, and both HI sessions, a pre-meal blood sample was taken. A plastic catheter (BD Vacutainer, Franklin Lakes, NJ, 0.9 x 25 mm) was inserted into the antecubital vein and an intermittent injection site was attached (Kawasumi

Laboratories Inc., Tokyo, Japan). The initial blood sample was drawn before the high-fat test meal and included into two 10 mL red top and one 4.0 mL purple top vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, 16 x 100 mm; BD Vacutainer, Franklin Lakes, NJ, 13 x 75 mm, K2 EDTA 7.2 mg). Next, the participant consumed a high-fat milk shake containing 20 tbsp (255 mL) of whipping cream and1/2 cup (74 g) of ice cream. The milk shake provided approximately 1010 calories, 100 g fat, 99 g saturated fat, 17 g carbohydrate, and 3 g protein (29,34,72). Participants were asked to consume the meal within 15 minutes.

A timer was started after the participant completed the meal and blood was sampled at 2, 4, and 6 hours postprandially. Following each blood draw sodium heparin was injected to maintain patency (Heparin Lock Flush, 10 USD units/mL, APP Pharmaceuticals, Schaumburg, IL). Prior to sampling at 2, 4, and 6 hours a small amount of blood was removed and discarded to ensure that no sodium heparin solution diluted the samples. Immediately after each draw 4 microcapillary tubes were filled with blood and centrifuged at 3900 X g for 15 minutes (75 mm Hematocrit Tubes, Drummond, Broomall, PA; ZipOcrit, LW Scientific, Lawrenceville, GA). These samples were used to determine hematocrit, and to ensure that no substantial changes in fluid volume had occurred (140). Vacutainers were allowed to clot on ice for 30 minutes before being centrifuged at 3500 X g for 15 minutes (Clinical 50, VWR, Radnor, PA). Serum and plasma were aliquoted into 2.0 mL plastic ultracentrifuige tubes and stored at – 80 ºC.

A total of 16 blood samples were obtained with a minimum of 5 needle sticks. Approximately 17 mL of blood was drawn at each sampling time point. The maximum amount of blood drawn in one day was 68 mL. This resulted in a total blood sample

volume of 272 mL. In total, 6 lab visits amounting to approximately a 42 hour time commitment was required.

Analysis of Dependent Variables

Dependent variables included triglyceride, insulin, HDLC, NEFA, non-HDLC, total cholesterol, ApoB, and ApoA1. Homeostatic model assessment (HOMA) and glucose to insulin ratio (G/I ratio) were calculated to assess insulin resistance in the fasted state [HOMA = fasting glucose (mg/dl)/fasting insulin (mU/mL) * 22.5; G/I ratio = fasting glucose (mg/dl)/fasting insulin concentration (mU/mL)] (141). Independent variables included the experimental condition and the time point at which blood was sampled. Triglycerides, total cholesterol, LDLC and glucose were determined enzymatically (Siemens Vista Autoanalyzer, Malvern, PA). NEFA was determined enzymatically as described by Wako Diagnostics (Wako Diagnostics, Richmond, VA). The intra-assay coefficients of variation for triglycerides, total cholesterol, LDLC, glucose and NEFA were 1.5%, 2.5%, 3.1%, 1.8%, and 2.9%, respectively. HDLC was determined by immunoinhibition colorimetrically as described by Siemens (Siemens Vista Autoanalyzer, Malvern, PA). The intra-assay coefficient of variation for HDLC was 3.1%. ApoB and ApoA1 were determined by immunoinhibition, and the ApoB/A1 ratio was calculated by dividing Apo B by ApoA1. The intra-assay coefficients of variation for ApoB and ApoA1 were 2.4% and 2.6 %. The non-HDLC was calculated by subtracting HDLC from total cholesterol. Insulin was determined by enzyme linked immunosorbent assay (ELISA) (Siemens Vista Autoanalyzer, Malvern, PA). The intra-assay coefficient of variation for insulin was 2.1%.

Statistical Procedures

The postprandial changes in triglycerides were analyzed as the mean values at each time point, and by calculation of the total and incremental areas under the curve (142). The following equations were used to calculate these variables:

$$PPL\ (mg * dl^{-1} * 6\ h) = n_B + 2[n_2 + n_4] + n_6 \qquad \text{(Total)}$$

$$PPL\ (mg * dl^{-1} * 6\ h) = 2[n_2 + n_4] + n_6 - 5n_B \qquad \text{(Incremental)}$$

The baseline triglyceride is represented by n_B, and n_2 through n_6 and are equal to the triglyceride response after the meal.

A cross-over design was used where each participant served as his own control. The "Proc Univariate" procedure (SAS, Version 9.2, Cary, NC) was employed to determine if data were normally distributed. Separate 1 (group) x 4 (condition) repeated measures ANOVA's were used to determine differences in fasting triglyceride concentration and triglyceride incremental and total AUC. To examine temporal alterations in these variables 4 (condition) x 4 (time point) and 4 (condition) x 6 (time point) repeated measures ANOVAs were employed. Follow up was performed with Duncan's New Multiple Range Test when significant differences were observed between groups. A comparison wise alpha level of $p < 0.05$ will be considered significant.

CHAPTER FOUR

Results

Participants

Twenty-seven men responded to study advertisements. Seven of the 27 did not respond to follow-up telephone calls or e-mails, and 3 volunteers declined participation due to the required time commitment. Six volunteers were excluded following the telephone screening due to orthopedic injuries, prescription medications, regular physical activity, lactose intolerance, or normal BMI. One volunteer displayed lightheadedness and dyspnea immediately following a maximal graded exercise test, which was qualified as an abnormal cardiovascular response to exercise. This volunteer was excluded from the study and referred to his physician for further testing. Ten volunteers met all entry criteria and were asked to complete the study. One of these volunteers completed the screening process, including all physiologic assessments, but withdrew before beginning the experimental conditions. Nine participants completed the entire study. Upon review of our triglyceride results, the primary dependent variable, 2 participants exhibited different pre-meal triglyceride concentrations and a refractory triglyceride response during the control condition. The pre-meal triglyceride concentration and triglyceride responses were dissimilar to all other experimental conditions and the refractory response has not been observed in the literature. While we cannot fully explain these abnormalities, we have identified confounding variables that may have contributed to these unexpected physiologic responses. One of these participants reported

gastrointestinal symptoms on the day of the control trial, while the other reported consuming a meal on the night before the trial that he was not able to replicate for the subsequent trials. Due to these extraneous variables that were outside of our control and our protocol, these 2 participants were not included in the statistical analysis. Of the 9 participants that completed the entire experimental protocol, data is presented for 7 participants. All of the participants were of Caucasian decent.

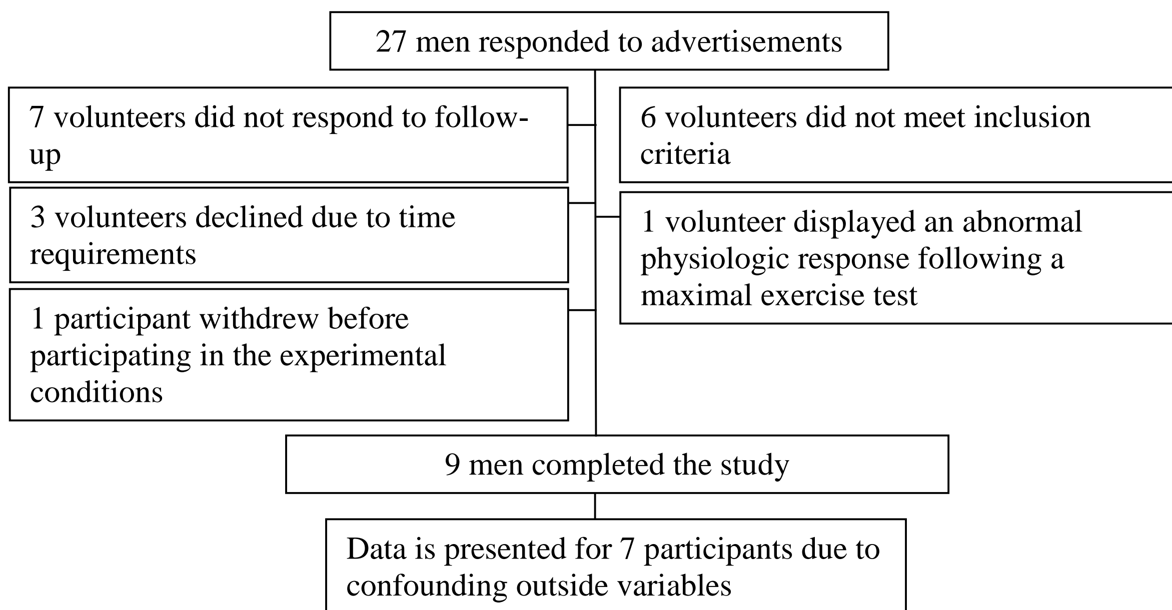


FIGURE 2. Participant selection.

Baseline physiologic and anthropometric characteristics are presented in Table 1. Ages ranged from 28 to 55, with a mean of 43 $\pm$ 10 years. Participants were overweight and obese, with a mean BMI of 31.8 $\pm$ 4 kg/m^2 and body fat percent of 30 $\pm$ 6%. All participants reported engaging in less than 2.5 hours per week of low to moderate physical activity. VO_2peak for the group averaged 31.1 $\pm$ 7.5, representing the 10th percentile for men between the ages of 40 and 49 (143). Three of the 7 participants met the criteria for MetS designated by NCEP guidelines (15). The mean fasting triglyceride

TABLE 1
Baseline anthropometric and physiological characteristics

Variable	Mean + SD	Minimum	Maximum
Age (yrs)	43 ± 10	28	55
Height (in)	69.8 ± 2.4	67	73.5
Weight (kg)	100.6 ± 17.7	78.2	118.7
BMI (kg/m^2)	31.8 ± 4.5	25.6	36.6
Body Fat (%)	30 ± 6	24	41
Waist (cm)	107.2 ± 14.9	81.3	120.7
SBP (mmHg)	128 ± 15	114	158
DBP (mmHg)	81 ± 9	70	100
VO_2peak (L/min)	2.9 ± 0.3	2.52	3.16
VO_2peak (ml/kg/min)	31.1 ± 7.5	21.4	40.4
Glucose (mg/dl)	98 ± 5	89	106
Triglycerides (mg/dl)	160 ± 84	62	291
Total Cholesterol (mg/dl)	169 ± 34	135	219
HDLC (mg/dl)	40 ± 13	24	57
LDLC (mg/dl)	97 ± 23	72	133
NHDLC (mg/dl)	129 ± 31	92	172

Values are presented as means ± standard deviation along with minimum and maximum values. SBP = systolic blood pressure; DBP = diastolic blood pressure.

concentration was 160 ± 84, with 3 of the 7 participants displaying elevated fasting triglyceride levels above the recommended cut-point of 150 mg/dl. Four participants had a waist circumference greater than 102 cm, and 2 participants were being treated for hypertension with Lisinopril. Additionally, 4 participants had HDLC levels below 40

mg/dl. Fasting glucose levels ranged from 89 to 106 $\pm$ mg/dl, with 3 of the 7 displaying elevated fasting glucose above 100 mg/dl. All of the participants were overweight or obese with reduced cardiovascular fitness. All met the criteria for "moderate risk" based on the American College of Sports Medicine guidelines, possessing 2 or more risk factors for cardiovascular disease (143).

Diet and Physical Activity

All participants reported averaging less than 2.5 hours per week of low- to moderate- physical activity before being admitted to the study. Participants were asked to categorize the physical activity completed in each 24-hour period for 3 days leading up to the first experimental condition. Categories 1 through 4 included activities of daily living ranging from sleeping to slow dressing and showering, while categories 5 through 10 included more rigorous endeavors such as housework, manual labor, and planned exercise. Four of the 7 participants were able to replicate their physical activity with only minor modifications that fell within categories 1 through 4. Three of the 7 participants reported slight deviations in activity level before testing that were categorized into levels 5 through 7. These activities were limited to yard-work (a riding lawn mower), and moving and loading goods at work. Finally, 1 participant engaged in light gardening and yard-work on the 3 days leading up to each trial, but the time spent in activity varied slightly between conditions.

Table 2 details the reported dietary intake leading up to each trial. There were no significant differences between conditions for total calories, macronutrients, or the polyunsaturated/saturated fat ratio (Kcals, $F_{3,18} = 1.54$, $p = 0.239$; fat, $F_{3,18} = 0.28$, $p =$

0.842; carbohydrate, $F_{3,18} = 1.43$, $p = 0.262$; protein, $F_{3,18} = 0.14$, $p = 0.937$; saturated fat, $F_{3,18} = 1.19$, $p = 0.342$; P/S ratio, $F_{3,18} = 1.11$, $p = 0.370$).

TABLE 2
Average energy and macronutrient intake

Variable	CON	LI	HI	HI+EERM
Kcals	2427 ± 216	2655 ± 137	2373 ± 245	2450 ± 257
Fat (g)	96 ± 13	97 ± 14	95 ± 13	97 ±15
CHO (g)	282 ± 32	290 ± 37	273 ± 33	283 ± 34
PRO (g)	98 ± 11	97 ± 11	98 ± 12	97 ± 11
SatFat (g)	40 ± 8	41 ± 9	42 ± 9	41 ± 9
P/S ratio	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1

Values are presented as means ± standard error. Calorie and macronutrient averages for 2 days leading up to each experimental condition are shown. No statistically significant differences were found between any of the conditions for any variable. Kcals = calories; CHO = carbohydrate; PRO = protein; SatFat = saturated fat; P/S ratio = polyunsaturated/saturated fat ratio. CON = control, LI = low-intensity, HI = high-intensity, HI+EERM= high-intensity + EPOC energy replacement.

Analysis of resting variables before each of the experimental conditions confirmed that participants began each trial under similar physiologic conditions. Body weight, glucose, insulin, HOMA score, G/I ratio, and resting energy expenditure were not significantly different between the experimental conditions. HOMA score and G/I ratio were used to estimate insulin resistance and sensitivity under fasting conditions, and were no different between any of the trials (body weight, $F_{3,18} = 1.87$, $p = 0.173$; glucose, $F_{3,18} = 0.22$, $p = 0.881$; triglyceride, $F_{3,18} = 0.61$, $p = 0.619$; NEFA, $F_{3,18} = 0.66$, $p = 0.586$; non-HDLC, $F_{3,18} = 0.32$, $p = 0.813$; HOMA, $F_{3,17} = 0.44$, $p = 0.729$; GIR, $F_{3,17} = 0.62$, $p = 0.609$; resting energy expenditure, $F_{3,18} = 0.68$, $p = 0.576$).

TABLE 3
Baseline physiologic variables across conditions

Variable	CON	LI	HI	HI + EERM
Weight (kg)	97.7 $\pm$ 7.1	100.1 $\pm$ 6.5	100.3 $\pm$ 6.5	100.5 $\pm$ 6.7
REE (L/min)	0.235 $\pm$ 0.02	0.232 $\pm$ 0.02	0.262 $\pm$ 0.01	0.251 $\pm$ 0.02
Glucose (mg/dl)	105 $\pm$ 3	106 $\pm$ 1	104 $\pm$ 2	104 $\pm$ 2
Insulin (mU/L)	15.2 $\pm$ 3.6	16.0 $\pm$ 4.4	17.4 $\pm$ 4.4	15.9 $\pm$ 4.1
TG (mg/dl)	165 $\pm$ 30	151 $\pm$ 22	170 $\pm$ 21	164 $\pm$ 37
NEFA	0.377 $\pm$ 0.05	0.469 $\pm$ 0.08	0.450 $\pm$ 0.07	0.446 $\pm$ 0.05
NHDLC (mg/dl)	125 $\pm$ 10	127 $\pm$ 12	128 $\pm$ 10	124 $\pm$ 11
HOMA	3.86 $\pm$ 0.90	4.18 $\pm$ 1.13	4.49 $\pm$ 1.11	4.09 $\pm$ 1.08
G/I ratio	8.46 $\pm$ 1.62	9.56 $\pm$ 2.55	8.03 $\pm$ 1.51	8.91 $\pm$ 1.73

Values are presented as means $\pm$ standard error. No significant differences were found for body weight, resting energy expenditure (REE), or any of the blood variables measured. HOMA = homeostatic model assessment, G/I ratio = glucose/insulin ratio. CON = control, LI = low-intensity, HI = high-intensity, HI+EERM= high-intensity + EPOC energy replacement.

Exercise Sessions

The caloric expenditures of the LI, HI, and HI + EERM exercise trials were each approximately 500 calories, with no significant differences between the conditions ($F_{2,12} = 0.03$, $p = 0.975$). The exercise time for both high-intensity trials averaged 47 $\pm$ 2 minutes, and, by design, the mean exercise time for the LI session was significantly longer, at 74 $\pm$ 2 minutes ($F_{2,12} = 1271.98$, $p < 0.0001$). Participants achieved intensities of 39.1 $\pm$ 0.6 for LI, and 69.3 $\pm$ 1.5, and 70.3 $\pm$ 2.9% of VO_2peak during the high-intensity trials ($F_{2,12} = 145.35$, $p < 0.0001$). The relative exercise intensities and average RER during the high-intensity trials were statistically similar, and were significantly

higher than those measured in the LI trial as expected (Intensity, $F_{2,12} = 56.37$, $p < 0.0001$; RER, $F_{2,12} = 17.39$, $p < 0.001$). Average heart rate was significantly different between the three conditions: 112.1 ± 5.2 (LI), 148.9 ± 5.5 (HI), and 140.2 ± 5.2 (HI + EERM) ($F_{2,12} = 102.1$, $p < 0.0001$). All participants were able to complete each of the exercise trials with no adverse events.

TABLE 4
Exercise Session Data

Variable	LI	HI	HI + EERM
Energy Expenditure (Kcal)	500.8 ± 0.6	500.4 ± 0.6	502.4 ± 11.5
Time (min)	74 ± 2	47 ± 2*	47 ± 2*
Avg VO_2 (ml/kg/min)	13.8 ± 1.0	21.6 ± 1.6*	22.0 ± 2.0*
% of VO_2peak	39.1 ± 0.6	69.3 ± 1.5*	70.3 ± 2.9*
Avg HR (bpm)	112.1 ± 5.3	148.9 ± 5.5*	140.4 ± 5.2*†
Avg RER	0.83 ± 0.02	0.89 ± 0.01*	0.88 ± 0.02*

Values are presented as means ± standard error. Values with similar superscripts are statistically similar. * = significantly different than LI. † = significantly different from HI. LI = low-intensity, HI = high-intensity, HI + EERM= high-intensity + EPOC energy replacement meal.

EPOC

Excess post-exercise oxygen consumption was measured following LI and HI exercise for 2 hours, or until the average VO_2 of 10 minutes was equal to pre-exercise oxygen consumption values. Following LI and HI exercise, oxygen consumption was elevated above rest for an average of and 24 ± 17 and 27 ± 16 minutes. EPOC was more than 2-times higher following HI when compared to LI exercise (9.1 ± 4.3 L vs. 4.4 ± 2.0 L), yet there was no statistically significant difference between the conditions ($F_{1,6}$ =

3.83, $p = 0.098$). The energy expenditures resulting from EPOC following HI and LI exercise were equal to 45.3 ± 21.7 and 22.0 ± 10.0 calories. Only 1 of the 7 participants demonstrated oxygen consumption values that remained above rest for the entire 120 minute post-exercise period. There were no statistically significant differences in EPOC time or calories expended during EPOC between exercise conditions (time, $F_{1,6} = 3.31$, $p = 0.119$; calories, $F_{1,6} = 3.81$, $p = 0.099$).

TABLE 5
Characteristics of EPOC Measures

	Mean	Min	Max	Range
Low-Intensity				
EPTM (min)	24 ± 17	1	120	119
EPOC (L)	4.4 ± 2.0	1.4	16.3	14.9
EPOC Kcals	22.1 ± 10.0	7.2	81.4	74.2
High-Intensity				
EPTM (min)	27 ± 16	3	120	117
EPOC (L)	9.1 ± 4.3	3.4	35.1	31.7
EPOC Kcals	45.3 ± 21.7	16.9	175.4	158.5

Values are presented as mean ± standard error along with minimum and maximum values. No significant differences were found for EPOC time, total EPOC, or EPOC energy expenditure between low- and high- intensity exercise. EPTM = EPOC time, EPOC Kcals= EPOC energy expenditure above rest. Min = minimum value, Max = maximum value.

Postprandial Responses

Blood Lipid Responses

There were no statistically significant changes in plasma volume across conditions or time points ($F_{15,89} = 1.24$, $p = 0.256$). Blood responses were analyzed as unadjusted and adjusted for plasma volume changes, and no differences were found between these analyses. Therefore, values presented are derived from unadjusted data.

TABLE 6
Plasma volume changes

Condition	PRE	POST	0-hr	2-hr	4-hr	6-hr
CON	0	-0.2 ± 3.1	-0.2 ± 3.1	-2.5 ± 2.3	-2.5 ± 2.8	-0.9 ± 2.0
LI	0	2.4 ± 2.5	4.9 ± 3.2	4.5 ± 4.3	5.8 ± 4.6	3.4 ± 4.3
HI	0	-0.2 ± 1.4	2.4 ± 2.1	1.6 ± 3.0	2.3 ± 1.5	4.1 ± 1.3
HI + EERM	0	-3.9 ± 1.4	1.0 ± 2.0	-0.5 ± 1.6	-2.2 ± 1.8	-4.8 ± 3.1

Values are presented as mean % change from PRE ± standard error. There were no statistically significant differences observed across time or condition. PRE = preliminary blood sample before each condition, CON = control, LI = low-intensity, HI = high-intensity, HI + EERM = high-intensity + EPOC energy replacement.

The temporal triglyceride responses for each condition are presented in figure 4. At 4 hours, triglyceride concentrations were significantly reduced below control values for both the LI and HI exercise trials, with no significant difference between the control and HI + EERM. Six hours after LI, a significantly reduced triglyceride response was observed when compared to control (triglyceride by time, $F_{9,54} = 30.23$, $p < 0.0001$).

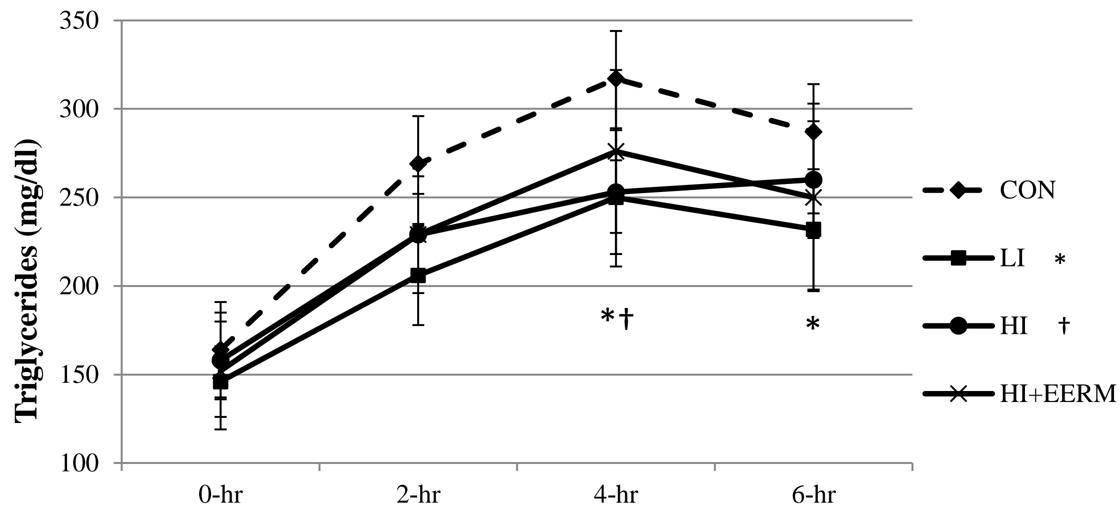


FIGURE 3. Temporal triglyceride response by condition. Means ± standard error are presented. * = low condition is significantly lower than control; † = high condition is significantly lower than control. All values were increased significantly at 2-hr when compared to baseline. 0-hr = pre meal.

Total and incremental areas under the curve for triglycerides are presented in figures 5 and 6. For both total and incremental areas under the curve, the LI, HI, and HI + EERM trials were significantly lower when compared to the control trial (AUC_T, $F_{3,17}$ = 3.59, $p < 0.05$; AUC_I, $F_{3,17}$ = 5.15, $p < 0.05$). No statistically significant differences were found for total or incremental triglyceride responses between the 3 exercise conditions.

Both non-HDLC and LDLC showed non-significant changes across the postprandial period (non-HDLC, $F_{9,53}$ = 1.26, p = 0.280; LDLC, $F_{9,53}$ = 1.27 p = 0.273). The temporal NEFA responses are presented in figure 7. NEFA concentrations decreased at 2 hours, and rose at hours 4 and 6 under all conditions. At 0 and 2 hours, NEFA concentrations were significantly higher during each exercise condition when compared to control.

The temporal responses of TC, HDLC, ApoB, ApoA1, and the ApoB/A1 ratio are presented in table 7. Total cholesterol showed minimal variation across time points ($F_{9,54}$ = 4.46, $p = 0.0037$). HDLC was decreased during the postprandial period significantly at both 2 and 4-6 hours when compared to baseline ($F_{9,54}$ = 16.07, $p < 0.0001$). Apo B and the ApoB/A1 ratio rose significantly across time points as early as 2 hours into the postprandial period (ApoB, $F_{9,54}$ = 11.12, $p < 0.0001$; ApoB/A1 ratio, $F_{9,54}$ = 4.84, $p < 0.05$). ApoA1 was significantly elevated at 4 and 6 hours postprandially ($F_{9,54}$ = 3.85, $p < 0.05$).

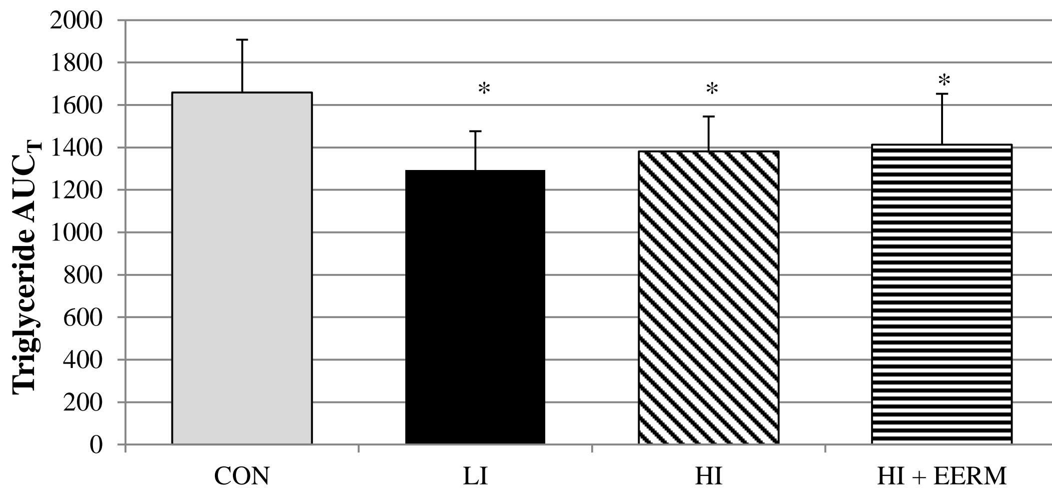


FIGURE 4. Total triglyceride area under the curve by condition. Means ± standard error are presented. AUC_T was significantly lower following the exercise conditions when compared to control. CON =control, LI = low-intensity, HI = high-intensity, HI + EERM = high-intensity with EPOC energy replacement. * = significantly different from control.

Glucose and Insulin Responses

Insulin concentrations were statistically similar between the conditions, but a main effect was found for time, with the 2-hour postprandial insulin concentrations significantly higher than all other time points across conditions. Likewise, glucose levels did not differ significantly across conditions, but a significant interaction was found for

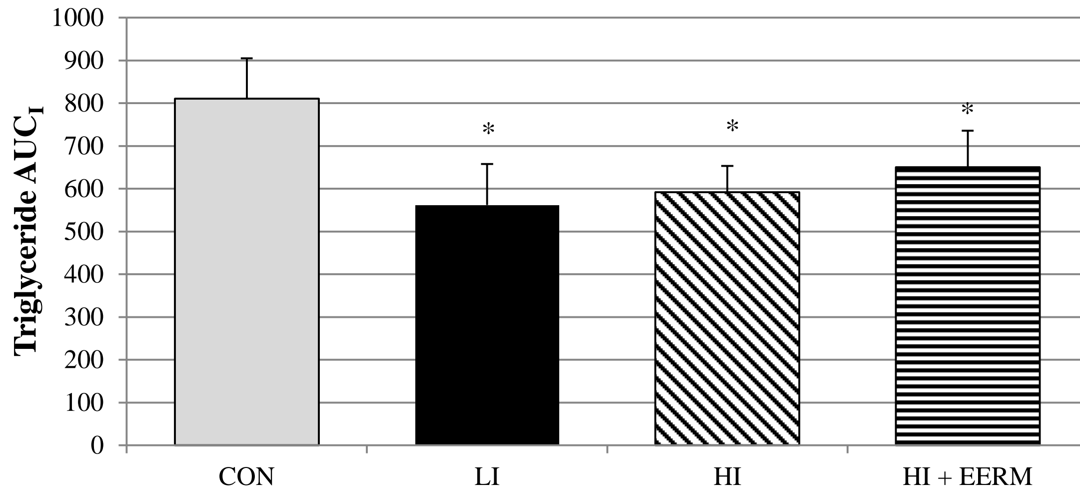


FIGURE 5. Incremental triglyceride area under the curve response by condition. Means + standard error are presented. AUC_I was significantly lower following the exercise conditions when compared to control. CON =control, LI = low-intensity, HI = high-intensity, HI + EERM = high-intensity with EPOC energy replacement. * = significantly different from control.

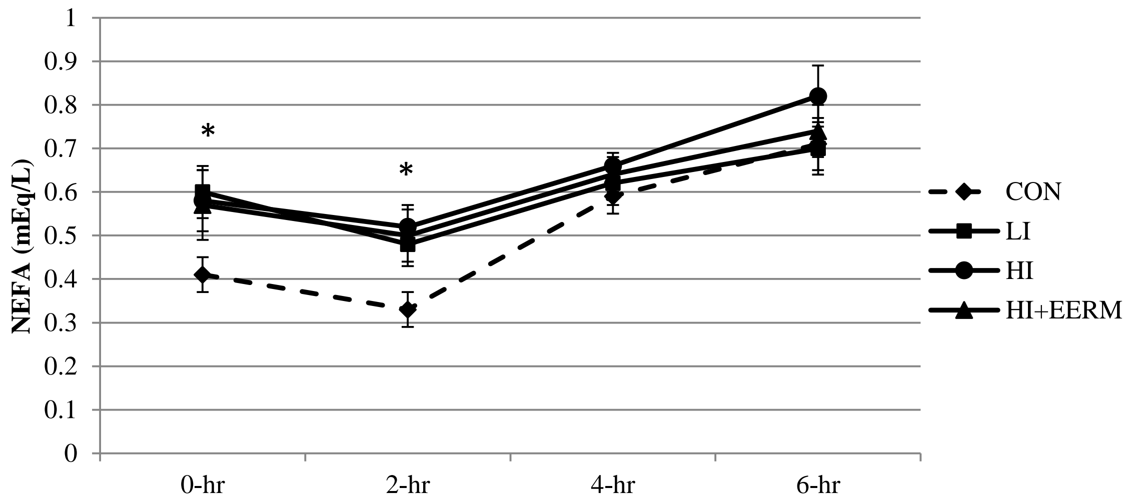


FIGURE 6. Temporal NEFA response by condition. Means + standard error are presented. * indicates significantly difference from control, $p < 0.001$.

time, with significantly lower values observed at 4 and 6 hours when compared 0 and 2 hour time points ($F_{9,54} = 23.23$, $p < 0.0001$). Mean glucose and insulin values across time are presented in figures 8 and 9. Total and incremental areas under the curve for insulin

were not significantly different between any of the 4 conditions (total, $F_{3,17} = 0.30$, $p = 0.824$; incremental, $F_{3,17} = 3.05$, $p = 0.061$).

TABLE 7
Temporal changes in blood lipid variables

Variable	0-hr	2-hr	4-hr	6-hr
TC (mg/dl)	163 ± 5	162 ± 5	160 ± 5	163 ± 5
HDLC (mg/dl)	38 ± 2[a]	36 ± 2[b]	34 ± 2[c]	34 ± 2[c]
ApoB (g/L)	0.88 ± 0.03[a]	0.96 ± 0.4[a,b]	0.99 ± 0.04[c]	0.98 ± 0.04[c]
ApoA1 (g/L)	1.32 ± 0.04[a]	1.33 ± 0.4[a]	1.36 ± 0.04[b]	1.36 ± 0.04[b]
ApoB/A	0.70 ± 0.03[a]	0.73 ± 0.03[b]	0.74 ± 0.04[b]	0.74 ± 0.04[b]

Values are presented as means ± standard error. Means with similar letters are statistically similar. TC = total cholesterol; ApoB/A ratio = ratio of Apo B/ Apo A1.

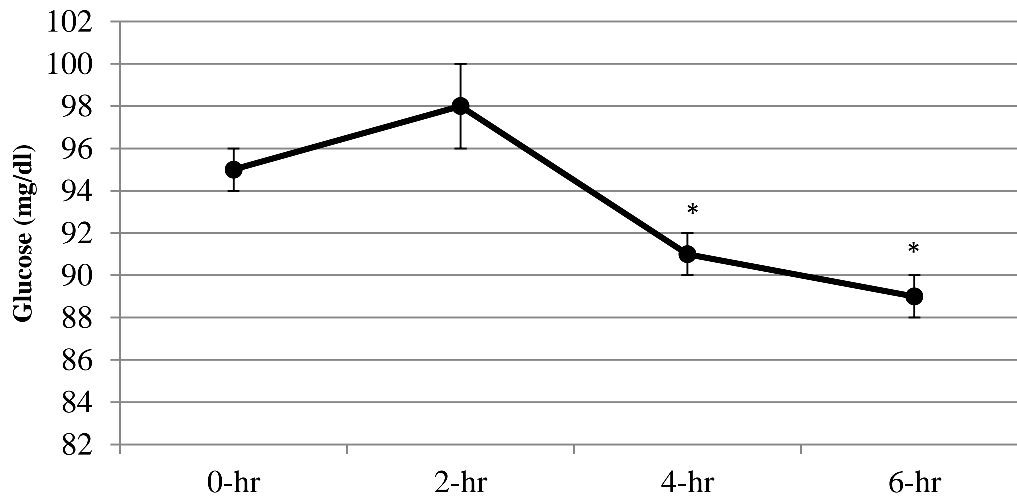


FIGURE 7. Temporal postprandial glucose response. Means ± standard error are presented. * = significantly different than 0 and 2 Hr. 0-hr = pre-meal blood sample.

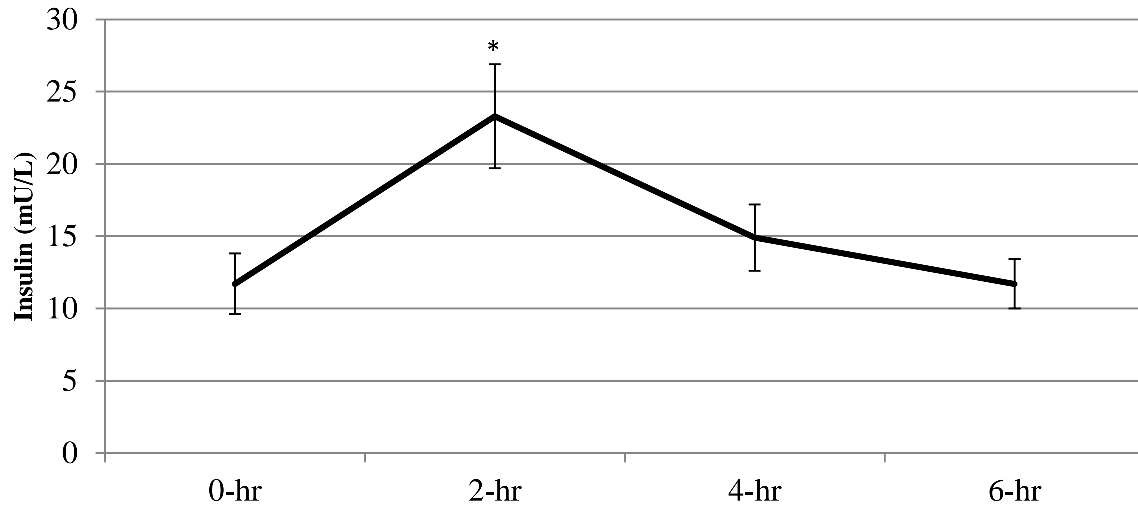


FIGURE 8. Temporal postprandial insulin response. Means + standard error are presented. * = significantly different from 0,4, and 6- hr. 0-hr = pre-meal blood sample.

CHAPTER FIVE

Discussion

Primary Findings

The purposes of this study were to determine the effects of LI and HI exercise on postprandial lipemia in sedentary, overweight men and to quantify the contribution of EPOC to reductions in postprandial triglycerides. Our findings demonstrate that LI and HI favorably and similarly reduce postprandial triglyceride levels below non-exercise control values. This observation is supported by a similarly reduced AUC_T and AUC_I following all exercise trials when compared to control. It may be argued that the small, subtle difference observed at 6 hours postprandially distinguishes LI from HI to attenuate postprandial triglycerides. Additionally, the reduction in AUC_T was 4% lower following LI when compared to HI. However, there is little to no evidence supporting that the lower triglyceride response at 6 hours, or the small differences in AUC_T were of any meaningful physiological significance. Our results suggest that LI and HI reduce postprandial lipemia to a similar extent in men of low cardiovascular fitness. These findings are in agreement with other studies that have shown reductions in postprandial lipemia following aerobic exercise, however, our results do not support our primary hypothesis that HI would produce a significantly lower triglyceride response when compared to LI (25-26,28,34,36). HI did not confer additional benefits toward reducing postprandial lipemia.

Increased EPOC following HI does not result in additional reductions in postprandial triglycerides. Our interpretation is based on the fact that no significant differences in AUC_T or AUC_I were observed between HI and LI trials. Moreover, the triglyceride lowering effects of exercise were not altered by re-feeding the difference in post-exercise energy expenditure between LI and HI. Both LI and HI produced a postprandial triglyceride response at 4 hours that was significantly lower than the control or HI + EERM conditions, and AUC_I was 11% and 7% lower than HI+EERM in the LI and HI trials. An argument could be made that that LI and HI confer a small benefit above HI + EERM based on these results. However, our results do not indicate any significant benefit of LI and HI above HI+EERM, evidenced by the non-significant differences in AUC_T and AUC_I between the 3 exercise conditions.

Our primary hypothesis was based on work that has demonstrated that the energy expenditure of the exercise session partially explains decrements in postprandial lipemia (30-31,33-34). Our exercise sessions were isocaloric, and we did not find significant differences in total EPOC, EPOC energy expenditure, or EPOC time between the LI and HI trials. Thus changes in EPOC between LI and HI did not account for alterations in postprandial lipemia. While we cannot support our hypotheses, our findings support the contention that the reductions in postprandial triglycerides are dependent on exercise energy expenditure, as EPOC did not significantly alter the caloric expenditures of the exercise sessions.

EPOC

Although the differences were not statistically significant, EPOC was 210% higher following HI when compared to LI, increasing from 9.1 to 4.4 L. These findings

are similar to others reported in the literature. In women, EPOC was significantly different following isocaloric moderate- and high-intensity exercise (50 and 75% of VO_2max), and was equal to 4.8 and 9 L (128). Gore and Withers (130) have also shown that EPOC increases with increasing intensity, demonstrating that 50 minutes of moderate- (50% of VO_2max) and high- (70% of VO_2max) intensity exercise produced EPOC values of 5.19 and 10.04 L. In the latter study, however, exercise sessions were not isolcaloric, and thus a potential effect of caloric expenditure cannot be ruled out. It is possible that, had our exercise been of a greater duration or intensity, the difference in EPOC would have been greater between LI and HI. Indeed, Borsheim and Bahr (68) have conducted an extensive review of literature on EPOC and have concluded that exercise intensity makes the greatest contribution to EPOC. Furthermore, our participants were of low cardiovascular fitness, and thus the absolute VO_2 that each participant was able to maintain continuously during HI was relatively low compared to those of average or high-fitness (143). Thus, HI for these participants may have produced a smaller EPOC than would have been observed for an individual capable of maintaining a higher oxygen consumption continuously. Based on our results, it can be concluded that, although EPOC is indeed elevated to a greater extent following HI when compared to LI exercise, the differences are not robust enough to drastically increase energy expenditure. Our findings may seem contradictory to those of Katsanos, et. al., (61) who reported finding a significantly lower triglyceride response following moderate- (65%) when compared to low-intensity (25%) exercise. However, in the former study physically active participants with a substantially higher VO_2peak were examined, and the intensities used differed by 40% of VO_2peak. Thus the absolute differences in oxygen

consumption between the low- and moderate- intensity trials were likely greater than that achieved in our study.

Only 3 studies have reported the effects of re-feeding on postprandial lipemia and have shown that re-feeding the energy that was expended during exercise reduces the positive effect of prior exercise on triglycerides (36-38). These examinations included protocols where the entire exercise energy expenditure or oxidized carbohydrate was re-fed, and thus the participants ingested a substantially greater caloric load than was given in our study. Burton, et.al, (36) re-fed 110% of expended calories in attempt to account for EPOC, however none of these investigations quantified EPOC. Our study contributes to our understanding of the effects of re-feeding on postprandial lipemia by showing that EPOC energy expenditure is not sufficient to abolish the exercise induced reductions in postprandial triglycerides.

We observed no change in NHDLC across conditions, supporting the current recommendations that NHDLC values are a useful index in both fasted and postprandial states (1,5). ApoB and ApoA1 rose across time points in all trials, with no difference between exercise and control conditions. The ApoB to A1 ratio rose at 4 and 6 hours postprandially, indicating that, as expected, apoB lipoproteins increased to a relatively greater extent than did ApoA1 containing lipoproteins after the high-fat meal.

We observed a main effect of time for insulin, with the 2-hour concentration being elevated under all conditions. We did not observe any significant differences in insulin across conditions. Mestek, et.al., (34) observed similar results in a cohort of men with MetS. However, our findings are contrary to some studies in which prior exercise significantly reduced insulin concentration below non-exercise control values during the

postprandial period (36,61). These discrepancies may be due to differences in the macronutrient composition of the test meal, as our meal was high-fat, and only contained approximately 17 g of carbohydrate.

NEFA concentrations responded to exercise as anticipated. We observed higher NEFA concentrations at times 0 and 2-hr following exercise, indicative of increased fatty-acid mobilization during and in the hours following exercise when compared to control. However, by 4 and 6-hr into the postprandial period, there were no differences in NEFA concentrations between conditions. These results are in agreement with others that have shown NEFA to increase following exercise conditions (36).

Participants

As intended, our participants represent young and middle-aged, sedentary, overweight men. Our results are in agreement with those from other studies where aerobic exercise has significantly lowered postprandial triglycerides in sedentary, obese individuals and those with MetS (34-36). Each of our participants was sedentary, reporting averaging less than 2.5 hours per week low- to moderate- physical activity. Their below-average fitness was confirmed by the group mean VO_2 peak of 31.1 ± 7.5 ml/kg/min, representing the 10th percentile for age (143). The mean BMI for the group categorized these individuals as obese, with body fat percentage confirming that excess body weight was chiefly due to adiposity. Each had 1 or more criteria for MetS, and, despite lack of physical activity and obesity, were otherwise healthy. None reported signs or symptoms suggestive of cardiovascular or metabolic disease.

Limitations

The results of this study are limited to Caucasian, sedentary and overweight men of low cardiovascular fitness. Participants were asked to maintain regular diet and physical activity habits through the course of the study, and to our knowledge, were able to comply with our request. Their compliance was supported by the lack of difference in body weight, resting oxygen consumption, and all blood lipid variables before each experimental condition. None-the-less, it is possible that variation in these outside factors could have confounded our results.

Our high-fat test meal was primarily composted of milk-fat. It is possible different results would have been obtained for the variables of interest, specifically triglyceride and insulin, had our meal contained a greater amount of unsaturated fat, carbohydrate and/or protein. Additionally, our results are limited to an intensity range of 40 and 70% of VO_2 reserve, and a 500 calorie energy expenditure. While it is possible that greater energy expenditure would have produced greater decrements in postprandial triglyceride response, our caloric expenditure is well above the suggested threshold needed to elicit a positive response (30).

Summary and Conclusions

This the first study to quantify the contribution of EPOC to changes in postprandial lipemia. Our findings indicate that, both LI and HI, producing 500 calories of energy expenditure, similarly alter postprandial lipemia. Despite a two-fold greater EPOC following HI when compared to LI, the contribution of EPOC energy expenditure to alterations in postprandial lipemia is not substantial. Our results suggest that exercise can be performed at low-, or high-intensity to achieve reductions in postprandial lipemia,

and that EPOC does not significantly contribute to exercise energy expenditure in sedentary, overweight men.

APPENDICES

APPENDIX A

Flyer

RESEARCH STUDY

THE EFFECTS OF EXERCISE INTENSITY ON POSTPRANDIAL
BLOOD LIPID AND VASCULAR FUNCTION

Be part of an important study that will help us determine the effects of exercise intensity on cardiovascular and metabolic health.

If you are selected to participate you will be required to:
1. Walk or run on a treadmill at different intensities (moderate or high)
2. Be monitored for 2 hours after exercise
3. Provide blood samples for 6 hours after consuming a milkshake

All participants will receive:
1. A comprehensive health profile (cardiovascular fitness, blood work, body composition assessment, health risk information, dietary profile, and exercise prescription based on personal information)
2. A free one-year follow-up assessment of cardiovascular fitness and body composition
3. A final report of our study results

You may be eligible to participate if you:
1. Are a male between the ages of 25 and 55
2. Do not smoke or use tobacco
3. Are not currently exercising on a regular basis

For more information please contact:

Laurel Littlefield or Zach Papdakis
254-710-3243
Laurel_Littlefield@baylor.edu
Or
Dr. Peter Grandjean
Peter_Grandjean@baylor.edu
This study is being conducted at the Baylor Laboratories for Exercise Science and Technology,
Marrs McLean Gym #127
Department of Health, Human Performance and Recreation,
Baylor University

APPENDIX B

Initial Screening Survey

Initial Screening Survey

Instructions: Progress through these questions until an exclusionary response is given.

1. Name: ____

2. Age: _______________ Ht: _______ Wt: _______BMI: __

3. Do you have a history of cardiovascular disease, lung disease, diabetes, or hypothyroidism?____
____
____

4. Are you currently taking any prescription medications, vitamins, or dietary supplements? ____
____
____

5. Are you a cigarette smoker or have you quit within the last 6 months?_______

6. Have you participated in any form of leisure-time physical activity or structured exercise in the past six months? _______________

Frequency/Intensity/Duration: ____
____
____
____

Describe the type and amount of physical activity required by your job: ____________
____
____
____

7. Do you have any orthopedic (or other) problems that would interfere with exercise? ___
____
____

8. Are you currently practicing a diet or are you involved in a weight loss program? _____
____
____

9. Do you consume dairy products on a regular basis? _____ Are you lactose intolerant? _

10. Contact Information:

Home Phone: __________________________

Cell Phone: ___________________________

Work Phone: __________________________

e-mail: ______________________________

APPENDIX C

Informed Consent

Baylor Laboratories for
Exercise Science & Technology
HHPR / One Bear Place 97313
Waco, Texas 76798-7313

Lab: 254-710-7199
Office: 254-710-3909
Fax: 254-710-3527
Peter_Grandjean@Baylor.edu

BAYLOR
UNIVERSITY

CONSENT DOCUMENT
for a Research Study entitled

"THE EFFECTS OF EXERCISE INTENSITY ON BLOOD LIPIDS AND VASCULAR FUNCTION AFTER A HIGH-FAT MEAL"

INVITATION and PURPOSE

You are invited to participate in a research study that is being conducted to investigate the influence of aerobic exercise, performed at different intensities, on blood lipid responses and blood vessel function that occurs in the hours after a high-fat meal.
Experts from the American College of Sports Medicine and American Heart Association recognize that higher-intensity exercise may impart some additional health benefits than lower-intensity exercise when the exercise of different intensities results in similar energy expenditure. In fact, these organizations recommend that high-intensity exercise be incorporated into exercise programs for most healthy adults. The effectiveness of higher-intensity exercise has been demonstrated for blood pressure and blood cholesterol. Greater improvements in cardiovascular fitness and body composition can occur at higher-intensities when exercise is regularly-practiced.
Reasons for the added health and fitness benefits that accrue with higher- versus lower-intensity exercise are not known. A primary reason for the greater effects of higher-intensity exercise may be due to the extra caloric expenditure that occurs after exercise, rather than solely due to the calories expended during the exercise session. This theory has not been directly assessed. Moreover, the influence of higher-intensity exercise on metabolic and vascular health after a meal (e.g., the postprandial period) has not been well-characterized in healthy middle-aged adults. *Results from this study will help us understand why high-intensity exercise can impart additional health benefits above and beyond what might result from a similar amount of lower-intensity exercise. It will also provide valuable information for health practitioners who prescribe exercise for adults with limited time to exercise.*

You have been asked to volunteer for this study because you are a male between 25 and 55 years of age and do not regularly engage in physical activity or exercise. You have

no physical conditions or medical considerations that would prevent you from walking or jogging on a treadmill or that prevent us from safely and reliably measuring responses in postprandial blood lipids and blood vessel reactivity (e.g., vascular function) following a high-fat meal.
We have two specific objectives for this study. First, we want to compare the effectiveness of higher-intensity versus lower-intensity exercise on postprandial blood lipids and vascular endothelial function. Second, we want to determine if the additional energy expenditure after higher-intensity exercise explains or accounts for the different responses in postprandial lipids and vascular function that occurs with higher-intensity exercise.

PARTICIPANT REQUIREMENTS and PRELIMINARY SCREENING

Participant Criteria

As a research volunteer, you may be eligible to participate in this study if you meet the following characteristics: **1)** you are a healthy male between the ages of 25 and 55; **2)** you are overweight or modestly obese – defined as having a body mass index (BMI) between 25 and 35 kg/m^2; **3)** you are physically-inactive – defined as engaging in no regular leisure-time or work-related physical activity over the last 6 months; **4)** you are a non-smoker; **5)** you are able to ingest dairy products without adverse gastrointestinal reactions (e.g., you are lactose tolerant), and; **6)** you are not currently taking medications known to influence blood pressure, lipids and lipoproteins, or glucose metabolism.

Preliminary Screening

Visit 1: We will verify some of your personal characteristics by phone, e-mail, or in-person interview. If you appear to meet entry criteria for the study, we will schedule an appointment to meet with you in order to verify your eligibility for the study. During this initial visit, we will explain and review all of the experimental procedures and you will be given a copy of this consent document to review and sign. We will keep the original consent form and a copy of the signed document will be given to you for your records. If you agree to participate and after you sign the consent document, we will ask you to complete a *Health History Questionnaire* and we will measure your height and weight. We estimate that this first visit will take up to one (1) hour to complete
Between your first and second visit to our lab, your *Health History Questionnaire* responses will be reviewed by one of our medical consultants (Jackson Griggs, MD; Mike Hardin, MD; Sally Weaver, MD; Nicholas Schwedock, MD, or; Ron Wilson, MD) for possible contraindications to exercise. Review of your *Health History Questionnaire* by one of these physicians is merely a precautionary step to help insure your safety. Based on our medical consultant's guidance: **1)** we will invite you to return to the lab to undergo some additional physical measurements related to the objectives of this study; **2)** we will require you to obtain medical clearance from your doctor before scheduling a second visit to the lab, or; **3)** we will determine that you are ineligible to participate in this study.
Visit 2: You will be asked to report to the lab after an 8- to 10-hour fast (limited to water ingestion only). You will report with shorts, t-shirt, and a comfortable pair of sports

shoes. We will review all of the experimental procedures again with you and answer any questions you may have concerning this study.
We will obtain a small blood sample (17 ml, or just over 1 tbsp) and measure your height, weight, and waist circumference. Next, we will measure your body composition (e.g., your lean and fat tissue) by dual-energy x-ray absorptiometry (DXA).
Next, you will be fitted with three surface electrodes on your chest. These electrodes will be used to track your cardiac cycles (i.e., your heart rate). We will then ask you to lie supine at rest for 10 minutes. During your supine rest, we will locate the brachial artery on the medial side of one of your upper arms using ultrasound. We will document the location and use this same location to measure changes in blood flow and the diameter of your brachial artery throughout the investigation. After we determine the best location to measure changes in your brachial artery, we will measure changes in blood flow and brachial artery diameter by ultrasound.
You will then be asked perform a standardized maximal graded exercise test on a treadmill to determine your cardiovascular fitness. We will monitor your heart rate, blood pressure, and rating of perceived exertion (RPE) throughout the test. You will be asked to breathe into a mouthpiece that is connected to a computer while you are exercising. This will allow us to measure your respiratory gasses (VO_2 and VCO_2). The test will begin at a fairly easy pace and the speed and grade of the treadmill will be increased every 3 minutes. This is a maximal exercise test, and therefore the last few minutes of the test will be challenging. We will stop the test at your request, or should you experience any uncomfortable symptoms such as dizziness, chest pain, or lightheadedness. This test will usually last about 6 to 12 minutes. We will use the results to describe your cardiovascular fitness and to calculate the appropriate exercise intensities for your experimental exercise sessions.
We will describe and explain all procedures for completing your diet and physical activity records to complete this visit. You will complete these records for 2 days prior to each of the experimental conditions. We estimate that the second visit will take up to two (2) hours to complete.

Participant Benefits
For your participation, you will receive:

1) An individualized and confidential report of your preliminary fitness assessment. Your report will include a cardiovascular disease risk evaluation from your *Health History Questionnaire* responses and your blood work; a diet analysis; assessments of your body composition and cardiovascular fitness, and; a personalized exercise prescription based on your current fitness status and fitness goals.
2) A free 1-year follow-up assessment of cardiovascular fitness and body composition.
3) A final report of your personal responses and the group responses to the experimental interventions.

EXPERIMENTAL METHODS & APPROACH
Study Overview
There are four (4) experimental conditions. Therefore, you will be asked to visit the lab on four (4) different occasions in order to address the objectives of this study. One of the experimental conditions will be a non-exercise “control” condition”. Three of the

conditions include walking on a treadmill to expend a target of 500 calories. Except for the fourth condition, all conditions will be completed in random order. The control condition will measure diameter changes in your brachial artery due to increased blood flow and your resting oxygen consumption over a 2-hr period. Next, you will consume a test meal (a milk shake) and we will obtain blood samples and ultrasound measurements of your brachial artery at regularly-scheduled intervals for up to 6 hrs. The exercise experimental conditions will require that you walk or jog on a treadmill to expend 500 kcals at either a higher- or lower-intensity. In the second and third condition, exercise will be followed by 2 hrs of resting post-exercise oxygen consumption, after which you will consume a test meal and we will obtain blood samples and ultrasound measurements of your brachial artery at regularly-scheduled intervals for up to 6 hrs. In the fourth condition, you will exercise at higher-intensity to expend 500 kcals again. Immediately after this session, you will consume a meal that has a caloric content equal to the difference in calories spent in the hours after the lower- and higher-intensity exercise sessions. All experimental conditions will be completed at the same time of day.
All preliminary screening and experimental procedures will take place in the Baylor Laboratories for Exercise Science and Technology (rooms 127 and 233.11 in the Marrs-McLean Gym).

Experimental Procedures
Diet and Physical Activity Record-Keeping
We will ask you to maintain complete records of your diet and physical activity for two (2) days leading up to the each experimental condition. You will follow all diet and physical record-keeping instructions and deliver these daily records to the lab by e-mail attachment, fax, or in-person in the morning following each day. It is important that you follow the same daily diet and physical activities prior to and during each experimental condition so that differences in diet, nutrient intake, and physical activity do not affect our measurements. We will ask you to immediately contact the lab technician responsible for diet analysis if you have questions concerning your diet or meal composition during any record-keeping period.
Blood Sampling
After two (2) days on a standardized diet (see above) and an 8- to 12-hr fast, you will be asked to return to the lab to have your blood drawn. You will be asked to refrain from any moderate or strenuous physical activity for 72 hrs (3 days) prior to blood sampling. A blood sample, equal to about 1.1 tbsp (17 ml), will be obtained by inserting a small catheter with a needle into the most prominent vein site in your lower arm. (NOTE: We will use the arm that is not being used for brachial artery measurements to obtain blood samples. By using the catheter, we are able to take multiple blood samples with only one needle "stick". The venous catheter will stay in your arm for the remainder of the experimental condition. We will withdraw the catheter immediately after the 6-hr post-prandial blood sample.)
Postprandial Milkshake
You will be asked to drink a "milkshake" consisting of whipping cream (20 tbsp) and ice cream ($^1/_2$ cup) within 15 minutes. The milkshake is designed to be high in fat and contains approximately 1000 Calories, 100 g fat, 17 g carbohydrate, and 3 g protein. We will ask you to remain at the lab to measure blood samples at 2 hour intervals up to 6

hours after you drink the milkshake. You will be asked to remain in the lab over the 6-hr period but will be allowed to perform light activities such as reading, watching television, paperwork, computing, etc.

Brachial Artery Vessel Reactivity

We will use ultrasound to measure changes in the diameter of your brachial artery. In order to obtain these measurements, we will place electrodes on your chest to track your cardiac cycles. You will be asked to lie down and rest for 10 minutes prior to each measurement, after which we will measure your resting blood pressure using a stethoscope and inflatable cuff attached to an aneroid sphygmomanometer. We will place a small amount of gel on your arm and locate the brachial artery site using a transducer attached to ultrasound equipment. Next, we will place a blood pressure cuff around your lower arm and inflate it to 50 mmHg above your systolic blood pressure. We will leave the cuff inflated to this pressure for 5 minutes in order to completely occlude blood flow. The cuff will then be deflated rapidly to allow for maximal blood flow through the artery. We will measure blood flow, the shear stress of blood against the artery wall, and the change in artery diameter for up to four (4) minutes after releasing the cuff pressure. This procedure will initiate each experimental protocol and will precede each blood sample during the experimental condition.

Sequence of Experimental Procedures

Non-Exercise Control: You will report to the lab after two (2) days on a standardized diet and an 8- to 12-hr fast limited to water intake only. You will be measured for height and weight, be fitted with the chest electrodes, and then be asked to rest quietly in a supine position for 20 minutes. During your supine rest, we will obtain a blood sample (17 ml or 1.1 tbsp) by venipuncture. Next, we will locate the documented brachial artery site obtained during preliminary screening. After 10 minutes of supine rest, we will inflate a forearm cuff (to a pressure determined during preliminary screening) for 5 minutes. We will then measure changes in blood flow and the diameter of your brachial artery for 4 minutes after releasing the cuff pressure according to the procedures that have previously been described. Next, you will be asked to sit upright. We will fit you with a mask so that we can measure your respiratory gasses while you sit quietly for one (1) hr. Immediately following respiratory gas measurements you will drink/eat the high-fat milkshake and undergo the 6-hr postprandial blood sampling. We estimate that these procedures, in total, will take eight (8) hrs to complete.

Exercise Sessions: You will report to the lab after two (2) days on a standardized diet and an 8- to 12-hr fast limited to water intake only. We will obtain a blood sample, measures of brachial artery reactivity and respiratory gasses as described above. Next, you will be asked to walk or jog on a treadmill in order to expend 500 kcals of energy. The time it takes to expend this amount of energy will depend on the exercise intensity chosen for the session. We estimate that you will walk for 70 to 90 minutes with the lower-intensity exercise [40 to 50% of your heart rate reserve (HRR)]. The time will be somewhat shorter (45 to 60 minutes) to achieve the caloric expenditure with the higher-intensity walking or jogging (70 to 80% of HRR).

All exercise sessions will be carefully monitored by one or more of the investigators. We will measure your exercise heart rate continuously. We will measure your respiratory

gasses at regular intervals in order to maintain the appropriate exercise intensity and to achieve the target energy expenditure.

Immediately following the exercise session, we will measure your respiratory gasses while you sit quietly for up to two (2) hrs or until you reach your pre-exercise oxygen consumption levels. During this time period you will be allowed to drink water only, and will be asked to remain in the lab and complete only light activities as previously described. The post-exercise measurement of respiratory gasses will help us determine the additional energy expenditure that occurs after higher- versus lower-intensity exercise. *We will use the difference in post-exercise energy expenditure between the high- and low-intensity sessions to determine the caloric content of the meal you will be fed immediately after exercise in the fourth experimental condition.* Immediately following post-exercise respiratory gas measurements you will drink/eat the high-fat milkshake and undergo the 6-hr postprandial blood sampling. We estimate that these procedures, in total, will take ten (10) hrs to complete.

The fourth experimental condition will require you to replicate the higher-intensity exercise session as before. However, we will not measure respiratory gasses after this exercise session. Instead, immediately after exercise, you will be fed a small meal that has a caloric content equal to the difference in calories spent in the hours after the lower- and higher-intensity exercise sessions that you previously completed. This meal will be a portioned amount of a commercially-available sports nutrition bar. *The completion of this experimental condition will help us determine if the additional energy expenditure after higher-intensity exercise explains or accounts for the different responses in postprandial lipids and vascular function that occurs with higher-intensity exercise.*

The data collected under all experimental conditions will enable us to compare the effectiveness of higher-intensity versus lower-intensity exercise on postprandial blood lipids and vascular endothelial function.

Please note: You will not be able to use your cell phone during brachial artery measurements. Cell phone and computer use will be limited to texting during respiratory gas analysis. The mask you will wear during respiratory gas analysis will prohibit any verbal communication.

Summary

There are a total of four (4) experimental conditions: Control; Lower-Intensity Exercise, and; Higher-Intensity Exercise (2 sessions). Each condition will be separated by at least three (3) days and no more than fourteen (14) days. The total time commitment, including preliminary screening, will be approximately 42 hours over 10 visits to the lab. However, approximately 28 hours may be spent watching television, performing light activities such as reading or computing, and other non-physical activities in the lab. In addition, a total of 24 blood samples will be obtained from you for a total blood volume of 408 mL (28 tbsp) over a 4 to 5 week period. The experimental protocol for each condition is illustrated in the figure below.

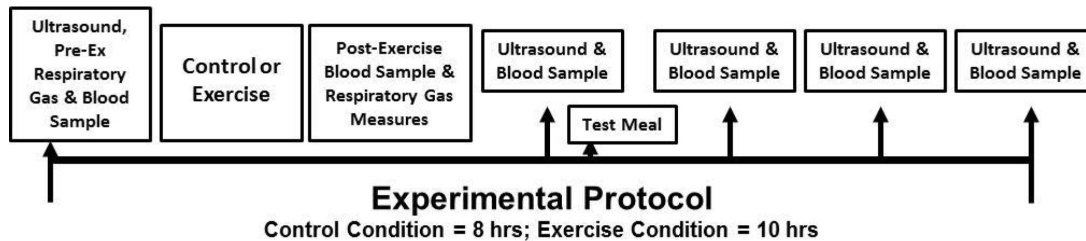


Figure 1. Experimental Protocol. We will obtain a blood sample and ultrasound measurements of your brachial artery followed by one hour of respiratory gas data (VO_2 and VCO_2) during the control condition. The exercise conditions will require you to walk or jog on a treadmill at either higher- or lower-intensity to expend 500 kcals of energy. We will measure your respiratory gasses for up to 2 hours after exercise. In all conditions, you will be asked to ingest a test meal (high-fat milk shake). We will obtain measures of your brachial artery and collect blood samples immediately prior to and every 2 hours after the test meal ingestion. We will collect data for up to 6 hrs after you consume the test meal. The fourth experimental session will be a replicate of your higher-intensity exercise condition followed by a small meal that is equal to the difference between the calories spent after lower- versus higher-intensity exercise. We will not measure respiratory gasses after exercise in the fourth experimental condition. There are a total of 4 experimental conditions: Control; Lower-Intensity Exercise, and; Higher-Intensity Exercise (2 sessions). Each condition will be separated by at least three (3) days and no more than fourteen (14) days. The control and all exercise sessions will be conducted at approximately the same time of day.

DISCOMFORTS and RISKS

1. The primary risk associated with dual-energy x-ray absorptiometry (DXA) is with radiation exposure. The radiation dose for one DXA scan to measure body composition is 1.5 millirem. This is similar to the amount of natural background radiation you would receive in one month while living in Waco, TX or less than what you would be exposed to during an airplane flight from New York City to Los Angeles (2 to 5 millirem). The maximal permissible x-ray dose for non-occupational exposure is 500 millirem per year.
2. The primary risk associated with respiratory gas analysis at rest, during graded exercise testing, the exercise sessions, and in the hours after exercise is with contamination of the mouthpiece and tubing.
3. The risks associated with maximal exercise testing are comparable to those faced when performing vigorous exercise. These include occasional abnormal blood pressure responses, the possibility of fainting, potentially abnormal heart beats, heavy and difficult breathing, and in rare instances heart attack or death. According to the reports published in the American College of Sports Medicine's Guidelines for Exercise Testing & Prescription (8th Edition, 2010) the mortality rate of maximal exercise testing is minimal with an observed rate of 0.5 deaths per 10,000 (0.005%) tests performed. The incidence of an untoward event or a medical injury is 8 events per 10,000 (0.08%).
4. According to the American College of Sports Medicine the risks associated with sub-maximal exercise are even lower than the risks associated with maximal exercise testing. Possible risks are the same as the maximal exercise test and are listed above.
5. Blood sampling may impose minor bruising, swelling, and itching of the affected area. As with any similar procedure that disrupts the skin barrier, there is an increased risk of infection.
6. There are no physical risks from ingesting the test meal (high-fat milk shake). The milk shake is similar to those purchased at popular fast food restaurants.

7. There is some physical discomfort associated with ultrasound measurement of the brachial artery. The discomfort may result from prolonged occlusion of brachial artery blood flow with an arm cuff. The cuff will be inflated to a pressure that exceeds systolic blood pressure by 50 mmHg for 5 minutes.
8. We will place electrocardiography electrodes on your torso during measurement of your brachial artery. This requires scrubbing the skin and wiping with an alcohol pad. The possible risks associated with this include an allergic reaction to the alcohol pad and abrasion or minor cuts to the scrubbed area. There is a slight possibility that you will be allergic to the gel used in the electrodes. This may cause some itching and redness of the area that might last for several days.

PRECAUTIONS TAKEN TO MINIMIZE DISCOMFORTS and RISKS

1. Our DXA equipment is checked by Baylor University's Risk Management and Safety on a regular basis to ensure radiation exposure remains at very low levels. In order to keep your exposure to a minimum, we will calibrate our DXA equipment before you arrive for your DXA assessment. You will undergo only one DXA scan.
2. All respiratory gas analysis will be performed with cleaned and disinfected pneumotachs, mouthpieces, masks, and tubes. This equipment will be cleaned with soap and water and disinfected with a Cydex® after each use. Mouthpieces, pneumotachs, masks, and tubing will be rinsed and allowed to air dry before reuse. Mouthpieces, pneumotachs, masks, and tubing will not be reused at the same testing session.

3 & 4. Every effort will be made to minimize all of the physiologic risks inherent with vigorous exercise through preliminary screening, identification of contraindications to exercise testing, adherence to standards of practice for graded exercise testing that are published by the American College of Sports Medicine (ACSM Guidelines 8th ed., 2010), and personal monitoring of each test by trained technicians. All graded exercise tests will be performed by Dr. Grandjean and research assistants working in the lab. Dr. Grandjean is an ACSM-certified Clinical Exercise Physiologist and has current ACLS Provider credentials. Mrs. Littlefield, Mr. Papadakis and Mr. Rigby hold current CPR certification.

5. Universal precautions will be observed for each blood sample and the following procedures will be used to help minimize any potential risks to the participant and investigator: the technician will use surgical latex gloves; the antecubital area will be cleansed with an alcohol pad prior to puncturing the skin; all blood draw equipment and instruments will be sterile; and all punctured sites will be properly dressed with antiseptic and bandage following each sample collection. Dr. Grandjean will oversee all phlebotomy procedures throughout the preliminary and experimental blood sampling.
6. We will screen all participants for lactose intolerance to minimize the risk of gastrointestinal discomfort associated with consuming dairy products.
7. The tolerance for the blood flow occlusion procedure increases with practice. We will review all procedures, including cuff placement and inflation pressure, with you prior to obtaining ultrasound measurements of your brachial artery.

8. We will use supplies and procedures typically employed in the clinical setting for the careful placement of electrocardiographic electrodes. We will ask you to report any adverse feeling or reaction to the electrodes.

If we find physical problems that, in our judgment, make completing the experimental procedures risky, for your own protection we will not allow you to continue in this study. Compensation for participating in the study will not be provided and will not include medical costs for physical injury or adverse effects. You, as the participant, will be responsible for the cost of medical care needed as a result of participating in the study.

YOUR RIGHTS TO PRIVACY

All individual information obtained in this study will remain confidential and your right to privacy will be maintained. Data collected will be used for research purposes only and will be limited to access by the investigators of this study. Only data reported as group means or responses will be presented in scientific meetings and published in scientific journals. Confidential data will be destroyed following the project.

QUESTIONS ABOUT THIS RESEARCH

As investigators, it is our obligation to explain all of the procedures to you. We want to make sure that you understand what is required of you and what you can expect from us in order to complete this research project. Please do not hesitate to inquire about the research, your rights and responsibilities as the participant, or our roles as the investigators now or at any time throughout the study.

YOUR CONSENT TO PARTICIPATE

Participation in this research is entirely voluntary. Your decision whether or not to participate will not jeopardize your future relations with Baylor University and/or the Department of Health, Human Performance and Recreation. You may withdraw your consent and discontinue participation at any time and for any reason without prejudice. Discontinuing your participation will involve no penalty of any kind.

Failure to comply with the procedures and to follow the instructions necessary for reliable and valid scientific measurements may result in termination of your participation in this study without your consent. You may be asked to withdraw if you fail to comply with the requirements for participation listed above. If you are withdrawn from participation by one of the investigators, our decision will not jeopardize your future relations with Baylor University and/or the Department of Health, Human Performance and Recreation.

ADDITIONAL INFORMATION REGARDING YOUR RIGHTS

If you have questions about your rights as a research participant, you may contact the Baylor University Committee for Protection of Human Subjects in Research. The current IRB Chair is Dr. David W. Schlueter, Baylor University, One Bear Place #97368 Waco, TX 76798-7368. Dr. Schlueter may also be reached at (254) 710-6920 or (254) 710-3708 or by e-mail at David_Schlueter@baylor.edu.

PRINCIPLE INVESTIGATOR'S CONTACT INFORMATION

Peter Grandjean, Ph.D.
Associate Professor, HHPR
Office: 254-710-3909
Cell: 334-444-4641
Peter_Grandjean@baylor.edu

CO-INVESTIGATOR'S CONTACT INFORMATION

Zach Papadakis, M.S.	**Laurel Littlefield, M.S.**	**Rhett Rigby, M.S.**
Doctoral Student	Doctoral Candidate	Doctoral Candidate
Lab: 254-710-7277	Lab: 254-710-7277	Lab: 254-710-7277
Zach_Papadakis@baylor.edu	Laurel_Littlefield@baylor.edu	Rhett_Rigby@baylor.edu

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES YOUR WILLINGNESS TO PARTICIPATE.

A COPY OF THIS DOCUMENT IS YOURS TO KEEP.

________________________ ______ ________________________

Participant's Signature **Date** **Investigator Obtaining Consent Date**

________________________ ______________________________

Printed Name **Printed Name**

APPENDIX D

Health History Questionnaire

HEALTH & LIFESTYLE HISTORY QUESTIONNAIRE

This information is being collected solely for use in the study entitled, ***"The Effects of Exercise Intensity on Postprandial Blood Lipids and Vascular Function".*** All information obtained in this document will be treated as privileged and confidential. Please complete this form as accurately and completely as possible. The information you provide will be used to evaluate your health by the principle investigator who will oversee the conductance of this study and the medical consultants listed in the consent document. All information will be treated as privileged and confidential.

1. IDENTIFICATION & GENERAL INFORMATION

Name			Today's Date
			/ /
Age	**Date of Birth**	**Gender**	**Occupation**
	/ /		

Home Address	City	State	ZIP

Home Phone	Work Phone	e-mail

Emergency Contact	Phone	Physician	Phone

Please check the box that applies to you:

Race or Ethnic Background

- ☐ White, not of Hispanic origin
- ☐ American Indian / Alaskan native
- ☐ Asian
- ☐ Black, not of Hispanic origin
- ☐ Pacific Islander
- ☐ Hispanic

2. ILLNESS & MEDICAL HISTORY

Check all of the conditions or diseases for which **you** have been diagnosed and/or treated. Also give the date of occurrence or diagnosis. If you suspect that you may suffer from one of the conditions, please indicate this in the right hand margin after the date.

Medical Condition	**Check if Applicable**	**Date Diagnosed (M / Yr)**	**Current?**
Allergies			
Arthritis			
Osteoarthritis			
Rheumatoid			

Medical Condition	**Check if Applicable**	**Date Diagnosed (M / Yr)**	**Current?**
Asthma			
Bronchitis (chronic)			
Bone Fracture			
Cancer of any kind			
Cataracts			
Cirrhosis (liver)			
Colitis (ulcerative)			
Depression			
Eating Disorders (anorexia, bulimia)			
Emphysema			
Epilepsy			
Frequent Bleeding			
Gallstones / Gallbladder Disease			
Glaucoma			
Gout			
Hearing Loss			
High Anxiety / Phobias			
Hepatitis / Other liver problems			
Osteoporosis			
Pneumonia			
Tuberculosis			
Renal / Kidney Problems			
Sleeping Problems			
Stomach / Duodenal Ulcer			

Substance Abuse Problems			
Rectal Growth or Bleeding			
Metabolic Problems Diagnosed	**Check if Applicable**	**Date Diagnosed (M / Yr)**	**Current?**
Thyroid Problems			
Diabetes			
Other			
Cardiovascular Problems Diagnosed	**Check if Applicable**	**Date Diagnosed (M / Yr)**	**Current?**
Angina			
Anemia (low iron)			
Coronary Disease			
Disease of the Arteries			
Enlarged Heart			
Heart Attack			
Heart Murmur			
Heart Rhythm Problem			
Heart Valve Problem			
Heart Problem (other)			
Heart Problem (other)			
High Blood Pressure (controlled)			
High Blood Pressure (uncontrolled)			
Peripheral Vascular Disease			
Phlebitis or Emboli			
Rheumatic Fever			
Rheumatic Heart Disease			
Pulmonary Emboli			

Other Health Problems			
Any other health problems (please specify and include information on any recent illnesses, hospitalizations, or surgical procedures			
Have you ever had:	**Check if Applicable**	**Date Diagnosed (M / Yr)**	
An abnormal chest x-ray?			
An abnormal electrocardiogram (ECG)?			
An exercise stress test?			
An abnormal exercise stress test?			
Orthopedic Problems	**Check if Applicable**	**Date Diagnosed (M / Yr)**	**Current?**
Low Back Pain			

Shoulder Pain			
Elbow Pain			
Wrist or Hand Pain			
Hip Problems			
Knee Problems			
Ankle or Foot Problems			
Is your work or any other activity limited by a current orthopedic problem? If so, please specify:			
Other Orthopedic Problems			
Any other orthopedic problems (please specify and include information on any recent illnesses, hospitalizations, or surgical procedures			

3. SYMPTOMS or SIGNS SUGGESTIVE of DISEASE

Do you presently have or recently had (Check if Applicable):

Yes	Description	Yes	Description
	Have you experienced unusual pain or discomfort in your chest, neck, jaw, arms, or other areas that may be due to heart problems?		Do you suffer from swelling of the ankles (ankle edema)?
	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling?		Have you ever experienced an unusual and rapid throbbing or fluttering of the heart?
	Have you had any problems with dizziness or fainting?		Have you ever experienced severe pain in your leg muscles during walking?
	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?		Has your doctor told you that you have a heart murmur?
	Have you ever experienced a seizure?		Have you ever had unexpected weight loss of 10 lbs or more?

4. CHRONIC DISEASE RISK FACTORS

Do you presently have or recently had (Check if Applicable):

Yes	Description	Yes	Description
	Are you a male over 45 years of age?		Is your total serum cholesterol greater than 200 mg/dL, or has your doctor ever told you that your cholesterol is at high-risk level?
	Has your father or brother had a heart attack, cardiac revascularization surgery, or died suddenly of heart disease before age 55; has your mother or sister experienced these heart problems before age 65?		Is your HDL cholesterol low (< 40 mg/dL for males) , or has your doctor ever told you that your HDL cholesterol is at high-risk level?
	Are you a current cigarette smoker?		Are your triglyceride levels > 150 mg/dL, or has your doctor ever told you that your triglycerides are at high-risk level?
	Has a doctor told you that you have high blood pressure (more than 140 / 90 mmHg), or are you on medication to control your blood pressure?		Are you physically inactive and sedentary (little physical activity on the job or during leisure time)?
	Do you have diabetes mellitus?		Do you weigh more than 20 lbs more than you should?

Additional Family History Information

Check all of the conditions or diseases for which **any member of your immediate family, including grandparents**, have been diagnosed and/or treated. Also provide their age and the date of occurrence or diagnosis if known.

Medical Condition	List Relative & Age at Diagnosis	Date Diagnosed (M / Yr)
High Blood Pressure before age 40		
High Cholesterol		
Obesity		
Diabetes		
Stroke under age 50		
Heart Attack under age 50		
Heart Operation		
Cancer under age 60		

Physical Activity Information

Please check the box that best describes you.

1. In general, compared to other persons your age, rate how physically fit you are:

Not at all fit	Slightly below average fitness	Average fitness	Slightly above average fitness	Extremely fit
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. Outside of your normal work, or daily responsibilities, how often do you engage in physical exercise?

☐ 5 or more times per week ☐ 3 - 4 times per week ☐ 1 - 2 times per week

☐ Less than 1 time per week ☐ Seldom or never

3. On average, how long do you exercise on each occasion?

☐ 10 - 20 min ☐ 20 - 30 min ☐ 30 - 40 min ☐ 40 - 50 min ☐ > 50 min

4. On a scale of 1 to 10 (1 being the lowest, 10 being the highest), how would you rate your exercise intensity ?

☐ Very Low (1 - 2) ☐ Low (3 - 4) ☐ Moderate (5 - 6) ☐ Mod. - High (7 - 8) ☐ High (9 - 10)

5. How much strenuous physical work is required on your job?

☐ A great amount (> 60%) ☐ A moderate amount (30 - 50%)

☐ A little (< 30%) ☐ None

6. How often does your work entail repetitive pushing and pulling or lifting while bending or twisting, leading to back pain?

☐ All of the time ☐ Most of the time

☐ Some of the time ☐ Rarely or never

Body Weight Information

1. What is the most you have ever weighed? ___________ When? ______________

2. Are you currently trying to:

- ☐ Lose weight
- ☐ Gain weight
- ☐ Stay the same
- ☐ Not trying to do anything

Substance Use

1. How would you describe your tobacco use habits?

- ☐ Never smoked
- ☐ Used to smoke (How long ago did you quit?):
- ☐ Still smoke (How many cigarettes / day?): ____

2. How many alcoholic drinks do you consume? (A "drink" is one glass of wine, a wine cooler, a bottle / can of beer, a shot glass of liquor, or a mixed drink).

- ☐ Never use alcohol
- ☐ Less than 1 per week
- ☐ 1 - 6 per week
- ☐ 1 per day
- ☐ 2 - 3 per day
- ☐ More than 3 per day

5. MEDICATIONS

Please indicate any medications, prescription or "over the counter" by providing the name and dosage:

Medication Type	Name of Medication	Dosage
Heart Medicine		
Blood Pressure Medicine		
Blood Cholesterol Medicine		
Insulin		
Other Medicine for Diabetes		
Thyroid Medicine		
Medicine for Breathing / Lungs		
Medicine for Weight Loss / Weight Control		
Hormones		

Painkiller Medicine		
Arthritis Medicine		
Medicine for Depression		
Medicine for Anxiety		
Medicine for Ulcers		
Allergy Medicine		
Other (please specify)		

In addition to the above information that you have listed, are you aware of any other conditions, symptoms, or special circumstances that might be related to your overall health and well being or that may influence your ability to participate in this study? _____
If so, please give an explanation below. ____

____

____

____

____

____

____

APPENDIX E

Physical Activity Record Sheet

Daily Food Record

This record will be used to determine your current caloric intake and the nutrient composition of your diet. Your accuracy and attention to detail will help us monitor your diet during experimental procedures.

Experimental Record: *Please record your diet beginning 2 days before reporting for your scheduled lab visit.*

- RECORD EVERYTHING YOU EAT AND DRINK INCLUDING SNACKS AND BEVERAGES.
- RECORD IMMEDIATELY AFTER FOOD IS CONSUMED
- INDICATE PORTION SIZES. MEASURE AMOUNTS OF EACH FOOD USING MEASURING CUPS OR SPOONS WHEN IT IS PRACTICAL. RECORD PORTION SIZES IN GRAMS, OUNCES, CUPS, TABLESPOONS, TEASPOONS, OR PIECES. (example: 8 oz. orange juice, 1 piece wheat bread, 1 tbsp. butter) YOU MAY ALSO USE OBJECTS OF KNOWN SIZE TO HELP QUANTIFY PORTION SIZES (your hand, a deck of cards, a tennis ball, etc.) YOU MAY USE YOUR CELL PHONE CAMERA TO HELP WITH THIS ASPECT OF YOUR RECORDKEEPING.
- INDICATE THE BRAND NAME. (3 oz. Ruffles BBQ Potato Chips, 1 cup Uncle Ben's Long Grain Rice, McDonald's Large French Fries)
- INDICATE FORM OF PURCHASE. (fresh, frozen, canned, etc.)
- RECORD TIME OF DAY MEAL WAS EATEN
- PLEASE INCLUDE THE FOOD PACKAGE LABEL WHENEVER POSSIBLE AND/OR INFORMATION FROM THE RESTAURANT'S WEBSITE. COPY RECIPIES FOR HOME-COOKED MEALS.

Ms. Laurel Littlefield will serve as the point-of-contact for dietary record keeping. If you have any questions, please contact us:

Ms. Laurel Littlefield	Dr. Peter Grandjean
Lab: 710-3243	Office: 710-3909
Laurel_Littlefield@baylor.edu	**Peter_Grandjean@baylor.edu**

Return your completed record to our lab in one of 3 ways:

1) hand-deliver it to our lab (MMG 127)

2) send it as an e-mail attachment to one of the e-mails listed above

3) fax it to 254-710-3527 (c/o Dr. Peter Grandjean)

Name: ___________________ Office Phone**:** ______________ **Cell Phone:** _____________

e-mail: ____

Week Day: _________________________ Date: _________________________

Time	Food Description	Portion Size	Quantity	Preparation (fried, grilled, baked, broiled)

APPENDIX F

Physical Avtivity Record Sheet

Daily Physical Activity Record

Name: ______________________ Office Phone: _____________ Cell Phone: ____________

e-mail: ____

Day: ________________________ Date: _____________________

Please complete the following Physical Activity Record as accurately as possible. Estimate the total number of hours you spend per day performing activities from the categories listed below. Report time spent in the activity to the nearest minute. Similar activities are grouped together. If you perform an activity that is not already included in **Categories 1-9**, choose a category which lists similar activities. If no category applies to your activity, use **Category 10** and specify the activity performed.

Category	Physical Activity Description	Time Spent in Physical Activity
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Category 1 = sleeping, resting in bed, sitting quietly
Category 2 = eating, writing
Category 3 = washing dishes, combing hair, cooking, driving
Category 4 = slow walking, dressing, showering
Category 5 = floor sweeping, mopping, slow cycling (5.5 mph), recreational volleyball
Category 6 = recreational golf, baseball, rowing, bowling, walking at moderate speed (3mph)
Category 7 = yard work, loading and unloading goods
Category 8 = jumping, canoeing, bicycling (9mph), dancing, skiing, tennis
Category 9 = weight training, jogging / running (less than 12 minutes per mile), racquetball, swimming, hiking, bicycling (>15 mph)
Category 10 = any activity that does not seem to fit in any of the categories listed above

Adapted from the CEAD Project, 2003

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