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GARRETT C. WARDLOW
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EFFECTS OF POSTPRANDIAL HYPERTRIGLYCERIDEMIA ON ISCHEMIC-REPERFUSION INJURY

A THESIS APPROVED FOR THE
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BY

_____
Dr. Christopher Black, Chair

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Dr. Rebecca Larson

_____
Dr. Jason Campbell

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Abstract

Background: Cardiovascular disease, including ischemic-reperfusion injury, is the number one cause of mortality in the United States. Among numerous contributing factors, a westernized high fat diet can negatively affect cardiovascular health or compound existing cardiovascular disease. To date, studies have focused on the effects of ischemic-reperfusion injury and high fat meal(s) on the endothelium separately, but have not investigated the impact they have together. **Primary Aim:** The primary aim of this study was to assess the impact of a single high fat meal followed by an ischemic-reperfusion injury on endothelial-dependent vasodilation. **Methods:** Subjects consumed either a single high fat meal or placebo, at least seven days apart. The high fat meal was appropriated to each individual and contained 1.5 grams of fat per kg of body weight. Endothelial function was assessed with a flow-mediated dilation technique via ultrasound measurements of the right brachial artery in both the placebo and high fat meal conditions before and after ischemic reperfusion injury. The ischemic-reperfusion period consisted of 20-minutes of occlusion distal to the ultrasound measurement followed by 20-minutes of reperfusion. Occlusion was achieved in both the ischemic-reperfusion period and flow-mediated dilation periods with use of rapid blood pressure cuff inflation. **Results:** The high-fat meal by itself significantly impaired the flow-mediated dilation of the brachial artery. After the ischemic-reperfusion injury there was still significant impairment in the dilation of the endothelium. These results suggest that the high fat meal has a lasting effect in impairment of the endothelium that prolongs the endothelial health following a negative

cardiovascular event such as ischemic-reperfusion injury. This can help to explain how a normal population is directly affected by the consumption of even one high fat meal, and how the effect can carry a lasting impact on their endothelial health.

Chapter 1: Introduction

Globally, cardiovascular disease is the number one cause of death, with many of those attributed to the myocardial injury associated with an ischemic-reperfusion insult. The development of coronary artery disease and subsequent myocardial infarction is due to atherosclerotic plaque development within the coronary vasculature. Several risk factors such as smoking, insulin resistance, diabetes, lack of physical activity, unhealthy diet, and high levels of triglycerides may play a role in the development of this atherosclerotic plaque[1]. If untreated, this coronary plaque will result in a thrombotic coronary artery occlusion and subsequent myocardial ischemia. While the myocardium can tolerate brief periods (~15 min) of severe myocardial ischemia, prompt reperfusion of the tissue is required. However, with increasing duration of ischemia the act of tissue reperfusion can elicit significant myocardial injury, known as ischemic reperfusion (IR) induced injury.

Mitochondria are one of the primary cell components affected by IR-induced injury and both endothelial and myocardial cells rely on mitochondria as an energy producer and regulator of programmed cell death[2]. During a period of IR, permeability transition pores in the mitochondria open leading to a consumption of ATP, excessive water entry, and ultimately death. Such permeability transition pores are susceptible to changes in calcium levels, redox, voltage, and pH. Furthermore, reperfusion introduces additional reactive oxygen species (ROS) that can further increase permeability transition pore opening.

IR-induced injury can worsen cardiac cell damage after the removal of an occlusion in a coronary artery through myocardial stunning and microvascular and endothelial injury[3]. During arterial occlusion within the heart the highly aerobic cardiac myocytes are deprived of the oxygenated blood necessary to sustain function and health, bringing the cells into an ischemic condition[4]. Consequences of this ischemic period include increases in hydrogen ion concentration, a drop in blood pH, and near exhaustion of the glycolytic and creatine phosphate systems[3, 4]. As such, if occlusion lasts beyond 20 minutes, permanent damage will be sustained by the cardiac myocytes[2]. However, if reperfusion of the myocardial tissue occurs the ischemic damage can be minimized, but not without consequence. With reperfusion the ischemic tissue experiences an increase in ROS that not only impacts the cardiac myocytes, but can also decrease arterial function. Following IR-injury, a decreased endothelium-dependent, nitric oxide (NO) mediated vasodilation is observed within the arteries[5] due primarily to an altered balance between NO bioavailability and ROS production. Additionally, reductions in microvascular perfusion may follow IR-injury[6]. This could be due in part to capillary plugging, endothelial swelling, and edema-driven capillary compression that had built up during the ischemic period[6].

Currently, the Western diet contains an excess amount of fat consumed in everyday life[7]. High-fat meals consumed in succession lead to a decrease in endothelium-dependent dilation, due in part to an increase in oxidative stress. This suggests that compounding one high fat meal after another leads to even higher levels of endothelial dysfunction[8]. However, even after the consumption of a single high-fat meal blood triglyceride levels significantly increase above that seen with a low-fat

meal. During this acute hypertriglyceridemic state, increases in blood triglyceride levels lead to oxidative stress and the inhibition of nitric oxide bioavailability. Over generation of the superoxide anion (O_2^-) leads to the inactivation of nitric oxide. This inhibition of nitric oxide production and bioavailability diminishes the ability of the endothelium to dilate and in some cases can result in endothelial cell death[9].

Accordingly, the primary aim for this investigation was to determine the magnitude of IR-injury, following a single high-fat meal, on vascular function. The rationale for this investigation was that because IR-induced injury is, in part, elicited via increases in ROS, the IR-induced injury following a high fat meal would be increased. It was therefore hypothesized that postprandial hypertriglyceridemia will result in an increased ischemic reperfusion injury as determined by the degree of endothelial impairment. To test this hypothesis, the effects of IR-induced endothelial function were examined via endothelium-dependent brachial artery flow-mediated dilation, before and after a single high-fat meal. Brachial artery endothelial function was chosen as a surrogate for the coronary circulation because 1) of the danger associated with direct occlusion of the coronary arteries, 2) brachial artery endothelial function is strongly associated with gold-standard measurements of coronary artery function[10], and 3) the forearm model of IR-induced injury has previously been used successfully in determining the level of change in flow mediated dilation[11, 12].

Research Aims

1. To determine the effects of postprandial hypertriglyceridemia, via high-fat beverage replacement, on vascular endothelial function following ischemic reperfusion injury.

Hypothesis

1. Postprandial hypertriglyceridemia will augment the decrease in vascular endothelial function following ischemic reperfusion injury.

Significance of the Study

Examining the effect of a high-fat meal on ischemic reperfusion injury could provide insight into potential consequences for individuals who have high-fat meals consistently included in their diets. In addition, this study could provide additional information about how exactly a high-fat meal affects endothelial dilation as it pertains to ischemic reperfusion. Considering CVD is the leading cause of death worldwide, information provided by this study could assist in altering the traditional westernized diet leading to a possible increase in life expectancy and/or quality of life.

Assumptions

1. All subjects will be fasted prior to the ingestion of the high fat meal.
2. All subjects have a similar and consistent westernized diet.

3. Subjects do not consume excess water or other materials after the ingestion of the high fat meal but before the post testing.

Delimitations

1. All subjects were free of cardiovascular disease.
2. All subjects were healthy individuals between 18 and 35 years of age.

Limitations

1. Subjects were only sampled from in and around the University of Oklahoma
2. Brachial artery endothelial function was used as a surrogate for coronary artery endothelial function.
3. Due to the texture and taste of the high fat meal, participants could not be blinded to the meal.

Operational Definitions

Cardiovascular Disease (CVD) – a group of disorders of the heart and blood vessels including: coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism[13].

Myocardial Infarction (MI) – heart attack[14] (Mayo Clinic)

Atherosclerosis – a disease characterized by the deposition and buildup of plaque inside arteries[1].

Ischemia – An inadequate blood supply to an organ or part of the body.

Cardiac Myocytes – Muscle cells of the heart.

Ischemic Reperfusion (IR) – Re-entry of oxygenated blood past a removed blockage in a blood vessel that results in tissue dysfunction, injury, or death.

Chapter 2: Literature Review

Ischemic Reperfusion Injury

Myocardial Infarction (MI) accounts for 17 million deaths worldwide with the majority of those deaths being related to Ischemic Reperfusion (IR) –induced MI[15]. Usually MI is due to a thrombotic coronary artery occlusion, ischemic conditions and cell death that will begin to develop past the blockage[16]. If reperfusion is achieved prior to ischemic cell death this will cause further damage that leads to apoptosis and necrosis of the cardiac myocytes[2, 3, 15, 17].

Cardiac myocytes are highly aerobic cells that rely primarily on oxidative phosphorylation to meet their energy needs[4]. Therefore, these cells require blood flow to be constant to avoid any cellular damage and to meet metabolic needs. This depravation of oxygenated blood can be sustained for up to 20 minutes before causing permanent damage to the myocytes[16]. In the case of MI, when myocytes are deprived of oxygenated blood, hydrogen ion concentration increases, pH drops to 5.5-6, and the glycolytic and creatine phosphate systems are nearing exhaustion[4]. An increase in cystolic hydrogen ions causes cystolic sodium to increase followed by an overload of cystolic calcium[3, 4]. This overload of calcium into the cell can cause the heart muscle to contract without a period of relaxation. With the heart unable to relax, there is little filling of the ventricles with blood. Ultimately this leads to an inability to pump blood throughout the body and leads to further cell death.

If there is reperfusion of blood into the ischemic cardiac myocytes, reactive oxygen species (ROS) flood the tissue in addition to the increased calcium levels[18, 19]. This overflow of ROS mediates mitochondrial damage and dysfunction that leads to the death of the cells[20]. In normal cells, small amounts of ROS are produced in the mitochondria in the form of superoxide, which can actually drive cell proliferation and hypertrophy[20]. However, it is the overflow of ROS that can cause the mitochondria to trigger necrosis and apoptosis. A key factor in triggering necrosis and apoptosis is the opening of mPTP, a multiprotein complex that forms a nonselective pore in the mitochondria. An increase in calcium, ROS, acidosis, etc., all increase the amount of mPTP in the mitochondria leading to that eventual apoptosis or necrosis of the myocyte[2, 17, 20].

Ischemic Reperfusion Injury and Endothelial Function

In addition to cardiac myocyte damage accrued from IR injury there can also be injury to endothelial cells. Consequences of hypoxia such has a disturbance in membrane potential, increase in reactive oxygen species, decrease in nitric oxide production, intracellular volume, and decreased membrane fluidity are exacerbated upon reperfusion[21]. Endothelial cell dysfunction in arterioles following IR is primarily a result of impaired endothelium-dependent, NO-mediated relaxation of smooth muscle[21]. Overproduction of superoxide anions are linked to this decrease in endothelial function[21].

In a study by Gross et al., they sought to determine if these superoxide anions were primarily responsible for endothelial damage resulting from IR-injury. The left anterior descending coronary artery of a dog heart was examined for its endothelium-dependent responses. They found that after multiple 5 minute occlusive and reperfusion periods oxygen-derived free radicals play a key role in diminishing endothelial function in the coronary arteries[22]. A similar study involving cat hearts reported superoxide anions as the primary cause for endothelium injury and dysfunction[23]. The endothelial dysfunction from IR can be seen at two and a half minutes following reperfusion but not before reperfusion[23] . This suggests that the impairment occurs during the reperfusion as a result of the chain of events caused by superoxide anions[23]. Tsao et al., examined the effects of separate superoxide dismutase and nitroglycerin injections after a 30 minute ischemic period. They found that endothelial injury was blocked with the application of superoxide dismutase and compared to no protection with nitroglycerin. This would suggest that superoxide free radicals are responsible for some of the endothelium dysfunction[24].

Age may also play a great factor in an individual's response to IR-injury. To determine the impact of age on endothelial-dependent dilation FMD and circulating markers of nitric oxide breakdown of both young (18-40 yrs) and middle aged (41-65 yrs) individuals were measured in a study by DeVan et al. The FMD response after IR-injury from sedentary middle aged subjects was significantly less than the response from sedentary young adults[25]. Additionally, they reported that the middle aged individuals had no significant difference in the circulating inflammatory markers[25].

However, they did suggest that middle-aged individuals had lowered NO bioavailability as indicated by lower serum nitrite levels[25]. Another study focusing on IR-injury in young adults examined the effect of resistance training on IR-injury to the endothelium. In the study by DeVan et al., those who were resistance trained were more protected from IR-injury as compared to the non-resistance group. This protection from IR-injury is reflected by a greater FMD response from the resistance trained group[26]. The repeated bouts of IR caused from muscle contractions during resistance training may play a role in this protection and an increase in NO bioavailability[26].

High Fat Meal(s) and Endothelial Function

More than 64 million Americans have one or more types of cardiovascular disease (CVD), which represents the leading cause of mortality in the United States of America[7]. Currently, the Western diet contains an excess amount of total fat as compared to previous years[7]. The total amount of fat being consumed has been consumed has been considered more important than the type of fat that is being consumed by the everyday American[7]. This consistent elevation of fat in the everyday diet can lead to some of the CVD seen today.

CVD such as atherosclerosis is caused by the buildup of plaque that is associated with fat, cholesterol and other substances found in the blood. High fat meals overtime can continue to put more triglycerides into the blood and potentially increase plaque buildup[27]. In addition, impairment of the vascular endothelium through

oxidative stress can also contribute to the progression of atherogenesis and atherosclerosis[27]. Oxidative stress may occur when there is an over generation of superoxide anion (O_2^-), which can then inactivate nitric oxide (NO)[9] . The inhibition of NO can lead to a decrease in the ability of the endothelium to dilate (REF – Ade). Furthermore, NO and O_2^- can interact and produce peroxynitrite (indicated by nitrotyrosine, NT) which is an oxidant. This type of oxidant can lead to depletion of antioxidant defenses, inactivation of enzymes, and apoptosis of myocytes and endothelial cells[9]. In a study by Ceriello, et. al., subjects ingested a high fat meal, glucose alone, and a high fat plus glucose meal and then were measured for their level of glycemia, triglyceridemia, nitrotyrosine, and endothelial function. They found that both postprandial hypertriglyceridemia and hyperglycemia cause an elevation of nitrotyrosine and therefore a decrease in endothelial function[9]. Additionally, their study found that hypertriglyceridemia and hyperglycemia have an independent and cumulative effect on endothelial impairment.

After the ingestion of a high-fat meal, several studies site that there is an increase in oxidative stress which can contribute to endothelial dysfunction[8, 28-30]. Anderson et. al. and Saxena et. al. tested subjects who were type 2 diabetic. Their studies suggest that increased oxidative stress during the postprandial state (which is higher in diabetics) correlated with hypertriglyceridemia[28, 30]. Tushuizen et. al. took a different approach by giving subjects two consecutive high-fat meals. Furthermore, they suggest that this increase in triglyceridemia along with the oxidative stress plays an important role in the development of endothelial dysfunction and therefore increased

the risk for vascular disease[8]. In the first meal plasma tricyglycerol elevations were considered primarily responsible for the subjects' increased endothelial dysfunction. However, Tushuizen found that postprandial oxidative stress correlated highest with endothelial dysfunction after the ingestion of the second meal A study by Wei-Chuan et. al. reported that endothelial function was impaired following the high-fat meal in healthy subjects. However, unlike the previous studies they did not find significant correlation between oxidative stress markers and endothelial dysfunction[8]. This insignificant correlation between oxidative stress markers and endothelial dysfunction could be attributed to their small sample size.

Once a high fat meal is ingested, the body can go into an acute hyptertriglyceridemic state. Several studies have shown that this acute hypertriglyceridemic state leads to impairment of endothelial function[30-33]. In a study by Giannattasio et. al., dyslipidemic and normolipidemic subjects were given a high-fat meal. Subjects' endothelial dysfunction was measured through the use of FMD in the radial artery. They found that there was decreased endothelial function in the dyslipidemic subjects but not the normolipidemic controls[33]. The study could have used the dyslipidemic subjects as their own control by giving them a no/low fat meal instead of comparing between subjects. In a study by Shimabukuro et. al., subjects' forearm blood flow was measured at 3 separate and different meals (high-fat, high-carbohydrate, and a "standard test meal"). Results showed the subjects' forearm blood flow and endothelial function decreased following the high fat meal but not the high-carbohydrate or "standard test meal". In addition to providing the macronutrient detail

of the meals, the type of meal should be described i.e., milkshake, fast food meal, etc.. Gaenzer et. al. found that endothelial function decreased following a high-fat meal, however the magnitude of post prandial lipemia significantly impacted the decrease of endothelial function[30]. There was a standardized liquid fat meal given to the subjects. However, the subjects were tested twice once from an 8 hour fast and another week off of a 12 hour fast.

Anderson et. al., sought to determine if this impaired endothelial function after a high fat meal can be attenuated with vitamin C, an antioxidant. Their study involved a double blind procedure with twenty type 2 diabetics serving as their own control by ingesting both a high fat meal and a placebo on separate occasions. They found that with 2 days of vitamin C supplementation there was no increase in oxidative stress in the postprandial state[34]. However, it should be noted there was not a decrease in oxidative stress and therefore endothelial function did not improve but rather sustained baseline endothelial function[34].

Flow Mediated Dilation

The vascular endothelium throughout the body serves as an anti-coagulant surface, regulates fluid and molecule movement between blood and tissue, contributes to vascular homeostasis and maintains vascular tone and blood flow regulation[35]. Assessment of these functions, and the overall endothelial health, can be achieved through the use of a Doppler ultrasound in conjunction with a flow-mediated dilation

technique. The use of flow mediated dilation provides an alternative invasive measurement of endothelial health as compared to an invasive and direct coronary angiography[35].

During the flow mediated dilation technique blood flow in a limb, typically the arm, is occluded via the inflation of a blood pressure cuff or other tourniquet device. When blood flow is restored there is an acute increase in flow that results in flow-mediated endothelium-dependent vasodilation via the production of NO and other endothelial derived vasodilators[12, 36]. Measurements of artery diameter are therefore measured both before and after the FMD protocol and displayed as a percent change from pre to post. This assessment of endothelial function is proposed to represent the endothelium-derived nitric oxide bioavailability of the subject[12].

A study by Anderson, et. al., measured changes in both coronary and brachial vasodilation in each subject to examine the relationship between the two arteries. The coronary artery's endothelial function was assessed through use of a catheter. Whereas, the brachial artery of each subject was measured through the ultrasound method described above. When comparing the two arteries endothelial function against one another they found little difference[10]. Thus they suggest that using an ultrasound technique measuring brachial vasodilation and function is closely related to coronary vasodilation and health[10].

The magnitude of a flow-mediated dilation response can vary between different experimental protocols and measurement locations on the body. In a study by Betik et. al., they examined blood flow and brachial artery diameter with occlusion at the following positions: at the wrist, forearm, brachial artery (proximal), at the wrist with exercise, and at the forearm with exercise. They found that there are differences in an FMD response when occlusion occurs at different places on the arm with or without exercise[37]. Occlusion at the proximal position provided an increased magnitude of reactive hyperemia which results in a greater FMD response. Thus they recommend distal occlusion be used when studying endothelial function to enhance reactive hyperemia and FMD responses[37].

Using an ultrasound technique, some studies have assessed the endothelial health of individuals who are considered at risk for atherosclerosis[38, 39]. Even in the absence of atherosclerosis those with hypertension, hypercholesterolemia, and/or diabetes showed decreased endothelial function when the coronary artery was measured through a catheter[38]. Similar results were found in a study by Celermajer et. al., when they measured the endothelial function of both children and adults known to be at risk for atherosclerosis. External ultrasound of the subjects' brachial and femoral arteries showed an impaired flow-mediated dilation in both children and adults who had risk factors for atherosclerosis but were not considered atherosclerotic[39]. In comparison, those who did not have any risk factors for atherosclerosis and were free of atherosclerosis had a normal flow-mediated dilation response[39].

Chapter 3: Methods and Material

Ethical Approval

All procedures were approved by the Institutional Review Board for Research Involving Human Subjects at the University of Oklahoma Health Sciences Center, which conformed to the Declaration of Helsinki. Each eligible participant provided verbal and written consent prior to experimental testing.

Subjects

A total of 5 men participated in the study. Eligibility was assessed through use of a Medical History Form. The subjects were recruited via campus wide emails, flyers, and newspaper ads placed within the surrounding communities.

Inclusion Criteria

1. Men and Women
2. 18 to 35 years Old

Exclusion Criteria

1. Known atherosclerotic cardiovascular disease (ASCVD) defined by the history of acute coronary syndromes, myocardial infarction, stable or unstable angina, coronary or other arterial revascularization, stroke, TIA, or peripheral artery disease
2. Diabetic
3. Known significant ventricular arrhythmias
4. Current sue of statin therapy
5. > 140 mmHg resting systolic blood pressure
6. Current use of antioxidant supplements (e.g., fish oil)
7. Current and chronic use of anti-inflammatory drugs (e.g., NSAIDS)
8. Current smoker or within last 6 months
9. Lactose intolerant

Experimental Design

The study utilized a randomized balanced single-blind placebo-controlled crossover protocol with two treatments (placebo and high fat meal). Each treatment occurred at the same time of day following a >4 hr fast while also abstaining from caffeine. The testing procedures occurred on two separate days, at least 7 days apart. At each visit plasma markers of oxidative stress along with measurements of endothelial-dependent flow mediated dilation of the brachial artery of the forearm were assessed under resting conditions before, after the treatment (placebo or high fat beverage), and after IR (20 min arm ischemia and 20 min arm reperfusion). The order of treatment was randomized.

The high fat beverage used in the present study consisted of heavy cream that provided a fat dose equivalent to 1.5 g per kg of body weight. This high fat beverage consisted of heavy whipping cream (Great Value Heavy Whipping Cream). Serving size was measured as milliliters (mL) of whipping cream = ((body weight in kg x 1.5)/15) x 5. The placebo was composed of water of equal volume to the high-fat beverage. Each beverage was ingested within twenty minutes.

To elicit IR-injury in the present study a rapid inflation/deflation pneumatic cuff (Hokanson) was positioned on the upper most proximal right arm. To induce ischemia of the arm the cuff was inflated to 220 mmHg for 20 min. Occlusion was confirmed by palpation of the radial artery. The 20 min occlusion period was immediately followed by a 20 min period of reperfusion. Given the strong association between brachial artery endothelial function and gold-standard measurements of coronary artery function, this procedure provides a surrogate for in vivo endothelial IR-induced injury[10].

Experimental Measurements

Anthropometric Measurements

Prior to experimentation subjects' height were measured on a standard digital scale [Detecto ProDoc Scale Digital Height Rod (Model: DHR/#809161147506) Webb City, MO]. Weight was measured with a Health-o-Meter scale (Model 349KLX McCook, IL). Shoes and any headwear were removed prior to obtaining the

measurements. Initial blood pressure measurements were taken with an Omron Automatic Blood Pressure Monitor [Model BP785 (HEM-7222-Z) Omron Healthcare, Inc., Lake Forest, IL].

Blood Analysis

Blood samples were collected in acid citrate dextrose (ACD) vacutainer tubes from the antecubital vein via a venipuncture. The blood samples were used to obtain measurements of plasma oxidative stress markers (Interleukin-6, IL-6). Immediately following the blood draw, each sample was centrifuged for 10 min at 3300 g. Next the upper part of the plasma sample was collected without disrupting the buffy layer coat and stored at -80º C before analysis. IL-6 concentrations were measured via commercially available Enzyme-Linked Immunosorbent Assay Tests (LabCorp, USA).

Vascular Function Measurements

Endothelial function was assessed non-invasively by endothelium-dependent flow mediated dilation (FMD) in the brachial artery with the subject in the supine position. The right arm was abducted to an angle of approximately 80º from the torso at heart level and placed on a foam pad. Simultaneous measurements of artery diameter and blood velocity were obtained with an ultrasound system equipped with a multi-frequency linear array transducer. Measurements were taken by multiple researchers. These measurements were recorded for 5 minutes prior to blood pressure cuff inflation

to determine a baseline of endothelial diameter and flow. After baseline measurements the blood pressure cuff was rapidly inflated to 220 mmHg to occlude blood flow. To determine the FMD response, and endothelial function, post measurements of the artery were recorded for 5 minutes during reperfusion that resulted from rapid cuff deflation.

Brachial artery diameters were measured off-line using a commercially available edge-detection and wall-tracking software package, which minimizes investigator bias [Vascular Research Tools 6, (Medical Imaging Applications, Coraville, Iowa, USA)]. This system obtains diameter measurements a 15 frames per second. The baseline brachial artery diameters were averaged over 1 min. The post-occlusion brachial diameters were averaged into 3 s bins. FMD was calculated as the highest absolute (mmΔ) and relative (%Δ) mean average 3-s diameter following cuff release in peak brachial artery diameter from the preceding baseline diameter. The averaged mean velocity (in centimeters per second) during the baseline and post-occlusion period were determined using the ultrasound manufacturer's on-screen software. From this velocity and diameter data the shear rate was calculated as shear rate (s^{-1}) = (4 × mean blood velocity (cm/s) / diameter (cm). Using the trapezoid rule the area under the shear rate curve was calculated to quantify the stimulus eliciting brachial artery dilation. The peak reactive hyperemia response was taken as the highest (peak) blood velocity response immediately following cuff release.

Statistical Analysis

A commercially available statistical analysis software package was used to perform all statistical analyses (Sigma-Plot/SigmaStat12.5, Systat Software, Point Richmond, CA). All data are presented as mean ± standard deviation. Brachial artery FMD and peak hyperemic responses were analyzed with a two-way repeated measures ANOVAs (meal x time). To identify significant changes within and between groups a Tukey post hoc test was performed. Statistical significance was declared when $P < 0.05$.

Chapter 4: Results

Subject Characteristics

A total of 5 men participated in this study. The average height (cm) and weight (kg) is 183.8 ± 6.61 and 98.56 ± 34.99, respectively. The average age (years) was 20.8 ± 2.949, with the oldest at 26 years and the youngest at 19 years. Mean body mass index was 28.735 ± 8.57.

Table 1: Subject Characteristics

Variable	Value
n	5
Sex (men/women)	5/0
Age (years)	20.8 ± 2.9
Height (cm)	183.8 ± 6.6
Weight (kg)	98.56 ± 34.99
Body mass index (kg m^{-2})	28.735 ± 8.57

Values are mean ± SD

Baseline Characteristics

Subjects began each testing day under the same conditions. The subject conditions were that they were fasted before beginning testing during the early to mid-

morning. Due to this, there were no significant differences for any variables between the Placebo and HFM conditions at baseline.

Systolic blood pressure means were 122.9 ± 12.8 mmHg for the placebo condition, and 119.8 ± 12.8 mmHg for the HFM condition ($P > 0.05$). Diastolic blood pressure means were 72.2 ± 9.9 for the Placebo condition, and 71 ± 7.9 for the HFM condition ($P > 0.05$). The FMD (mm) mean was 0.17 ± 0.10 for the Placebo condition and 0.26± 0.08 for the HFM condition ($P > 0.05$).

Figure 1 illustrates the typical changes in diameter during a FMD test. Notice that immediately following the 5-min cuff occlusion period a substantial increase in diameter occurs, which allows for the calculation of FMD. At baseline the FMD (%) average was 3.7 ± 2.4% and 6.6 ± 2.6% for the Placebo and HFM conditions, respectively ($P > 0.05$). Shear rate, AUC, means were 11336.5 ± 4034.0 and 10706.4± 2989.2 for the Placebo and HFM conditions, respectively ($P > 0.05$). Finally, the Peak Reactive Hyperemia means were 79.8 ± 12.7 cm s^{-1} and 72.3 ± 21.8 cm s^{-1} for the Placebo and HFM conditions, respectively ($P > 0.05$).

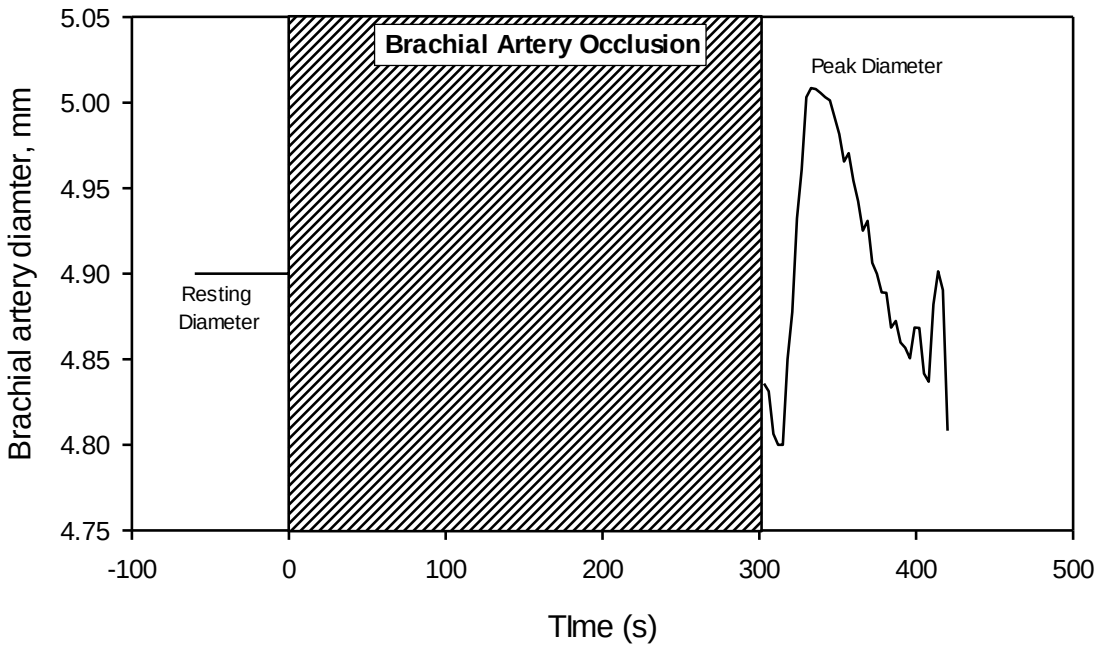
Table 2: Baseline Characteristics

Variable	Placebo	HFM
Systolic blood pressure, mmHg	122.8 ± 12.8	119.8 ± 12.8
Diastolic blood pressure, mmHg	72.2 ± 9.9	71 ± 7.9

FMD, mm	0.17 ± 0.10	0.26 ± 0.08
FMD, %	3.7 ± 2.4	6.6 ± 2.6
Shear rate, AUC	11336.5 ± 4034.0	10706.4 ± 2989.2
Peak Reactive Hyperemia, cm s^{-1}	79.8 ± 12.7	72.3 ± 21.8

Values are mean ± SD

Figure 1: Representative brachial artery response during a FMD test.



Flow Mediated Dilation Responses

The FMD (mm) Post-Meal mean value was 0.23 ± 0.09 mm for the placebo condition. This Post-Meal mean value was significantly greater ($p < 0.05$, $d=2.46$) from the HFM mean value of 0.07 ± 0.04 mm (Figure 2). Additionally, there was a significant difference ($p < 0.05$, $d=2.56$) in Post-Meal FMD (%) from placebo to HFM conditions at mean values of 5.27 ± 1.87% and 1.77 ± 0.86%, respectively (Figure 2).

Shear rate, AUC, mean values were not significantly different between the Post-Meal Placebo and HFM conditions. The mean values for shear rate for the Post-Meal placebo condition were 9744.2 ± 3700.2, and 10890.2 ± 2531.9 for the Post-Meal HFM condition. Also, Peak Reactive Hyperemia (cm s^{-1}) mean values were not significantly different between the Post-Meal Placebo and HFM conditions. Peak Reactive Hyperemia was at a mean value of 72.7 ± 18.1 cm s^{-1} at the Post-Meal placebo condition. Peak Reactive Hyperemia was at a mean value of 83.6 ± 13.14 cm s^{-1} for the Post-meal HFM condition.

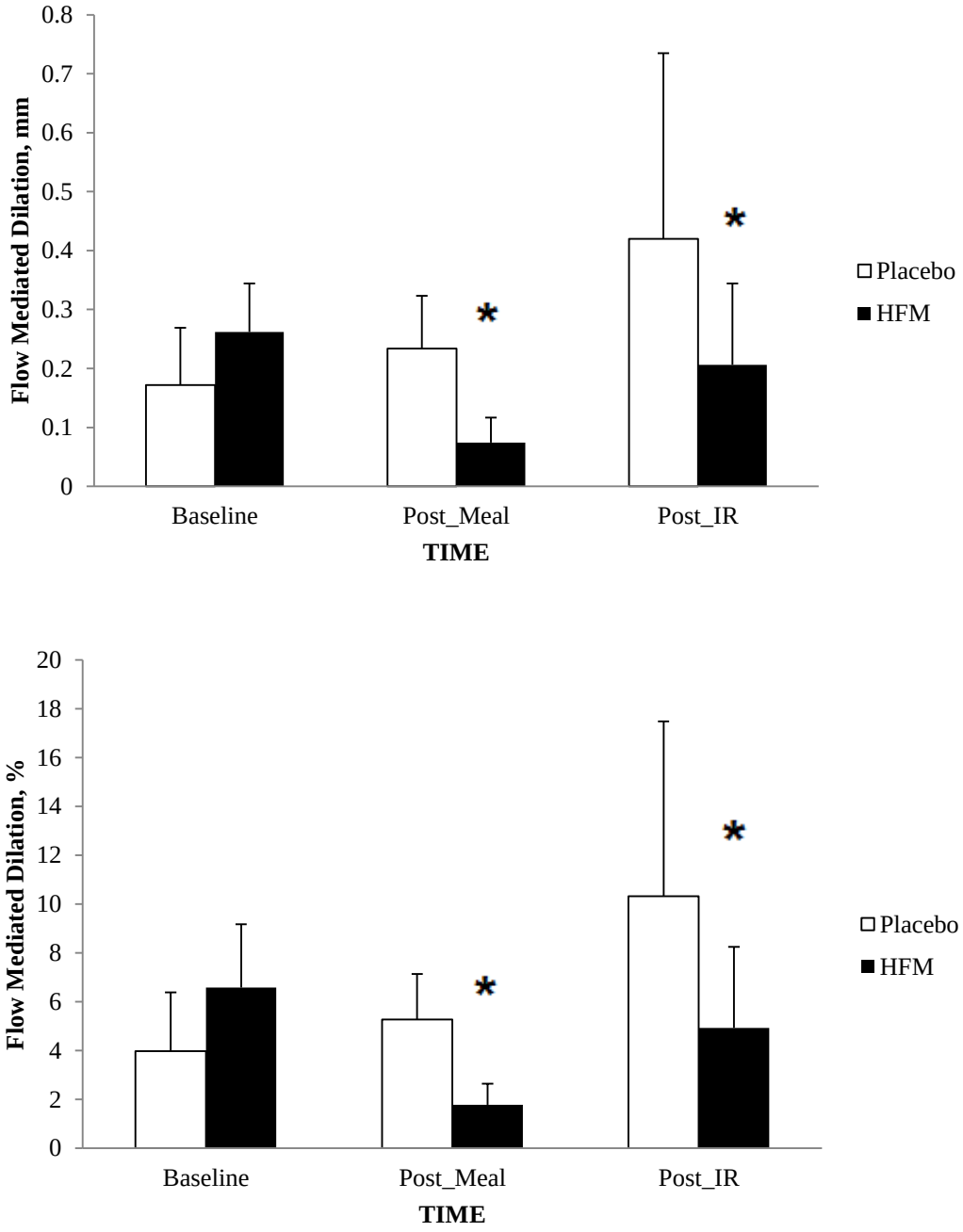
The FMD (mm) mean values were significantly different between meals ($p<0.05$, $d=0.91$) during the Post-IR conditions at 0.42 ± 0.32 mm and 0.21 ± 0.14 mm for Placebo and HFM conditions, respectively (Figure 2). Likewise, FMD (%) mean values showed significant decrease ($p<0.05$, $d=1.03$) during the Post-IR conditions at 10.32 ± 7.16% and 4.92 ± 3.32% for the Placebo and HFM conditions, respectively (Figure 2). The FMD response was not significantly different between Post-Meal and Post-IR time points for either the Placebo or HFM condition. The Post-IR Shear rate, AUC, values were not significantly different between the placebo and HFM conditions at 11409.4 ± 6174.3 and 134712, respectively. Finally, the Post-IR mean values for Peak Reactive Hyperemia were not significantly different between placebo and HFM at 83.36 ± 21.1 cm s^{-1} and 95.58 ± 11.378 cm s^{-1}, respectively.

Table 3: Effects of HFM and IR on brachial artery endothelial function

	Baseline		Post-Meal		Post-IR	
Variable	Placebo	HFM	Placebo	HFM	Placebo	HFM
FMD, mm	0.17 ± 0.10	0.26 ± 0.08	0.23 ± 0.09	0.07 ± 0.04*	0.42 ± 0.32	0.21 ± 0.14*
FMD, %	3.7 ± 2.4	6.6 ± 2.6	5.27 ± 1.87	1.77 ± 0.86*	10.32 ± 7.16	4.92 ± 3.32*
Shear rate, AUC	11336.5 ± 4034.0	10706.4 ± 2989.2	9744.2 ± 3700.2	10890.2 ± 2531.9	11409.4 ± 6174.3	13472 ± 2917.1
Peak Reactive Hyperemia, cm s^{-1}	79.8 ± 12.7	72.3 ± 21.8	72.7 ± 18.1	83.6 ± 13.14	83.4 ± 21.1	95.6 ± 11.4

Values are mean ± SD
* Significantly different compared to Placebo
+ Significantly different compared to Post-Meal

Figure 2: Flow-mediated dilation responses post-meal and post-IR

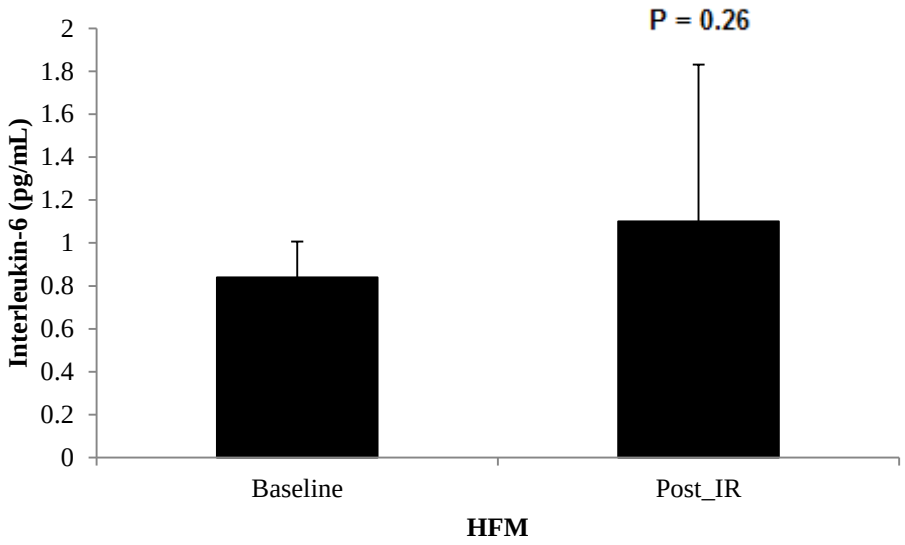


* Significantly different between Placebo and HFM at the specific time point

Interleukin-6 Response

Due to technical problems the IL-6 response could only be measured in the HFM condition. Prior to ingestion of the HFM, IL-6 at baseline was 0.84 ± 0.17 pg/ml and after post-IR was 1.10 ± 0.73 pg/ml. Blood could only be drawn from 3 participants on the placebo day due to difficulties in drawing their blood. Due to this, only the responses to the single HFM are included.

Figure 3: IL-6 response following HFM



Chapter 5: Discussion

Summary

This study examined the impact of a single high fat meal on brachial artery endothelial function of healthy individuals following an ischemic reperfusion event via measurements of flow mediated dilation responses. Both FMD measurements and Interleukin-6 concentrations were taken following baseline and ischemic reperfusion on each testing day after ingesting the placebo or high fat meal. FMD measurements were compared between the placebo and high fat meal at the baseline, post-meal and post-ischemic reperfusion conditions. To our knowledge, this is the first study to investigate the effects of a single high fat meal on ischemic-reperfusion injury measured via differences in FMD response. The results from FMD measurements between the placebo and high fat meal condition at baseline showed no significant difference. However, FMD measurements indicated a significant decrease in artery endothelial function from placebo to high fat meal in post-meal measurements, as well as a significant decrease in endothelial function from placebo to high fat meal in post-ischemic reperfusion measurements. This study hypothesized that a single high-fat meal that introduces a state of postprandial hypertriglyceridemia will augment the decrease in vascular endothelial function following ischemic reperfusion injury. Based on the data, the hypothesis presented was not rejected and suggests that artery endothelial function can be significantly hampered following a single high fat meal.

Effects of High Fat Meals on Cardiovascular Health

The typical Western diet of today contains an excess amount of total fat as compared to previous years[7]. One complication of increased fat intake is an increased risk of cardiovascular disease[1]. In the United States of America, cardiovascular disease happens to be the leading cause of mortality[1]. As seen in the results from this study, even a one-time acute increase in fat intake can result in dysfunction within the cardiovascular system. It may be possible that chronic high fat intake leads to a state of chronic damage to the cardiovascular system over time.

This constant introduction of high amounts of fat into the blood stream can lead to the build up of plaque, otherwise known as atherosclerosis. Aside from the plaque build up, this increase in triglycerides in the system results in an increase in oxidative stress on the endothelium[2]. Increases in oxidative stress can result in the inactivation of nitric oxide, ultimately leading to a decrease in endothelial function[3]. Though nitric oxide was not specifically measured it can be speculated that similar the study done by Piper, et al. ((2004), subjects in this study experienced a decrease in nitric oxide bioavailability which resulted in decreases in endothelial function. This study is in agreement with Ceriello, et. al. (2002), in that introducing a high fat meal will bring about a hypertriglycemic state resulting in a decrease in endothelial function.

The present study differed in design from several others in that the population tested consisted of healthy college aged males. However, similar results were found

between most studies in that no matter the population or number of high fat meals given, there was a decrease in endothelial function. The healthy subjects in this study had a similar response to type 2 diabetic patients in studies conducted by Anderson et al., and Saxena et al. (2001, 2005), which had decreases in endothelial function following the ingestion of the high fat meal[30, 34].

Other studies have examined oxidative stress of the endothelium through measurement of inflammation in the form of pro-inflammatory markers. One study by Peluso et at. (2012), found that healthy overweight subjects showed significantly greater levels of pro-inflammatory markers following the ingestion of a high fat meal[40]. These results are contrary to results found by this study, which found no significant increase in pro-inflammatory markers. This could possibly be due to the low sample size in this study or the fact that a blood sample was not feasible in some of the subjects tested. Additionally the study by Peluso et al. (2012), used a large meal rather than a single high fat meal used by this study. This could also contribute to the conflicting results found by the two studies.

Factors that Impact Ischemic-Reperfusion Injury

A variety of factors can affect Ischemic-Reperfusion injury including but not limited to, time length of infarction, type of tissue affected, and endothelial health prior to injury. In highly aerobic tissue, such as cardiac tissue, an ample amount of oxygen is required to function properly, and permanent damage can be seen at 20 minutes of

ischemic condition and beyond[4, 16]. These results of this study were contradictory to a study by DeVan, et al., which showed a decrease in FMD in young subjects following their IR-Injury[25]. It should be noted that in the study by DeVan., et al., that the subjects vascular function recovered fully at 45 minutes post cuff release[25]. This study was different in that the ischemic region was the arm, looking more specifically at the brachial artery to identify consequences of 20 minutes of ischemic conditions. Even though the arm is far less aerobic than cardiac tissue, ischemic conditions can still bring about reductions in pH as a result of hydrogen ion concentration increases[4]. Additionally, the deprivation of oxygen can bring about exhaustion of the glycolytic and creatine phosphate system[4].

More damage can be done to the endothelium during this period with disturbances in membrane potential, increase in reactive oxygen species, and a decrease in nitric oxide production/utilization as stated above[21]. This endothelial dysfunction of the artery can result in the impairment of endothelium, nitric-oxide mediated relaxation or dilation of the smooth muscle[21]. Results from this study are in disagreement with the study by Carden et al., in that we did not observe a significant decrease in FMD following the IR period. In fact, this study observed an increase in FMD following the IR period, though the values were not significant. This increase in mean values may be due in part to the relatively young age of our participants, the small sample size, or issues in achieving full occlusion of the artery for a 20 minute period. However, this study does clearly demonstrate that the introduction of the high fat meal can worsen the function of the endothelium following an ischemic-reperfusion event.

Gross et al., found that the ischemic conditions introduced oxidative stress on the endothelium following an ischemic reperfusion event[22]. The increased amount of oxidative stress on the endothelium resulted in the dysfunction of the endothelium's ability to dilate following the ischemic reperfusion event[22]. More specifically, the reactive oxygen species were a contributing factor in the inhibition of nitric oxide which is key in the dilation of the endothelium following the ischemic-reperfusion event[4]. In addition to ischemic conditions, oxidative stress can also be caused by the ingestion of a high fat meal and therefore can further damage the endothelium[9]. Like previous studies, this study found that the ingestion of a high fat meal will yield a decreased flow mediated dilation response, and therefore signal endothelial injury. This study also found that the depressed flow mediated dilation response, and thus endothelial injury, remained even after the ischemic reperfusion injury occurred.

In regards to age, this study tested a healthy and active population with a fairly young age range (19-26 years). Other studies found slight differences in flow mediated dilation responses between varying age groups and training conditions[25, 26]. More specifically, subjects who were middle aged showed a significant decrease in endothelial function following an ischemic-reperfusion injury as compared to those who are younger. Additionally, a similar study by DeVan et al., showed that those with resistance training were better protected from the endothelial impairment of an ischemic-reperfusion injury than are those who were not resistance trained[26].

Implications of the Present Study

Based on the results of this study, there may be broad clinical benefit to a specific population. When an individual ingests a single high fat meal, it clearly inhibits proper function of the endothelium for even the average healthy individual. When a high fat meal is coupled with an ischemic-reperfusion event the endothelium's function is even worse. Extrapolating this data out, if an individual has a chronic high fat diet their endothelium is experiencing this damage on a daily basis and chronic damage could possibly result over time.

This study did not test older populations or those with co-morbidities. If similar testing were done with other already diseased populations, similar results could possibly be found based upon previous research that tested the effects of ischemic-reperfusion and high fat meals separately. Clinicians or patients could take this information and apply it to their daily lives to reduce the amount of high fat meals consumed on a regular basis. A reduction of high fat meals from an individual's diet would likely result in less damage to the endothelium and by consequence result in healthier tissue.

Experimental Considerations

This study is not representative or applicable to a variety of populations. The sample size was small for this study, and the age range was very narrow. Based on the abnormal increase in FMD following IR-injury, there may not have been full occlusion

of the subject's artery for a 20 minute period. Similar results may not be found in an older population, obese population, or those with other health complications. Additionally, this study only tested males. Because of this results may not be applicable to females. This study did not control for training status. Based on previous studies an individual's training status could have an effect on the flow mediated dilation response. The meal given was limited in carbohydrates and proteins, while being rather high in fat. While this isolated the effect of the fat, it is likely not representative of an average high fat meal that would also include protein and carbohydrates.

Future Studies

Studies in the future should expand to different populations to determine if the effects seen from these results are replicated. Isolating different training statuses or health complications along with a similar protocol would further the knowledge on the subject. Comparing a similar protocol between populations of different age groups but similar health statuses would give better information as to the effects of age on high fat meals and ischemic-reperfusion injury. As mentioned above, future studies should test women to see if similar responses are present. Future studies may want to present a subject with a meal that is more representative of an average high fat western meal. More specifically, additional research could test to see if similar results are found with a solid meal, as compared to the liquid meal that was consumed by subjects in this study. Other markers for inflammation aside from IL-6 could also be taken to see if the amount or type of fat ingested leads to alterations in other inflammatory markers. Other

studies have looked at inflammatory markers such as: C-reactive proteins (CRP), tumor necrosis factor (TNF) α, IL-1β, and IL-8.[41]

Chapter 6: Conclusion

Previous studies have researched the impact of ischemic-reperfusion injury on the endothelium. Other studies have investigated the impact of high fat meal(s) on the function of the endothelium and have found that the meal significantly impacts the endothelium-dilation of the artery. However, to the best of our knowledge, no studies to date have evaluated the impact of a single high fat meal followed by ischemic reperfusion on endothelial function. Thus the aim of this study was to examine the effect of postprandial hypertriglyceridemia, via a high fat meal, on vascular endothelial function following ischemic-reperfusion injury. This study hypothesized that consuming a high fat beverage followed by an ischemic-reperfusion injury would impair endothelium-dependent vasodilation. Subjects were given either a placebo or high fat meal consisting of 1.5 grams of fat per kg of body weight. Impairment of the endothelium was assessed via ultrasound both before and after ischemic-reperfusion injury with use of a flow mediated dilation technique. Results from this study were in agreement with previous studies and showed that the high fat meal alone caused a significant decrease in endothelium dependent flow mediated dilation. Furthermore, results were in agreement with this study's hypothesis in that the dilation response of the endothelium was also impaired following the ischemic-reperfusion injury. More specifically these results show that consuming a single high fat meal can have a long lasting impairment of the endothelium considering the endothelial dilation remained depressed following the ischemic-reperfusion event. This is clinically relevant in that it can provide a healthy population with knowledge as to the impact of a single high fat

meal in conjunction with a negative cardiovascular event such as ischemic-reperfusion injury.

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Appendix A: IRB Outcome Letter

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Initial Submission – Board Approval

Date: January 22, 2016

To: Carl Ade, PhD

IRB#: 6228
Meeting Date: 01/04/2016
Approval Date: 01/21/2016
Expiration Date: 12/31/2016

Study Title: Effects of Postprandial Hypertriglyceridemia on the Ischemic-Reperfusion Injury
Reference Number: 646078
Study Status: Active - Open
Collection/Use of PHI: Yes

At its regularly scheduled meeting the IRB reviewed the above-referenced research study. Study documents associated with this submission are listed on page 2 of this letter. To review and/or access the submission forms as well as the study documents approved for this submission, open this study from the *My Studies* option, click to open this study, look under Protocol Items to click on the current *Application*, *Informed Consent* and *Other Study Documents*.

If this study required routing through the Office of Research Administration (ORA), you may *not begin your study yet*, as per OUHSC Institutional policy, until the contract through ORA is finalized and signed.

As principal investigator of this research study, it is your responsibility to:

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations at 45 CFR 46 and/or 21 CFR 50 and 56.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB Policy.
- Maintain accurate and complete study records for evaluation by the HRPP quality improvement program and if applicable, inspection by regulatory agencies and/or the study sponsor.
- Promptly submit continuing review documents to the IRB upon notification approximately 60 days prior to the expiration date indicated above.

In addition, it is your responsibility to obtain informed consent and research privacy authorization using the currently approved, stamped forms and retain all original, signed forms, if applicable.

If you have questions about this notification or using iRIS, contact the IRB @ 405-271-2045 or irb@ouhsc.edu.

Sincerely,

Karen Beckman, MD
Chairperson, Institutional Review Board

1105 N. Stonewall Avenue, Oklahoma City, OK 73117 (FWA00007961)

Study documents associated with this submission:

Study Consent Form			
Title	**Version Number**	**Version Date**	**Outcome**
Informed Consent - Final	Version 1.7	01/13/2016	Approved

Study Document			
Title	**Version Number**	**Version Date**	**Outcome**
CITI Training Documents	Version 1.0	11/20/2015	Approved
Health History	Version 1.0	11/20/2015	Approved
HIPAA 1 - Final	Version 1.1	11/20/2015	Approved
Flyer	Version 1.3	01/13/2016	Approved
Email/Letter Script	Version 1.2	12/30/2015	Approved
Protocol - Final	Version 1.4	01/13/2016	Approved

Information for Industry Sponsors: the columns titled Version Number and Version Date are specific to the electronic submission system (iRIS) and should not to be confused with information included in the Document and/or Consent title(s).

Appendix B: Informed Consent

701A Consent Version: IRB Number: 6228

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma, Norman

EFFECTS OF POSTPRANDIAL HYPERTRIGLYCERIDEMIA ON THE ISCHEMIC-REPERFUSION INJURY

Sponsor: Department of Health and Exercise Science
Principal Investigator: Carl J Ade, Ph.D.

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this study because you are a healthy person between the ages of 18 and 45.

Why Is This Study Being Done?
The purpose of this study is to determine the role a high fat beverage and the subsequent increase in blood triglycerides plays in regulating changes in cardiovascular health.

How Many People Will Take Part In The Study?
A maximum of 28 people between the ages of 18-45 will take part in this study.

What Is Involved In The Study?
Ischemic-reperfusion injury refers to the acute and reversible decreases in blood vessel function that follows a short period of ischemia (i.e., low-to-no blood flow) in your arm. To better understand how diet effects these changes you will consume a single high-fat beverage and a low-fat beverage consisting of water or low-fat milk on separate days. Before and after you drink each beverage a series of tests will be performed to determine how well the blood vessels in your arm work and how they respond to a short period of ischemia.

Procedures:
If you agree to be in this study you will be asked to complete health history and screening questionnaires prior to beginning the study. Additionally, you will also be asked to complete testing on two days that are roughly 7 days apart. On one day you will drink a single high-fat beverage. This will consist of heavy cream. On the other day you will drink a fat free beverage. This low-fat beverage will consist of water or low-fat milk.

The following procedures will be performed. Procedure 1 will be performed once. Procedures 2-6 will be performed before drinking the beverage (high-fat or low-fat), 2 hours after drinking the beverage, and after the ischemic-reperfusion protocol (Table 1).

Table 1: Protocol Timetable	
0:00 (hr:min)	Participants Arrives
0:00-0:05	Venous blood sample and occlusion familiarization
0:05-0:10	Rest
0:10-0:25	Baseline
0:25-0:35	Endothelium-dependent dilation test and Near-infrared spectroscopy test
0:35-0:55	Consume High-Fat or Low-Fat Beverage
2:55-3:05	Endothelium-dependent dilation test and Near-infrared spectroscopy test
3:05-3:45	Ischemic-reperfusion Protocol
3:45-3:55	FMD and MPOR

- **Ischemic-reperfusion (IR):** You will have a blood pressure cuff placed around the upper portion of the arm. The cuff will be inflated to no more than 220 mmHg for 20 minutes. This is similar to wearing a tight tourniquet for 20 minutes.

1. **Anthropometric measurements:** Your height and weight will be measured.

2. **Venous blood sample:** About 5 ml (one teaspoon) of blood will be drawn from your arm for the measurement of triglyceride and blood markers.

3. Occlusion familiarization: We will put a blood pressure cuff on your arm and inflate it for 60 seconds so that you can experience what will be felt in the following procedures.

4. **Resting blood pressure:** An average blood pressure will be taken after ten minutes of rest lying down. A blood pressure cuff will be inflated three individual times with a minute in-between each measurement.

5. **Endothelium-dependent dilation test:** A blood pressure cuff will be inflated to approximately 220 mmHg for 5 minutes. When five minutes have passed, the cuff will be deflated and measurement of your blood vessels will be taken for an additional 2 – 6 minutes.

6. **Near-infrared spectroscopy test**: A device placed on the surface of your skin at the forearm will send light signals into the tissue to give information about oxygen content in your blood.

What Are The Risks of The Study?

- The venous blood sample may include minor pain or a bruise where taken. This may also be associated with swelling of the vein and/or infection with a rare risk of fainting.
- Blood pressure cuff, when inflated for twenty minutes, will cause discomfort and may be painful. You may discontinue participation in the study if this procedure becomes too painful.
- The ischemic reperfusion may cause brief muscle discomfort that will subside within minutes of the test. No long-term or permanent damage will occur.
- The high-fat beverage may be displeasing and could cause some indigestion or slight discomfort but no significant risk to your health.

Are There Benefits to Taking Part in The Study?

There is no medical benefit to you by participating in this study. We hope the information learned from this study will benefit others by providing information on blood vessel function after drinking a high fat beverage.

What Other Options Are There?

You may choose not to participate in the study.

What about Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration, the Department of Health and Exercise Science, and the OUHSC Institutional Review Board.

What Are the Costs?

The study sponsor will pay for all costs related to your participation in this study.

Will I Be Paid For Participating in This Study?

You will be compensated with a free T-shirt or $15 gift card upon completion of the study.

What if I am Injured or Become Ill While Participating in this Study?

In case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company will be expected to pay for this care. No funds have been set aside by The University of Oklahoma Health Sciences Center or the University of Oklahoma Norman to compensate you in the event of injury.

What Are My Rights As a Participant?

Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. Please discuss leaving the study with the principal investigator. You may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?

If you have questions, concerns, or complaints about the study or have a research-related injury, contact **Carl J Ade, Ph.D.** at (785) 577-4098. He can be reached 24 hours a day, seven days a week.

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at (405) 271-2045.

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at (405) 271-2045.

Signature:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18) (*Or Legally Authorized Representative)*	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

IRB Last Revised: 05/23/2014

Appendix C: Subject Identification And Health History

I.D. _____ _____ _____ _____
Initials *Subject #*

Study ID:

Date _____/_____/_____

Name_____ **Age**_____ **Date of Birth**_____/_____/_____

Address_____**City**_____**State**_____**Zip**_____

Phone # (_____)_____ **Email** _____

Primary Physician _____ **Last Physical Examination** _____

Emergency Contact _____**Phone #** (_____)_____

If you answer "Yes" to any of the below questions, you will need a physician's approval before testing.

1.)	**Has a doctor ever said that you have a heart condition and that you should only do physical activity recommend by a doctor?**	**Yes**	**No**
2.)	**Do you feel pain in your chest when you do physical activity?**	**Yes**	**No**
3.)	**In the past month, have you had chest pain when you were not doing physical activity?**	**Yes**	**No**
4.)	**Do you lose your balance because of dizziness or do you ever lose consciousness?**	**Yes**	**No**
5.)	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**	**Yes**	**No**
6.)	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**	**Yes**	**No**
7.)	**Has a doctor ever said that you have microvascular or peripheral artery disease?**	**Yes**	**No**
8.)	**Has a doctor ever said that you have COPD, asthma, lung disease, or cystic fibrosis?**	**Yes**	**No**
9.)	**Has a doctor ever said that you have Diabetes (Type 1 or 2) or renal disease?**	**Yes**	**No**
10.)	**Do you know of any other reason why you should not do physical activity?**		

Have you ever had, or currently have any of the following? (please √)

___Asthma	___Arthritis
___Anemia	___Heart Disease/Heart Attack
___Chest Discomfort/Pain	___Light Headed/Dizziness/Fainting
___Diabetes	___Unusual Shortness of Breath
___Heart Murmur	___Stroke
Seizures	

If you answered yes or checked (√) any of the above, please explain in detail and list age of onset.

_____
_____
_____

1.)	Has your physician ever said you have high blood pressure?	Yes	No
	a. If "Yes" is your blood pressure controlled via medication?	Yes	No
2.)	You are a male 45 or over or a female 55 or over	Yes	No
3.)	Has your physician ever said you have high cholesterol?	Yes	No
	a. If "Yes" is your cholesterol controlled via medication?	Yes	No
4.)	Do you currently smoke?	Yes	No
5.)	Do you have a family history of heart disease? (Heart disease or sudden death before 55 for male first relative and before 65 for female first relative)	Yes	No

Please list any prescribed and/or over the counter medications and purpose for taking them.

_____
_____
_____

Please list any over-the-counter supplements and purpose for taking them.

_____
_____

Females:

1.) Are you pregnant? Yes No
2.) Do you have a regular menstrual cycle? Yes No
3.) Have you experienced menopause? Yes No

Recent Physical Activity History

Think about all the *vigorous* activities which take *hard physical effort* that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities?
   _____ Days per week

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?
   __ __ Hours per day
   __ __ Minutes per day

Now think about activities which take *moderate physical effort* that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities?
   ____ Days per week

4. How much time did you usually spend doing **moderate** physical activities on one of those days?
   __ __ Hours per day
   __ __ Minutes per day

Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?
   ____ Days per week

6. How much time did you usually spend **walking** on one of those days?
   __ __ Hours per day
   __ __ Minutes per day

Now think about the time you spent doing resistance/strength exercises in the last 7 days.

5. During the **last 7 days**, on how many days did you **resistance/strength exercises** for at least 10 minutes at a time?

   _____ Days per week

6. How much time did you usually spend **resistance/strength exercises** on one of those days?

   __ __ Hours per day

   __ __ Minutes per day

6. What exercises and limbs did you train?