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EFFECTS OF TRAINING ON POSTPRANDIAL FLOW MEDIATED DILATION

By

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B.S., University of Louisville, 2004

A Thesis
Submitted to the Faculty of the
Graduate School of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Health and Sport Sciences
University of Louisville
Louisville, KY

May 2006

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ACKNOWLEDGEMENTS

I would like to thank the following people for their support and assistance during the completion of this thesis project as well as my time spent here at UofL:

- My family, for all their support and help throughout my life
- Dr. Jennifer Olive, for her guidance and experience that she has given me over the past two years which will last far past my years spent at UofL
- Drs. Kara Gallagher and Bob Topp, for your insight and advice during this project and for serving on my thesis committee
- Dr. James Miller for his assistance in running the blood lipid assays
- Dr. Chong Lee for his help with statistical analyses
- All my fellow graduate students who assisted me with data collection (James, Melissa, Jess, Tiev, Jason, Jenn, Robb), especially for those long Saturdays spent in the lab
- My girlfriend, Shelby, for her understanding and patience during this process

ABSTRACT

EFFECTS OF TRAINING ON POSTPRANDIAL FLOW MEDIATED DILATION

Kevin D. Ballard

May 13, 2006

The purpose of this study was to determine if training has a protective effect on endothelial function following the consumption of a high-fat meal.

Twenty young males classified as trained or untrained underwent vascular and blood lipid testing pre and post (two- and four-hours) a high-fat meal.

Flow mediated dilation was significantly decreased at two- ($p < 0.001$) and four-hours ($p < 0.001$) in both groups, with no differences between groups ($p = 0.119$). Serum TG increased at two- ($p < 0.001$) and four-hours ($p < 0.001$) in both groups. LDL-C was reduced at four-hours ($p = 0.050$) in trained subjects, and two- and four-hours ($p \leq 0.01$) in untrained subjects. Two-hour HDL-C was reduced compared to baseline ($p = 0.024$) and four-hours ($p = 0.014$) in both groups.

This study demonstrates that a high-fat meal induces endothelial dysfunction for up to four hours in males, independent of training status.

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CHAPTER I

INTRODUCTION

Everyday, one out of four Americans eats at a fast food restaurant ¹. Regular consumption of high energy, high fat fast food meals can lead to serious health problems such as obesity and diabetes, both of which are important risk factors for cardiovascular disease ⁹⁸. Atherosclerosis, a cardiovascular disease, occurs when plaques accumulate on the walls of blood vessels which then block the delivery of essential nutrients such as oxygen. The predominant mechanism by which a high-fat diet leads to atherosclerosis is through an elevation of serum cholesterol ⁸¹. A second mechanism is caused by the direct impairment of vascular endothelial function, which is an important early process in the development of atherosclerosis ³³. Endothelial dysfunction has been shown to be an early event in atherogenesis and has been demonstrated in healthy subjects with risk factors for atherosclerosis many years before the appearance of atheromatous plaques ^{9, 56}. Endothelial dysfunction has been found to occur in many different diseased populations, including individuals with hypertension ⁷⁶, hypercholesterolemia ¹⁴, diabetes ⁴⁴, atherosclerosis ⁹, and in smokers ⁶⁷ and obese individuals ⁹⁷.

Nitric oxide (NO) is secreted by the endothelium and is the predominant substance by which vasodilation occurs ⁸³. Nitric oxide is released from the endothelium in response to mechanical factors such as shear stress. Endothelial function can be assessed repetitively and *non-invasively* in the peripheral circulation through flow

mediated dilation (FMD)⁹. Flow mediated dilation occurs due to an increase in blood flow, or shear stress, after temporary cuff occlusion of the brachial artery. The increase in shear stress causes the release of NO from the endothelium which then acts on the smooth muscle of the vessel causing dilation.

The consumption of a high fat meal (HFM) has been demonstrated to induce transient endothelial dysfunction^{5, 29, 65, 69, 81, 100, 102, 104}. The proposed mechanism for the decrease in endothelial function following the consumption of a HFM is thought to be due to the oxidation of postprandial triglyceride-rich lipoproteins^{5, 81, 100} which reduces the bioavailability of NO³¹. The inability to produce NO or the breakdown of NO is an important consequence of endothelial dysfunction⁸³.

Conversely, endurance training has been shown to improve endothelial dysfunction in many diseased populations^{34, 38, 56, 103} as well as in healthy, young men¹¹. A cross-sectional study demonstrated that young, endurance-trained men had significantly higher FMD responses of the brachial artery than sedentary young men⁴⁷. No reported literature was found examining the effects of a single HFM on endothelial function in endurance-trained young males in comparison to their sedentary peers.

Purpose of the Study

The purpose of this study was to determine if endurance training has a protective effect on endothelial function following the consumption of a single HFM.

Primary Aim:

- 1) To determine the effects of a single HFM on FMD of the brachial artery and on blood flow in the femoral artery in trained and untrained men.

Secondary Aims:

- 1) To determine between groups (trained and untrained men) the effects of a HFM on blood lipid levels (serum triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C)).
- 2) To compare the effects of a HFM on blood lipids over time (four-hours).
- 3) To determine if changes in FMD are correlated to changes in blood lipids.

Hypotheses

The following hypotheses will be tested:

Primary Hypothesis:

- 1) The ingestion of a HFM will impair FMD in the brachial artery of both groups, with the untrained group displaying a significantly greater impairment than the trained group.

Secondary Hypotheses:

- 1) Resting and peak femoral blood flow will not be significantly different between the two groups.
- 2) Half-time to recovery of the femoral artery will be significantly prolonged in the untrained compared to the trained group.
- 3) Blood lipid levels (TG, TC, and LDL-C) will be reduced significantly at baseline in the trained compared to the untrained group, while the trained group will demonstrate significantly higher HDL-C levels than the untrained group at baseline.

Significance of the Study

This study is significant as it will determine if there is a protective effect of chronic endurance training on endothelial function following a HFM. Several studies have indicated that consumption of a HFM significantly impairs endothelial function through a decrease in FMD. To date no literature has addressed the potential protective effect of exercise on endothelial function following a HFM. This research is critical in understanding ways to improve endothelial function and ultimately decrease cardiovascular disease and mortality rates.

CHAPTER II

LITERATURE REVIEW

The endothelium functions as an anatomical barrier between the blood and interstitium at the capillary level, as well as throughout the vascular tree⁴. Other roles of the endothelium include regulating vasoactivity, smooth-muscle cell proliferation, platelet aggregation, monocyte adhesion, inflammation, and free radical responses¹⁰¹. Vasoactivity is regulated by the release of substances from the endothelium. Endothelin, an endothelial vasoactive substance, causes arteriolar smooth muscle contraction and is considered one of the most potent vasoconstrictors yet identified⁹⁴. Another substance released by the endothelium, prostacyclin, inhibits platelet aggregation and promotes vasodilation³⁰. The vasodilator NO is also secreted by the endothelium and is the predominant substance by which vasodilation occurs⁸³. In addition to promoting vasodilation, NO also helps to stabilize platelets, inhibit smooth muscle migration and hyperplasia, and discourages the influx of activated monocytes and other inflammatory cells⁸². The inability to produce NO or the breakdown of NO is an important consequence of endothelial dysfunction⁸³.

Dysfunctional endothelium induces vasoconstriction, platelet aggregation, monocyte adhesion, thrombosis, and inflammation¹⁰¹, and has been shown to be a key early event in atherogenesis, appearing long before the formation of structural atherosclerotic plaques⁸³. Endothelial dysfunction has been studied in association with

various cardiovascular disease risk factors such as hypertension ⁷⁶, hypercholesterolemia ¹⁴, diabetes ⁴⁴, atherosclerosis ⁹, smoking ⁶⁷ and obesity ⁹⁷ and has been observed before the clinical manifestation of vascular disease. Dysfunctional endothelium has also been demonstrated in children and adolescents with risk factors for future cardiovascular disease ⁹.

All blood vessels are lined with a thin layer of endothelial cells that are in direct contact with the blood. A thick wall of smooth muscle and connective tissue surrounds the endothelial lining in arteries. Vasodilation of an artery can be classified as endothelium-independent or endothelium-dependent. Endothelium-independent vasodilation occurs due to a substance's effect directly on the smooth muscle, while endothelium-dependent vasodilation occurs due to the endothelium releasing a substance (i.e. NO) which then acts on the smooth muscle. Nitric oxide is released from the endothelium in response to substances such as acetylcholine and bradykinin, and in response to mechanical factors such as shear stress due to an increase in blood flow. Endothelial-dependent function can be assessed repetitively and *non-invasively* in the peripheral circulation through FMD ⁹. Flow mediated dilation occurs due to an increase in blood flow, or shear stress, after cuff occlusion of the brachial artery. The increase in blood flow in response to a transient cuff occlusion is termed reactive hyperemia and has been demonstrated to elicit an equal change in artery diameter compared to active hyperemia (i.e. exercise) ⁷⁵. The increase in shear stress on the vessel lumen causes the release of NO from the endothelium which then acts on the smooth muscle of the vessel causing dilation.

High-Fat Meals and Endothelial Function

Zilversmit first suggested the consideration of the postprandial effects of a meal as a risk factor for atherosclerosis and proposed the study of postprandial conditions and their relationship to endothelial dysfunction¹⁰⁷. However, it has only been recently that the influence of dietary factors on endothelial function has been examined. Consumption of a single HFM has been shown to induce endothelial dysfunction in several different populations, including healthy middle age men^{29, 81, 102, 104}, healthy middle age women^{81, 102}, healthy young men^{65, 69, 100}, and healthy older men and women⁵. Studies have found that peak impairment of FMD following a HFM has been shown to occur at two-^{65, 69, 100} and four-hour postprandial⁷⁵.

However, others have demonstrated that endothelial function, as measured by FMD, did not significantly decrease following HFM consumption^{19, 84}. Furthermore, it appears that there may be gender differences in FMD after a HFM as young, healthy women do not exhibit endothelial dysfunction as compared to men⁹¹. The discrepancies in results of a HFM on FMD may be explained by the different meals consumed, timing of vascular measurements, and dietary lipid composition of the meals¹⁶ or the gender of the subjects. The influence of gender on endothelial function may be explained by the presence of estrogen as this hormone has been shown to be the primary mechanism behind the preserved endothelial function seen in young women, and short-term estrogen therapy has been shown to improve endothelial function in postmenopausal women³⁶.

A possible mechanism for the impairment of endothelial function following a HFM is the transient increase in postprandial triglyceride rich lipoproteins^{60, 65, 81, 102}. Postprandial hypertriglyceridemia has been shown to cause endothelial dysfunction via

enhanced oxidative stress^{5,100,104}, thereby reducing the bioavailability of NO.

Postprandial triglyceride-rich lipoproteins, including chylomicron remnants and very low density lipoproteins (VLDL) have been shown to penetrate the vessel wall within the subendothelial space⁶³. It has been suggested that postprandial lipoproteins, particularly chylomicrons and VLDL, induce oxygen free radical generation at the endothelial surface reducing the bioavailability of NO³¹. The increase in oxidized LDL inactivates NO leading to endothelial dysfunction⁵⁹. The trapping of lipoproteins within the subendothelial space is considered to be the initiating event of atherogenesis⁶³. It has been demonstrated that remnant lipoprotein levels have been shown to be independently associated with abnormal endothelium-dependent vasomotor function in human coronary arteries⁵³ and in the peripheral arteries³. This mechanism has been supported by the findings of Plotnick et al. who demonstrated that pretreatment with antioxidant vitamins (Vitamin E and C) eliminated the decrease in FMD following a HFM⁸¹. An increased oxidative stress has also been reported to impair endothelial function in smokers³⁷.

Lundman et al. found that chronic mild-to-moderate hypertriglyceridemia in young men is associated with impaired FMD of the brachial artery and higher plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenous NO synthase (NOS) inhibitor⁶¹. Nitric oxide synthase is a precursor to the development of NO in the endothelium. Furthermore, it has been demonstrated in individuals with type 2 diabetes that a HFM increases ADMA levels, and that this is accompanied with impaired FMD²⁴. Although the subjects in the above mentioned research are different from those examined in the present study, these findings might suggest that acute hypertriglyceridemia

following a HFM in young, healthy men might increase ADMA levels, thereby reducing the bioavailability of NO and resulting in impaired FMD.

Lipoprotein lipase (LPL) is an enzyme found on the surface of the endothelial cells of the capillaries of fat and muscle, as well as in adipose tissue and in heart and skeletal muscle⁸. The action of LPL facilitates that clearance of most chylomicrons, including LDL-C and very low density lipoproteins⁸. Capillary LPL is activated following the ingestion of a meal and accelerates the hydrolysis of triglycerides in lipoproteins, allowing for the storage of free fatty acids in adipose tissue⁸.

Another type of LPL, muscle LPL (Type L-HSL), exists within muscle cells and is responsible for the hydrolysis of TG in circulating lipoproteins⁸. It is known that muscle LPL activity is increased following the consumption of a HFM⁴⁹. Plasma levels of LPL have been found to increase in middle-age male subjects following the ingestion of an oral fat load and to peak at six-hour postprandial⁴⁶. Kiens et al.⁴⁹ demonstrated that LPL activity was significantly increased in physically trained men following a four-week high-fat diet. The authors suggested that the increased LPL activity seen in this sample indicated a higher capacity for uptake of fatty acids from circulating serum TG⁴⁹. Therefore, increased lipoprotein levels following a HFM would result in an increase in LPL activity and would facilitate the clearance of circulating lipoproteins, thus helping to restore endothelial function several hours postprandial.

Exercise Training and Endothelial Function

Endurance training has been shown to improve endothelium-dependent vasodilation in patients with polymetabolic syndrome⁵⁶, chronic heart failure³⁴, hypertension³⁸, and acute myocardial infarction¹⁰³. Improved endothelial-dependent

vasodilation responses have also been reported in healthy, older endurance-trained men compared to their sedentary peers^{87, 88} and in young men following 10 weeks of whole-body exercise training¹¹. In a recent study, Kasikcioglu et al. found that young men who were endurance-trained athletes had significantly higher FMD responses of the brachial artery than young men who were sedentary, but otherwise healthy⁴⁷. The higher responses measured in the endurance-trained athletes suggests improved endothelial function with consistent aerobic exercise and demonstrates that aerobic exercise has the potential to improve systemic endothelial function. This study also demonstrated a significant positive correlation between FMD and maximal oxygen consumption (VO₂max). However, Franzoni et al. found that young, endurance-trained male athletes did not display a greater percent change in FMD compared to matched healthy sedentary men²⁶. This inconsistency might be explained by the possibility that FMD may approach a limit at which it is difficult to improve. Another difference between these two studies is the athlete's ages (33.4 ± 6.7 years vs. 23.2 ± 3.7 years, Franzoni vs. Kasikcioglu, respectively). Celermajer et al.¹⁰ found that aging is associated with progressive endothelial dysfunction in humans, and that this appears to occur earlier in men than in women. This finding might suggest that FMD measured in the athletes from the Franzoni study may be lower compared to the younger athletes from the Kasikcioglu study due to their increased age. In contrast, Moe et al. found that endurance-trained young women showed no significant improvements in FMD when compared to young sedentary females, suggesting that endothelial function is well preserved in young, healthy women and that endurance training does not improve the dilating capacity any further⁶⁶.

Whole body resistance training did not significantly improve FMD after 12 weeks in young, healthy men⁸⁵. The differences demonstrated in FMD between these two types of exercise training have been suggested to occur due to the different blood flow and pressure responses associated with each type of training. When performing aerobic exercise, blood flow is persistently elevated under low pressure thereby creating a constant shear stress on the endothelium. It was suggested that shear rates due to resistance training may be too small and transient to elicit adaptations of brachial artery endothelial function⁸⁵.

It has been postulated that physical training improves endothelium-mediated vasodilation via an enhanced endothelial release of NO²⁰. Exercise-induced increases in blood flow and subsequent shear stress on the vessel wall may result in mechanical alteration of the endothelium promoting NOS up regulation^{47, 11}. The gene expression of NOS has been found to be increased following chronic exercise¹⁰⁶.

Fewer studies have reported training-induced adaptations of the femoral artery. In a study by Kool et al., well-trained cyclists were found to have a higher distensibility and a larger diameter of the femoral artery compared to sedentary subjects⁵¹. These findings were supported by Huonker et al. who studied the size and blood flow of the common femoral artery using pulsed-wave Doppler⁴¹. They found that elite road cyclists had a larger diameter of the common femoral artery as compared to untrained subjects. These same elite road cyclists also demonstrated significantly larger stroke flow in the common femoral artery than untrained subjects. Gaenger et al. reported a significantly greater vasodilation of the femoral artery in response to submaximal cycling exercise in nonsmoking men compared to smokers²⁸. However, exercise-induced blood flow of the

femoral artery was not significantly different between the two groups exercising at the same workload. According to the reported literature, a sustained high blood flow in arteries supplying blood to exercising muscles causes an increased shear stress on the vascular wall and may play an important role in regulating the diameter of these arteries

40 .

Blood Lipids in Response to a Single HFM

The most characteristic feature of lipid metabolism after a HFM is an increase in postprandial TG. A single HFM has been shown to significantly increase TG levels up to six-hours¹⁹ and to peak at four-hours postprandial in young, healthy men⁶⁵. It has been demonstrated that a significant increase in TG occurs at two-^{5, 65, 81, 102} and four-hours^{65, 81, 102, 104} postprandial a commercially available HFM and that a significant inverse relationship exists between the mean change in FMD and postprandial TG levels¹⁰². These results are supported by Bae et al. who found a significantly negative correlation between changes in serum TG and impairment of brachial artery vasoactivity two-hours postprandial⁵. A novel finding of this study was a significant elevation in leukocyte superoxide production, a measure of oxidative stress, two-hours after the HFM. A potential mechanism for these findings is that hypertriglyceridemia increases oxidative stress, which results in the inactivation of NO production and leads to endothelial dysfunction.

Several studies have found that postprandial values of TC, LDL-C, and HDL-C remained unchanged compared to baseline^{5, 65, 81, 102}. In contrast, Tsai et al. found that total cholesterol, LDL-C and HDL-C were significantly decreased at both two- and four-hours postprandial following a single HFM¹⁰⁰. HDL-C remained significantly decreased

at six-hour post as well. It has been shown previously that a single HFM lowers LDL-C and HDL-C⁹⁰. It has been reported that the influx and lipolysis of triglyceride-rich lipoproteins following a HFM is associated with the transfer of cholesteryl ester from LDL-C and HDL-C to these triglyceride-rich particles through the action of cholesteryl ester transfer protein. The result of this transfer is a modest decrease in LDL-C and HDL-C after a single HFM¹³. A decrease in HDL-C following a HFM may suggest that plasma TG clearance would be impaired due to the finding that the clearance rate of plasma TGs are significantly related to HDL-C levels in male endurance athletes ($r = .75, p < 0.01$)⁸⁹. Therefore, a decreased HDL-C level after a HFM might impair TG clearance and could result in postprandial hypertriglyceridemia and subsequent impairment of endothelial function.

Blood Lipids and Exercise Training

The consensus is that regular exercise training decreases plasma triglyceride concentrations and LDL-C, while increasing HDL-C levels. The concentration of total plasma cholesterol is not consistently decreased compared to sedentary controls²¹.

Regular aerobic exercise has been shown to increase LPL activity and the clearance rate of plasma triglycerides in men⁹⁹. Kiens et al. found that muscle LPL activity was 48% higher in endurance-trained than in non-trained subjects, irrespective of gender⁵⁰. Mankowitz et al. also demonstrated a higher plasma LPL activity in endurance-trained individuals⁶⁴. Higher LPL activity seen in endurance-trained individuals would improve the clearance rate of lipoproteins following consumption of a HFM and may explain the low fasting triglyceride levels often seen in highly-trained individuals²⁷. Endurance-trained young men have also been shown to have a lower postprandial lipemia

than their sedentary counterparts following consumption of meals consisting of 40 and 140 g fat ¹². This reduction in postprandial lipemia seen in endurance-trained individuals was suggested to be partly due to a direct effect of chronic endurance exercise on the triglyceride removal system.

Another proposed mechanism by which lipoprotein clearance rates are enhanced with endurance-training may have to do with increases in muscle blood flow with training ³⁵. At least one study has demonstrated higher muscle blood flow in endurance-trained men compared to their sedentary peers ²², but the data is inconclusive. It is suggested that increases in muscle blood flow increase the exposure of LPL to lipoproteins, thereby facilitating their degradation ³⁵.

Regular physical activity has been shown to raise HDL-C and lower LDL-C and triglycerides levels ⁷⁸. Increased levels of HDL-C are a chronic adaptation to endurance training. Sady et al. found that elevated HDL-C levels in endurance trained men were directly related to the clearance rate of plasma triglycerides ⁸⁹. This finding suggests that elevated HDL-C levels seen in endurance-trained individuals could possibly contribute to the clearance rate of plasma triglycerides ⁸⁹ and therefore the lower postprandial lipemia demonstrated in this group. Kasikcioglu et al. found that total cholesterol and LDL-C levels were significantly lower in runners compared to sedentary subjects ⁴⁷; in contrast, HDL-C levels were significantly higher in runners. No significant difference between groups in triglycerides was found.

Acute endurance exercise performed the day prior to meal ingestion has been found to attenuate the postprandial lipemic response ⁷⁹. The energy expenditure of the exercise performed was determined as the major factor influencing postprandial lipemia.

In contrast, lipidemic profiles of athletes compared to non-athletes were not found to be significantly different, when body fat was similar between groups⁸⁰, thus providing further support to the role of endurance training on influencing postprandial lipidemia. The higher metabolic capacity of triglycerides seen in athletes can probably be attributed to not only differences in body fat levels, but possibly muscle fiber composition as well³⁵. Jacobs et al. demonstrated a significant positive correlation between the proportions of type I (oxidative) muscle fibers and muscle LPL activity⁴². Highly-oxidative muscle fiber types have been found to be the majority of fiber types in elite, distance runners⁸. This finding suggests that muscle fiber composition might contribute to the low levels of postprandial lipemia seen in athletes.

Summary

In summary, diets that regularly include high-fat meals have been shown to impair endothelial function through the inactivation of NO due to an increased oxidation of postprandial TG-rich lipoproteins by circulating free radicals. Conversely, an improvement in endothelial function has been shown in individuals who perform regular endurance exercise when compared to sedentary individuals. The mechanism behind this improvement has been suggested to be due to the increased expression of NOS, caused by an increased shear stress on the endothelial wall during endurance training. This adaptation would promote the development of NO and subsequent improvements in endothelial function. Improvements in the postprandial lipemic response have been demonstrated in endurance-trained individuals, and have been suggested to be due to increased levels of LPL that facilitate the clearance of circulating lipoproteins.

CHAPTER III

METHODS

Subjects

Twenty, healthy young men between the ages of 19 and 26 were recruited for this study. No subjects had a history of diabetes, hypertension, hypercholesterolemia, smoking or were presently taking any form of medication as determined through phone screening (Appendix A) and self-reported medical history (Appendix B). Subjects were screened and those with a body mass index (BMI) ≥ 32 kg/m² were excluded to decrease the risk of obesity acting as a confounder. Subjects were classified as trained (n = 10) or untrained individuals (n = 10) as determined by self-report. The trained subjects were recruited from University of Louisville athletic teams (9 cross-country runners, 1 tennis player) and had participated in vigorous aerobic-exercise training for greater than one year prior to the study. The untrained subjects were recreationally active but had not participated in a consistent aerobic-training program (< 3 days/week, 20 minutes/day) during the past six months. Before beginning the study, subjects were briefed on potential risks and benefits, and asked to sign a written informed consent (Appendix C) that was approved by the institutional review board of the University of Louisville.

Experimental Protocol

Day 1

Briefly, upon reporting to the laboratory height and weight were measured. Next, VO_2max was determined to verify the training status of each subject.

1.) Height and weight

Height was measured without shoes to the nearest $\frac{1}{4}$ inch using a wall mounted measuring tape. Weight was measured without shoes with the subject wearing shorts and t-shirt to the nearest 0.5 lbs using a properly calibrated Taylor lithium electronic scale (Taylor Precision Products, Las Cruces, New Mexico).

2.) Maximal oxygen consumption

VO_2max was measured to verify the self-reported training status of each subject and was determined through the use of a ramp protocol (Appendix D) via indirect open circuit spirometry (ParvoMedics, Sandy, UT). The speed of the treadmill was individualized to subjects based upon their reported one-mile pace. The ramp protocol consisted of a five-minute warm-up after which subjects ran at two miles per hour (mph) below their self-reported pace for two-minutes. Speed was increased by 1.0 mph at minutes two and four. At minute six, treadmill grade was increased 2.5% every two-minutes until the subject reached volitional exhaustion. Determination of VO_2max was ascertained if the subject met two of the following three criteria: 1) A plateau in oxygen uptake (or failure to increase oxygen uptake by 150 mL/min) with increasing workload; 2) a respiratory exchange ratio (RER) ≥ 1.15 ; or 3) a rating of perceived exertion (RPE) of >17 on the Borg 6-20 scale². VO_2max values were used to verify the trained from the untrained subjects (74.6 ± 5.2 ml/kg/min vs. 47.3 ± 7.1 ml/kg/min, trained vs. untrained,

respectively, $p < 0.001$). Following all measurements, subjects were scheduled for the second day of testing that occurred within two weeks of Day 1. Prior to the next visit all subjects were instructed to abstain from exercise for 24-hours to avoid any confounding influences of exercise on lipid metabolism of the HFM⁷⁹. Subjects were also instructed to fast and to not consume caffeine for 12-hours prior to the next visit in order to obtain fasting blood samples and accurate body composition measurements. Also, subjects were instructed not to ingest any supplements or vitamins 12-hours prior as to avoid any confounding influences on endothelial function⁸¹.

Day 2

Briefly, upon reporting to the laboratory body composition was measured using bioelectrical impedance analysis (BIA) to determine fat mass (FM). Fat-free mass (FFM), or lean body mass (LBM) was determined using a validated multiple regression equation⁹³. Next, FMD of the brachial artery and blood flow measures of the femoral artery were determined via Doppler ultrasound. A small blood sample was then obtained to determine fasting blood lipids. This was followed by the consumption of a commercially available HFM. Vascular measures and blood samples were again obtained at two- and four-hours postprandial to determine the effects of the meal on these variables.

1.) Anthropometric Measurements

Body composition was determined by use of BIA (RJL Systems, Clinton Township, MI). This method requires that a small electrode be placed on the hand, wrist, ankle and foot of the subject. A low-level electrical signal is transmitted between the electrodes.

Subjects were instructed to remove their right sock (if necessary), and to lie supine on the exam table. Electrodes were placed at the midpoint of the styloid process and the joint between the knuckles of the index and middle fingers of the right hand. The distance between electrodes was measured to verify a minimum distance of 8 cm. If the distance was found to be greater than 8 cm, the electrodes were adjusted as needed.

Two separate electrodes were then placed at the midpoint of the medial and lateral malleolus and the joint at the base of the big and second toes of the right foot using the same procedures as described above. The BIA cables were then attached to the electrodes at all four locations and the analyzer was turned on and measurements were allowed to stabilize for approximately 20 seconds. The displayed Resistance and Reactance values were recorded and were then used to calculate FM via computer software (Cyprus 2.7, Body Composition Analysis, RJL Systems, Clinton Township, MI). Due to errors in calculating LBM by the computer software, LBM was calculated using a validated multiple regression equation ⁹³:

$$\text{LBM} = -8.98751 + 0.36273(\text{Height}^2 \text{ (cm)})/\text{Resistance} + 0.21411(\text{Height}) + 0.13290(\text{Weight (kg)}) - 5.61911(\text{G}).$$

The “G” used in the equation refers to gender and is equal to zero if it is a male and one if it is a female. The sum of LBM and FM was equal to total body weight in all subjects studied.

2.) Vascular Measurements

Brachial

Endothelial function was measured *non-invasively* in the subject’s brachial artery using quantitative Doppler ultrasound (Philips HDI 5000, Seattle, WA). Measurements

were performed in a supine position on the right arm, in a quiet, dark room with a temperature of 22°C after a 10-minute equilibration period by a single investigator. The brachial artery was imaged longitudinally, 2-10 cm above the antecubital fossa by B-mode ultrasound, using a 12-5 MHz linear array transducer. Reactive hyperemia was induced by inflation of a pneumatic cuff (10 cm wide) at 60-80 mm Hg suprasystolic for five-minutes of the upper arm using a rapid cuff inflator (Hokanson E20, Bellevue, WA). A five-minute cuff inflation period has been shown to produce sufficient hyperemia to allow FMD, but not to compromise subject comfort⁸³. Complete cuff occlusion was verified visually via Doppler. Video images were recorded to a computer (Gateway E Series, Irvine, CA) for four-minutes at baseline and then five-minutes after the release of the cuff occlusion to determine percent change in diameter from baseline. The brachial artery was occluded above the image site because this method has been shown to elicit a greater percent change in diameter compared to that produced by the placement of the cuff on the forearm¹⁰¹.

Femoral

Blood velocity of the femoral artery was measured using a 7-4 MHz linear array transducer in a supine position on the right leg. The velocity of arterial flow was measured with a pulsed-Doppler signal using an insonation angle of 60°. All images were obtained on the right femoral artery 3 to 5 cm distal to the bifurcation of the common femoral artery. Doppler measurements were made proximal to the cuff to ensure that the vessel image was maintained throughout cuff occlusion. Hyperemia was induced by inflation of a pneumatic cuff (12 cm wide) at 80-100 mm Hg suprasystolic for four-minutes on the lower thigh. Velocity measurements were auto calculated every heart beat

by Philips vascular program software for the HDI 5000. Video images were recorded on a computer for four-minutes at baseline and then five-minutes after the release of the cuff occlusion to determine maximum, mean, and peak blood flow. Blood flow was calculated by the product of vessel cross sectional area and the time average maximum velocity. Resting femoral artery diameter was used for the calculation of blood flow as no vasodilation was measured in this vessel following cuff occlusion. This observation has been reported previously⁴⁵. Half-time to recovery of blood flow was calculated as the time at which blood flow dropped to one half the magnitudes between maximum flow and baseline flow by fitting the blood flow curve to a nonlinear regression curve fit with one phase exponential decay using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA). Half-time to recovery was used as an index of vascular reactivity as it indicates the ability of the vessel to return BF to resting values^{70, 77}.

All video images were saved by video recording software (Ulead Video Studio 7, Ulead Systems, Inc., Taipei, Taiwan) to a main computer by way of an S-video cable for future analysis. A custom-made software program using LabView version 7.1 (National Instruments, Austin, TX) was used for the collection of velocity data and the measurement of brachial artery diameter. This software downloaded diameter and velocity measurements every heart cycle to a Microsoft Excel spreadsheet.

Blood pressure was measured prior to and throughout all vascular measurements using an automated blood pressure monitor (Omron, HEM-907 XL, Vernon Hills, IL) to control for any alterations in blood pressure that might affect blood flow. No changes in blood pressure were found across the entire testing period.

3.) Blood Analyses

Approximately 10-ml of blood was collected from an antecubital vein with a 21-gauge needle to obtain fasting TG, TC, HDL-C, and LDL-C. Blood samples were obtained at three separate time periods: baseline, two- and four-hours postprandial. Samples were centrifuged (Fisher Scientific, Model 228) at 3300 rpm for \approx 30 minutes and the separated serum was transferred to labeled 0.5 ml vials and stored at -80° C until analyzed.

Triglycerides

Triglyceride levels were measured enzymatically by dissociating the TG from lipoprotein complexes present in the sample (VITROS Chemistry Systems, Johnson & Johnson Gateway, Piscataway, New Jersey). Triglyceride molecules were then hydrolyzed by lipase to yield glycerol and fatty acids. The glycerol was phosphorylated and oxidized to dihydroxyacetone phosphate and hydrogen peroxide, which was used to oxidize a leuco dye in the presence of peroxidase to generate a colored dye. Reflectance spectrophotometry was used to measure the TG concentration present in the sample, which was proportional to the density of the dye formed.

Total Cholesterol

Total cholesterol was measured enzymatically by dissociating the cholesterol and cholesterol esters from lipoprotein complexes present in the sample. The hydrolysis of the cholesterol esters was catalyzed by cholesterol ester hydrolase. The free cholesterol was then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide, which was used to oxidize a leuco dye in the presence of peroxidase to generate a colored dye. Reflectance spectrophotometry was used to measure the

cholesterol concentration present in the sample, which was proportional to the density of the dye formed.

HDL-Cholesterol

High density lipoprotein-cholesterol was measured enzymatically using a non-HDL precipitation method, followed by enzymatic detection. Phosphotungstic acid and magnesium chloride were used to separate HDL by the precipitation of non-HDL. Similar to the procedures used to measure TC, the oxidation of a leuco dye in the presence of peroxidase by hydrogen peroxide is used to generate a colored dye. The density of this formed dye is measured by reflectance spectrophotometry and is proportional to the HDL-C concentration present in the sample.

LDL-Cholesterol

Low density lipoprotein cholesterol was calculated by the Friedewald Formula:
$$\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{Trig}/5)^{86}.$$

4.) High-fat meal

Following the first blood sample, subjects consumed a commercially available fast-food meal consisting of an Enormous Omelet Sandwich® (740 kcals, 46 g fat, 16 g saturated fat, 330 mg cholesterol) and a medium order of hash browns (310 kcals, 20 g fat, 5.5 g saturated fat, 0 mg cholesterol) (Burger King Corporation, Miami, FL). This test meal was well tolerated by all subjects. Water was consumed ad libitum throughout the day. Vascular measurements and blood samples were again obtained at two- and four-hours postprandial to determine any effects of the meal on these variables. Between testing periods subjects were instructed to rest quietly in the laboratory while reading and were not allowed to consume any other food or drink except for water.

Statistical Analyses

SPSS (Version 10.0) was used for all statistical analyses with an alpha level of 0.05. An independent samples t-test was used to determine any mean differences between group characteristics. A repeated measures ANOVA was performed to assess differences within subjects in vascular measurements and blood lipid levels prior to and following the HFM. Correlational analyses were used to determine any relationship between changes in vascular reactivity and changes in blood lipid levels. If any significant correlations were found, linear regression analyses were conducted to predict FMD from blood lipid levels.

CHAPTER IV

RESULTS

The primary purpose of this study was to determine if endurance training has a protective effect on endothelial function following the consumption of a single HFM. A secondary purpose was to determine between groups and across time the effects of a HFM on blood lipid levels and any relationships that may exist between changes in FMD and changes in blood lipids.

Descriptives

Subject descriptives can be found in Table 1. The trained and untrained groups were not significantly different in age, height, weight or lean body mass. Significant differences were found between groups for $VO_2\text{max}$ ($t(18) = 9.808, p < 0.001, d = 4.384$), thereby confirming the differences in training status between groups. Body mass index ($t(18) = -2.738, p = 0.014, d = 0.520$) and fat mass ($t(18) = -2.370, p = 0.029, d = 1.059$) were also found to be significantly different between groups. An inverse association has been shown between high BMI and FMD⁶. Since neither group approached a BMI considered overweight/obese, these covariates were not included in statistical analyses of FMD. The data analysis revealed that the single tennis player was an outlier for $VO_2\text{max}$, but was not on any other measure. When statistics were conducted with this subject removed from the analysis there were no significant differences in the results. Thus, the subject was included in all data analysis.

Brachial Measurements

Baseline measurements of the brachial artery diameter were not significantly different between groups (0.48 ± 0.04 cm vs. 0.46 ± 0.05 cm, trained vs. untrained, respectively) ($F(1, 18) = 0.750$, $p = 0.398$, $\eta^2 = 0.040$) and did not change over time ($F(2, 36) = 0.080$, $p = 0.923$, $\eta^2 = 0.004$).

Brachial artery responses are displayed in Table 2. Flow mediated dilation was not significantly different between groups ($F(1, 18) = 2.677$, $p = 0.119$, $\eta^2 = 0.129$) (Figure 1). However, a significant time effect was found after the HFM ($F(2, 36) = 26.64$, $p < 0.001$, $\eta^2 = 0.597$). Significant time differences were determined between baseline and two-hours postprandial ($F(1, 18) = 57.97$, $p < 0.001$) and between baseline and four-hours postprandial ($F(1, 18) = 27.32$, $p < 0.001$). No significant time differences were found between two- and four-hours postprandial ($p = 0.179$).

Flow mediated dilation was significantly decreased from baseline to two-hours postprandial by approximately 35% in both groups ($p < 0.001$). Flow mediated dilation from baseline to 4-hours postprandial was significantly decreased by 21% in the trained group ($p < 0.001$) while the untrained group remained at a 35% reduction ($p < 0.001$).

Femoral Measurements

Femoral artery responses can be found in Table 3. Baseline femoral artery diameter was 0.71 ± 0.07 cm and 0.69 ± 0.04 cm for the trained and untrained subjects, respectively. Femoral artery diameter size was not significantly different between groups ($F(1, 18) = 0.870$, $p = 0.363$, $\eta^2 = 0.046$) nor did it change over time ($F(2, 36) = 0.392$, $p = 0.678$, $\eta^2 = 0.021$). No significant differences were found between groups in maximum blood flow ($F(1, 18) = 0.775$, $p = 0.199$, $\eta^2 = 0.079$), mean blood flow

($F(1, 18) = 0.402, p = 0.534, \eta^2 = 0.765$), peak blood flow ($F(1, 18) = 3.007, p = 0.100, \eta^2 = 0.179$), or half-time to recovery ($F(1, 18) = 0.798, p = 0.383, \eta^2 = 0.042$).

Furthermore, no significant differences across time were found in maximum blood flow ($F(2, 36) = 0.356, p = 0.703, \eta^2 = 0.028$), mean blood flow ($F(2, 36) = 1.301, p = 0.285, \eta^2 = 0.028$), peak blood flow ($F(2, 36) = 0.107, p = 0.899, \eta^2 = 0.008$), or half-time to recovery ($F(2, 36) = 0.356, p = 0.703, \eta^2 = 0.019$).

Blood Lipids

Blood lipid responses are shown in Table 4. No significant differences were found between groups in TG levels ($F(1, 18) = 3.147, p = 0.093, \eta^2 = 0.149$) (Observed power = 0.390) (Figure 2). However, a significant time effect was found for TG ($F(2, 36) = 22.945, p < 0.001, \eta^2 = 0.560$). Compared to baseline levels, TG significantly increased by approximately 43% ($p < 0.001$) and 52% ($p < 0.001$) at two- and four-hours postprandial, respectively. There was no significant difference in TG levels between two- and four-hours postprandial ($p = 0.323$). Total cholesterol (Figure 3) was not significantly different between groups ($F(1, 18) = 1.089, p = 0.310, \eta^2 = 0.057$) (Observed power = 0.167) or across time ($F(2, 36) = 2.345, p = 0.138, \eta^2 = 0.115$).

A significant interaction effect was found between time and group ($F(2, 36) = 3.921, p = 0.049, \eta^2 = 0.179$) in LDL-C levels. Levels of LDL-C were found to be significantly different across time ($F(2, 36) = 10.684, p = 0.002, \eta^2 = 0.372$) (Figure 4). Baseline LDL-C was found to be significantly greater than both two- ($p = 0.002$) and four-hour postprandial ($p < 0.001$) while no significant difference was found between two- and four-hours postprandial ($p = 0.177$). Thus, separate post hoc analyses were conducted to determine where differences existed across time for each group. In the

trained group, postprandial LDL-C levels were found to be decreased compared to baseline by approximately 5.4% ($F(1, 9) = 3.14, p = 0.110$) and 6.0% ($F(1, 9) = 5.11, p = 0.050$) for two- and four-hours, respectively. Significant decreases of approximately 19.3% ($F(1, 9) = 10.47, p = 0.010$) and 10.9% ($F(1, 9) = 28.93, p < 0.001$) from baseline were found in the untrained group at two- and four-hours postprandial, respectively. Thus, the trained group exhibited a decrease in LDL-C that remained reduced across all time points. The untrained group had a larger decrease in LDL-C from baseline compared to the trained group at two-and four-hours. However, by four-hours LDL-C began to rise back towards baseline in the untrained group. No significant differences were found between groups at any time period ($F(1, 18) = 0.577, p = 0.457, \eta^2 = 0.031$) (Observed power = 0.111).

No significant differences in HDL-C were found between groups at any time point ($F(1, 18) = 0.235, p = 0.634, \eta^2 = 0.013$) (Observed power = 0.075) (Figure 5). However, a significant time difference was found in HDL-C levels across time ($F(2, 36) = 4.728, p = 0.015, \eta^2 = 0.208$). Post hoc comparisons indicated that the two-hour postprandial period was significantly reduced compared to baseline ($p = 0.024$) and four-hour postprandial ($p = 0.014$). No significant differences were determined between baseline and four-hours postprandial ($p = 0.572$).

Correlational Analyses

Correlations between FMD and blood lipids across all time points can be found in Table 5. No significant correlations were found between FMD and blood lipid levels at any time point. Correlation analyses were also conducted controlling for baseline lipids and FMD and no significant correlations were found.

Table 1. Mean characteristics of trained and untrained subjects.

	Trained (n=10)	Untrained (n=10)	p-value
Age (yrs)	20.8 ± 1.8	20.9 ± 2.2	0.914
Height (cm)	180.7 ± 7.5	181.6 ± 10.0	0.826
Weight (kg)	67.3 ± 7.3	77.5 ± 14.0	0.057
BMI (kg/m²) *	20.5 ± 1.1	23.4 ± 3.1	0.014
LBM (kg)	60.5 ± 5.0	63.9 ± 6.5	0.207
FM (kg) *	6.9 ± 3.3	13.7 ± 8.5	0.029
VO₂max (ml/kg/min) *	74.6 ± 5.2	47.3 ± 7.1	< 0.001

Values are mean ± SD. BMI, body mass index; LBM, lean body mass; FM, fat mass; VO_{2max}, maximal oxygen consumption. * p < 0.05, significant difference between group.

Table 2. Flow mediated dilation (% change)

Time Period	Trained	Untrained
Baseline	10.0 ± 2.0	8.8 ± 1.9
Two-hour	6.5 ± 2.6 *	5.6 ± 1.5 *
Four-hour	7.9 ± 3.2 *	5.6 ± 2.5 *

Values are mean ± SD. FMD = flow mediated dilation. * p < 0.001, significant difference compared to baseline value.

Table 3. Baseline femoral artery blood flow and blood flow response to 4-minute cuff occlusion

Trained:				
Time Period	Maximum BF (ml/sec)	Mean BF (ml/sec)	Peak BF (ml/sec)	½ time (sec)
Baseline	14.1 ± 10.0	8.2 ± 6.2	40.4 ± 6.0	15.7 ± 6.7
Two-hour	15.1 ± 10.7	8.7 ± 6.9	40.6 ± 5.4	16.6 ± 5.2
Four-hour	15.0 ± 8.4	8.7 ± 5.2	39.7 ± 6.1	16.2 ± 5.0
Untrained:				
Time Period	Maximum BF (ml/sec)	Mean BF (ml/sec)	Peak BF (ml/sec)	½ time (sec)
Baseline	10.2 ± 5.2	5.9 ± 3.7	36.7 ± 6.1	18.6 ± 9.4
Two-hour	11.4 ± 5.6	6.5 ± 3.6	36.6 ± 9.0	18.6 ± 9.0
Four-hour	11.5 ± 4.3	6.7 ± 2.9	34.9 ± 7.9	20.0 ± 9.6

Values are mean ± SD. BF = blood flow, ½ time = half-time to recovery.

Table 4. Blood Lipid Responses

Trained:				
Time Period	TG (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Baseline	66.6 ± 18.0	139.7 ± 29.4	77.0 ± 21.1	49.4 ± 11.7
Two-hour	95.5 ± 28.3*	140.4 ± 23.3	72.8 ± 21.6	48.6 ± 10.6 ^α
Four-hour	102.3 ± 38.0*	142.8 ± 22.9	72.4 ± 22.8 [#]	49.9 ± 10.9 [†]
Untrained:				
Time Period	TG (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Baseline	75.2 ± 32.3	154.0 ± 28.9	90.5 ± 25.9	48.4 ± 7.5
Two-hour	134.6 ± 57.6*	145.9 ± 20.9	73.0 ± 18.3 ^{\$}	46.0 ± 8.9 ^α
Four-hour	144.4 ± 66.3*	156.8 ± 32.9	80.6 ± 23.8 [*]	47.3 ± 8.3 [†]

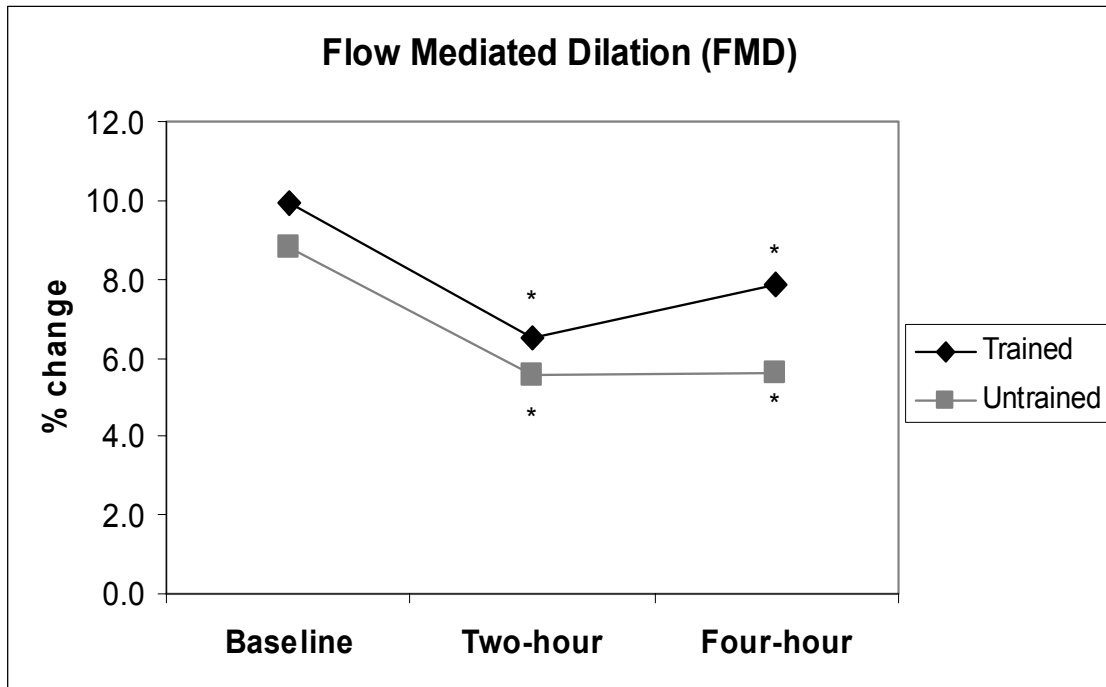
TG = triglycerides, TC = total cholesterol, LDL-C = low density lipoprotein-cholesterol, HDL-C = high density lipoprotein-cholesterol. * p < 0.001, significantly different compared to baseline. # p = 0.05, significantly different compared to baseline. \$ p = 0.010, significantly different compared to baseline. ^α p = 0.014, significantly different compared to baseline. [†] p = 0.024, significantly different compared to two-hours.

Table 5. Pearson product moment correlational analyses for comparative time points (r) (2-tailed)

	Baseline FMD	Two-hour FMD	Four-hour FMD
Triglycerides			
r	-0.422	-0.038	-0.210
Sig.	0.064	0.874	0.374
Total Cholesterol			
r	0.145	0.178	0.137
Sig.	0.542	0.453	0.565
LDL-C			
r	0.088	0.153	0.103
Sig.	0.714	0.520	0.666
HDL-C			
r	0.385	0.128	0.410
Sig.	0.094	0.592	0.072

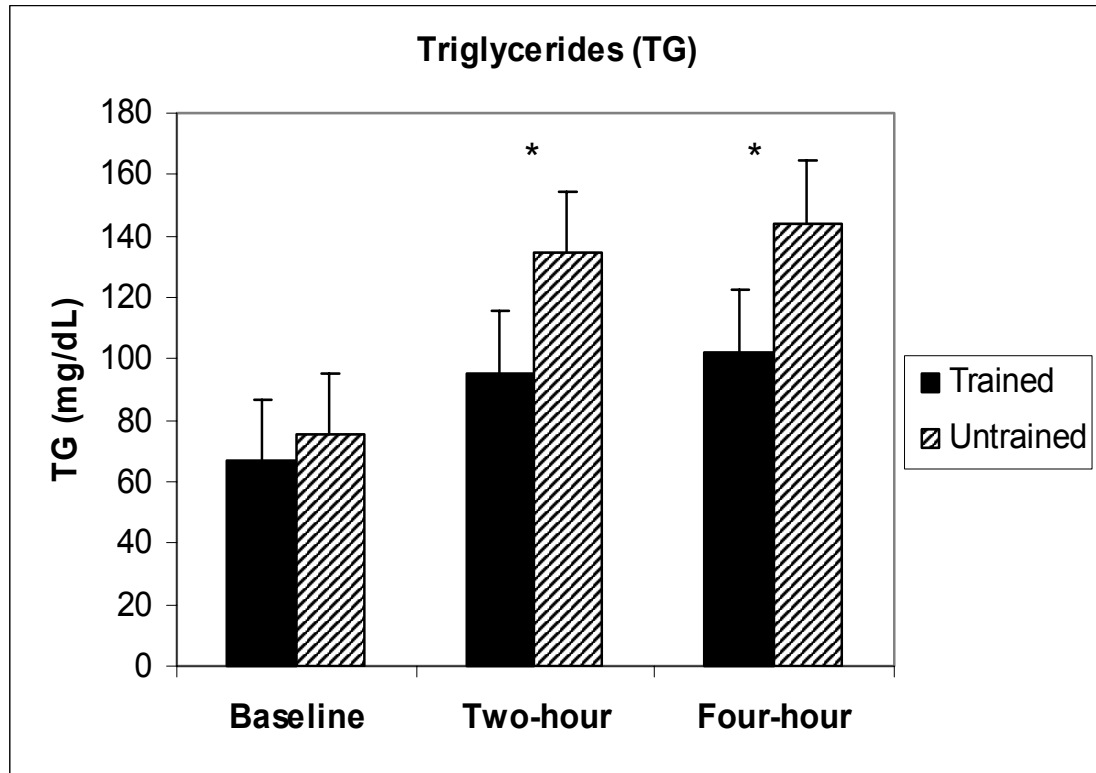
FMD = flow mediated dilation, LDL-C = low density lipoprotein-cholesterol, HDL-C = high density lipoprotein-cholesterol.

Figure 1



Flow mediated dilation (FMD) of the brachial artery in trained and untrained individuals as determined by Doppler ultrasound (means \pm SD). * Significantly different from baseline for both groups at $p < 0.001$.

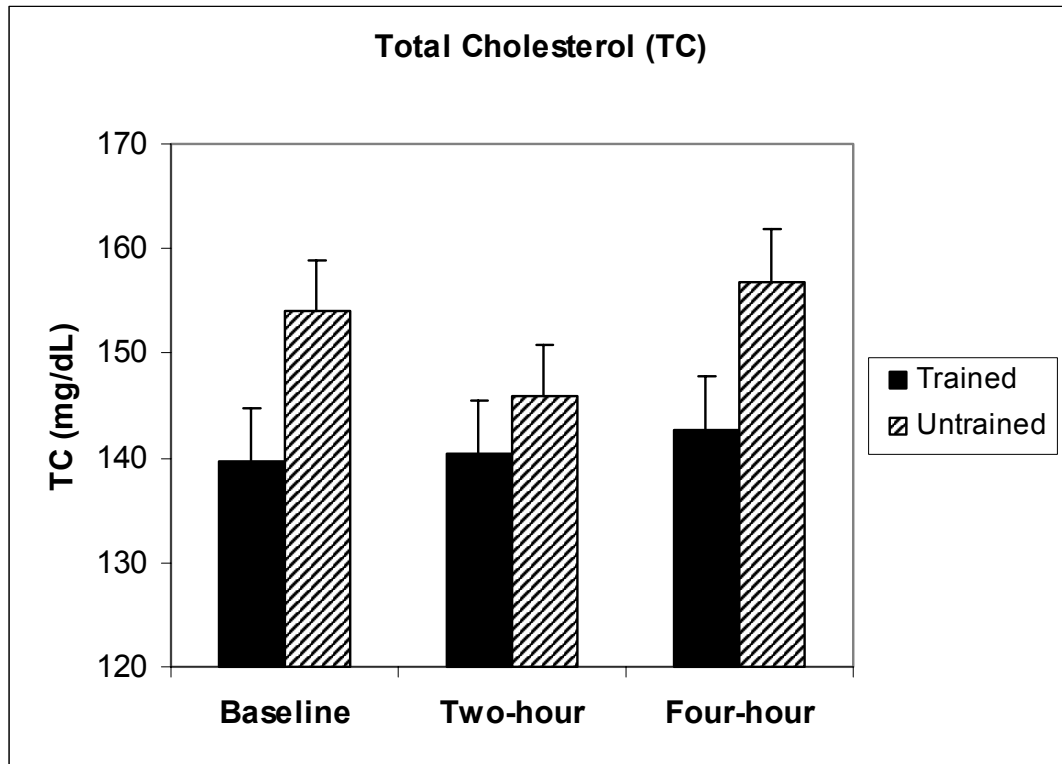
Figure 2



Serum triglyceride levels in trained and untrained individuals (means \pm SD).

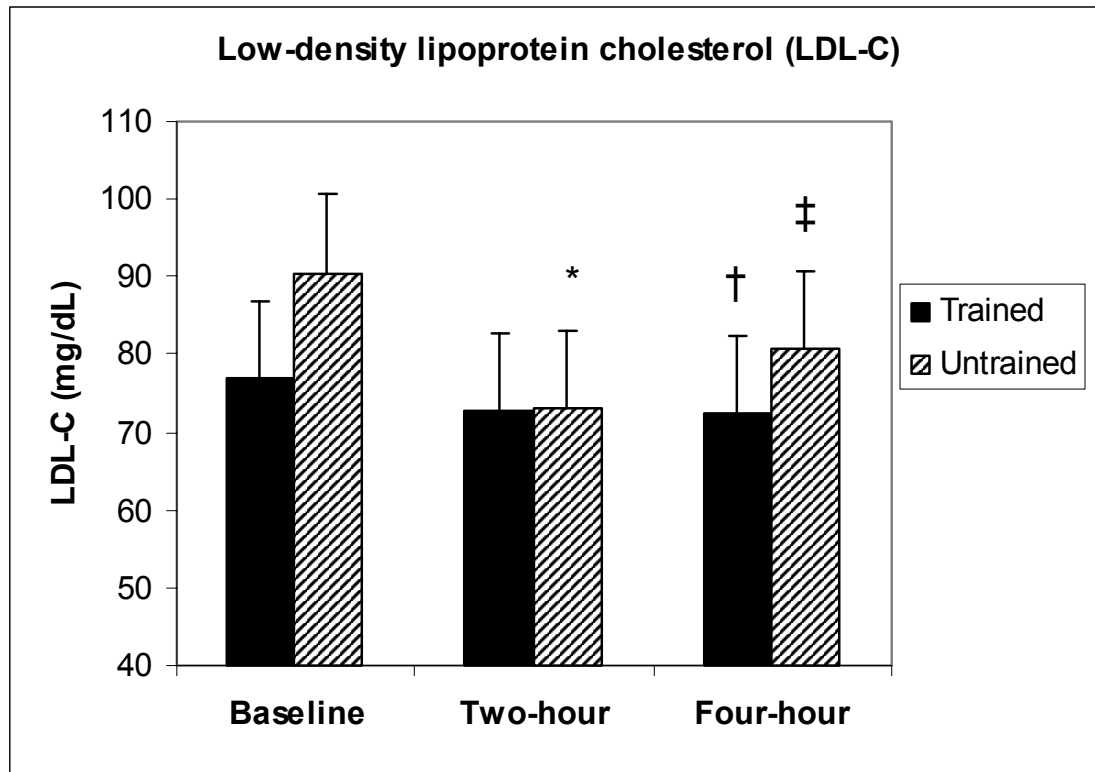
* Significantly different from baseline for both groups at $p < 0.001$.

Figure 3



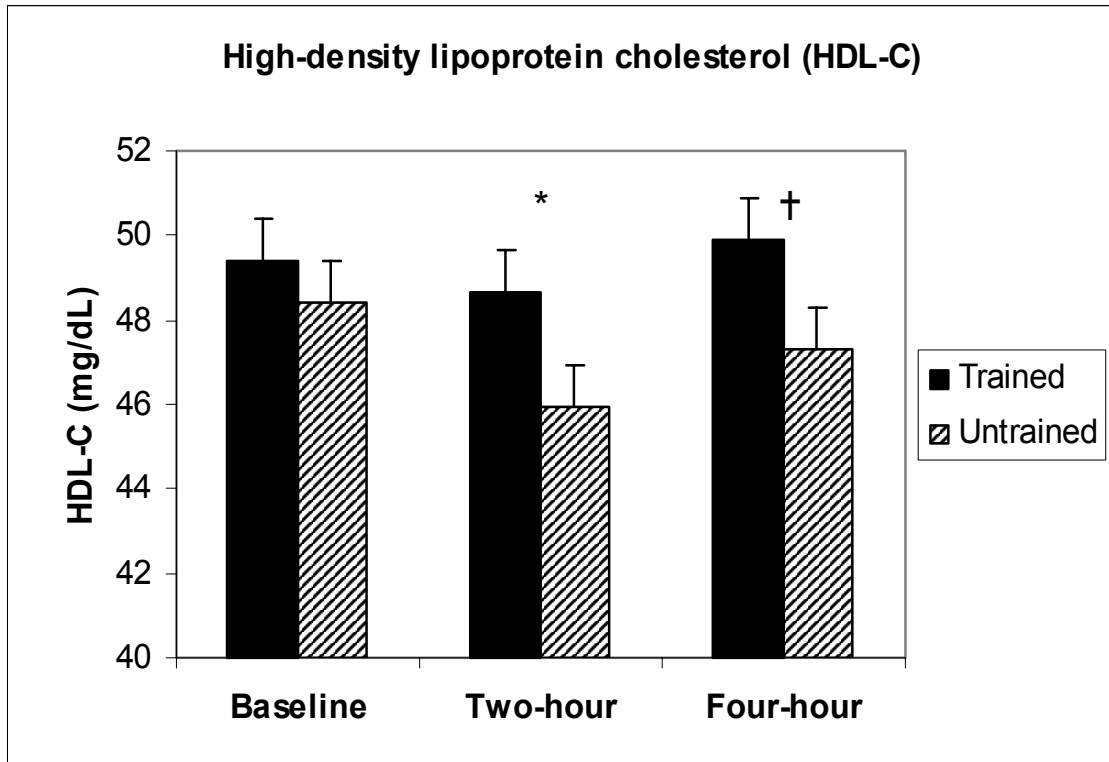
Serum total cholesterol levels in trained and untrained individuals (means \pm SD).

Figure 4



Serum low-density lipoprotein cholesterol levels in trained and untrained individuals (means \pm SD). * Significantly different from baseline in untrained group at $p = 0.05$. † Significantly different from baseline in trained group at $p = 0.010$. ‡ Significantly different from baseline in untrained group at $p < 0.001$.

Figure 5



Serum high-density lipoprotein cholesterol levels in trained and untrained individuals (means \pm SD). * Significantly different from baseline for both groups at $p = 0.024$. † Significantly different from two-hours postprandial for both groups at $p = 0.014$.

CHAPTER V

DISCUSSION

The primary finding of this study was that FMD of the brachial artery was significantly impaired following the consumption of a single HFM, independent of training status. To our knowledge, this is the first study to investigate the vascular changes that occur with a single HFM in highly-trained endurance athletes compared to untrained individuals. No differences in FMD were detected between groups after a HFM. However, these findings are not conclusive due to low power associated with the between groups comparisons. Our secondary aims were to determine the blood lipid responses to a single HFM and to determine if these responses were correlated to FMD. No significant correlations were found between FMD and any blood lipids across any time point.

Brachial

Flow mediated dilation was significantly reduced by approximately 35% at two-hours postprandial in both trained and untrained subjects. Furthermore, at four-hours postprandial FMD was reduced by 21% and 35% in the trained and untrained subjects, respectively. Several studies have found FMD to be significantly reduced after the consumption of a single HFM in healthy, young men^{65, 69, 100}. A recent study by Tsai et al. support our findings as they found that FMD was reduced by approximately 50% and 40% at two- and four-hours postprandially, respectively in healthy, young men (30 ± 5

years)¹⁰⁰. These reductions demonstrated by this group are similar to the reductions found in the untrained group. Another study by Marchesi et al. tested the acute effects of a HFM on FMD after the subjects were instructed to consume a typical Mediterranean diet for four-weeks (< 10% fat)⁶⁵. This study found that a single meal consisting of 65 g of fat significantly reduced FMD in healthy, physically active young men (23 ± 2 years) by 76% and 72% at two- and four-hours postprandial, respectively⁶⁵. The reductions in FMD were larger than in our study and suggest that chronic dietary habits may also influence endothelial function, as there is evidence that a Mediterranean diet may have a positive effect on endothelial function¹⁵.

On the other hand, several studies have found that FMD was not decreased following the consumption of a HFM in healthy, young (≈ 33 years old) men and women^{19, 84}. The discrepancies between the results of these studies and our study may be explained by age, differences in the type of meal consumed, the dietary lipid composition of the meals¹⁶, the timing of the vascular measurements, and/or the gender of the subjects studied⁶⁶.

Previous literature has shown that peak impairment in FMD after a HFM occurs at two-hour postprandial^{65, 100, 102} but may remain impaired for up to six-hours¹⁰⁰. Our study findings are in agreement with the literature in that we found significant peak impairment in FMD at two-hours postprandial with the impairment being maintained in the untrained group at four-hours postprandial. Our results for the untrained subjects are supported by Vogel et al. who compared the effects of a high and low fat meal on FMD each hour in healthy, physically active men and women (39 ± 10 years) for six-hours¹⁰². This study found that FMD decreased maximally ($\approx 50\%$) by two-hours and remained

reduced from two- to four-hours postprandial¹⁰². These findings are consistent with our untrained subjects. Flow mediated dilation measured at five- and six-hours postprandial was not significantly reduced compared to baseline in contrast to the study by Tsai et al.^{100, 102}. Flow mediated dilation was also measured after an isocaloric low-fat meal and no changes were measured across the six-hour time period¹⁰².

A lower baseline FMD ($10.0 \pm 2.0\%$ and $8.8 \pm 1.9\%$, trained and untrained, respectively) was found in this study compared to previous literature ($14.5 \pm 6.6\%$)⁶⁵. This finding can be explained by the greater resting diameters found in this study (0.48 ± 0.04 cm and 0.46 ± 0.05 cm, trained and untrained, respectively) compared to Marchesi (0.39 ± 0.06 cm). A reduced FMD has been found to be significantly related to larger vessel size¹⁰.

Despite a significant difference in VO_2max between our groups, baseline FMD was not significantly different between trained and untrained subjects ($10.0 \pm 2.0\%$ vs. $8.8 \pm 1.9\%$, trained vs. untrained, respectively, $p = 0.119$). Our findings are supported by research that has shown that endothelium-dependent vasodilation was not different regardless of training status in healthy, young men¹⁸. However, the data on the influence of training on FMD is mixed as another cross sectional study has found that young male runners demonstrated significantly greater FMD ($\approx 35\%$) compared to young, sedentary males ($17.1 \pm 2.3\%$ vs. $11.2 \pm 1.7\%$, runners vs. sedentary, respectively, $p = 0.002$)⁴⁷. Furthermore, a longitudinal study by Clarkson et al. found that 10-weeks of aerobic and anaerobic exercise training significantly improved FMD by approximately 44% in healthy male military recruits¹¹. A possible explanation for the lack of difference in FMD between our groups may be that exercise training may have little or no impact on

endothelial function if the endothelium is fully functional at baseline as could be possible in young, healthy men^{68, 85}. Endurance exercise has been shown to improve endothelial function when it is reduced by age¹⁸, chronic heart failure³⁹, coronary artery disease⁵⁵, hypertension³⁸ or type 2 diabetes⁶². These findings suggest that improvements in endothelial function with endurance training may occur only when impairments are demonstrated at baseline due to aging or disease⁶⁶. In our study we found that we had inadequate power to determine differences between groups on FMD (alpha = 0.341), thus, it is not possible at this time to determine if training influences FMD after a HFM. To determine if training impacted FMD in our population we would need to increase our sample size or use a longitudinal design instead of a cross sectional study to increase our power.

Resting baseline diameter of the brachial artery did not significantly change following the HFM and was not different between groups. These findings are supported by several studies that found that baseline brachial artery diameter was not altered after the consumption of a single HFM^{29, 65, 81, 102} or after exercise training⁴⁷.

Femoral

No significant differences in blood flow measures of the femoral artery were observed between groups at baseline or at any time period following the HFM meal. To our knowledge, no other studies have examined femoral artery responses following a single HFM. The lack of difference in femoral artery blood flow between our groups is most likely explained by the fact that FFM was similar between our groups. Peripheral blood flow is correlated to muscle mass⁷¹. Furthermore, cardiac output has been shown to be closely related to body mass, mainly due to the metabolic requirements of FFM¹⁷.

Several studies have found no significant change in baseline and post-hyperemic brachial artery blood flow following a single HFM^{65,81,102}. These results suggest that the consumption of a HFM does not alter peripheral blood flow in healthy individuals despite a reduction in FMD of the brachial artery. Thus, it appears that a HFM does not negatively impact blood flow or nutrient delivery to the peripheral tissues. Based on these results it would seem reasonable that blood flow could be maintained regardless of diet during times of increased need (i.e. exercise, response to cuff ischemia, stress, etc.). More interestingly, these results may provide insight into the correlation of FMD to disease risk and mortality after a HFM⁴³. Blood flow is calculated as the product of velocity and cross sectional area (CSA). If total blood flow is maintained despite a decrease in vessel CSA then velocity must increase. The increase in velocity will result in increased shear stress on the vessel walls. This increase in velocity and hence shear stress results in damage to the vessel wall which will further promote the atherosclerotic process, especially at a time when there are high levels of lipids present.

Half-time to recovery was found not to be significantly different between groups or over time. This measure has been used as an index of vascular reactivity as it indicates the ability of the vessel to return blood flow to resting values^{70,77}. Reduced vascular reactivity has also been associated with atherosclerosis⁹, type 2 diabetes¹⁰⁵, obesity⁹⁷, and in individuals who have suffered spinal cord injuries^{72,73}. These results suggest that this measure of vascular reactivity may be more sensitive to disease or age status than to dietary influences. Compared to young subjects, older individuals have been shown to have a prolonged half-time to recovery, indicating reduced vascular reactivity associated with aging that was independent of training status⁷⁰. These results would suggest that

age is more important than training status on half time to recovery and may explain why no between group differences were found in our study. Another possible explanation for why we may not have seen between group differences could be due to the cuff duration that was chosen for this study. Reactive hyperemia induced by a four-minute cuff occlusion results in subsequent shear stress on the vessel wall and the release of NO from the endothelium but does not result in significant metabolic alterations. However, a cuff duration of 10-minutes or longer has been associated with muscle metabolic alterations. Thus, had the cuff duration been longer we might have been more likely to detect between group differences in half-time to recovery due to the fact that the trained individuals would have had greater metabolic capacity⁴⁸.

Femoral artery size was not significantly different between the trained and untrained group across all time points. These results are in contrast to several other studies that have shown that elite road cyclists demonstrated a larger femoral artery diameter and a greater stroke flow compared to untrained, able-bodied individuals^{40, 51}. We did not find significant differences in femoral artery diameter size which is most likely due to the fact that we did not find significant differences in FFM as femoral artery size has been shown to be dependent on muscle mass⁷¹. However, it should be noted that we did not measure lower extremity muscle mass but rather whole body LBM. Other reasons to explain why our findings may be different from previous work may be differences in training mode or due to low statistical power ($\alpha = 0.341$).

Blood Lipids

Serum TG levels significantly increased at both two- and four-hours postprandially compared to baseline levels. This postprandial increase has been observed

in several other studies^{5, 19, 65, 81, 102}. Vogel et al. found that serum TG levels peaked at four-hours postprandial and that the mean change in postprandial FMD at two-, three-, and four-hours was significantly correlated with the change in two-hour serum TG levels¹⁰². These findings suggest that postprandial TG-rich lipoproteins are a cause of the impairment in FMD following a single HFM. Postprandial hypertriglyceridemia has been shown to cause endothelial dysfunction in healthy subjects^{5, 81}. Bae et al. demonstrated that endothelial dysfunction in healthy subjects induced by postprandial hypertriglyceridemia, was significantly correlated with leukocyte superoxide anion production, a measure of oxidative stress⁵. Another study found a transient decrease in endogenous glutathione peroxidase, an important antioxidant in the protection against oxidative stress in the atherogenic process, following the consumption of a HFM¹⁰⁰. Flow mediated dilation was also found to be significantly impaired for up to six-hours following the HFM. These findings suggest that postprandial hypertriglyceridemia induced by a HFM increases oxidative stress or causes direct injury to the vascular wall by TG-rich lipoproteins⁸¹, thereby inactivating NO and impairing FMD. Young male endurance athletes have been shown to have a 92% higher clearance rate of plasma TG compared with sedentary males⁸⁹ likely mediated by an upregulation of LPL activity³². Even though group differences were not detected in TG levels, a higher TG clearance rate could possibly be detected with a larger sample size.

The results of our study are in agreement with those of Vogel et al. in that serum TG levels peaked at four-hours postprandial. However, no significant correlations were found between postprandial FMD and postprandial serum TG, or any other blood lipid level. The lack of correlation between TG and FMD may possibly be attributed to low

power in the study. Another possible explanation might be due to the fact that our subjects were younger than the Vogel study (21 ± 2 years vs. 39 ± 10 years, respectively)¹⁰². Aging is associated with progressive endothelial dysfunction in healthy humans¹⁰. Thus, it is possible that in individuals who are older, dietary factors become more important at influencing FMD than in a younger population. Baseline values of TC and TG levels were higher in the subjects reported by Vogel compared to these same values in the present study (164 ± 21 mg/dL and 94 ± 55 mg/dL, TC and TG, respectively)¹⁰². Two- and four-hour TG values were also higher in the Vogel subjects compared to our subjects (147 ± 80 mg/dL and 158 ± 97 mg/dL, two- and four-hours, respectively)¹⁰². These higher values may suggest that the subjects used in the Vogel study had a chronically higher lipid level which has been shown to negatively influence FMD⁵².

No significant changes in TC were demonstrated by either group after a HFM. This finding is in agreement with others who found no significant change in TC levels during the postprandial phase^{19,65,81,102}. Dietary lipids that are ingested during a meal are absorbed from the small intestine and are transported to the liver for the production of cholesterol lipoproteins²³. The production of cholesterol and lipoproteins takes approximately eight hours to complete following a meal, and may explain why we did not see any changes within four hours in TC after our meal²⁵. Furthermore, cholesterol absorption at the intestines has been shown to be significantly reduced (15.6%) with a modest increase in cholesterol dose (412 mg)⁷⁴. Thus, it is possible that the amount of cholesterol used in the present study (330 mg) could have blunted cholesterol absorption into the bloodstream and may explain why TC levels did not change following the HFM.

Low density lipoprotein-cholesterol was found to be significantly reduced by approximately 20 and 10% at two- and four-hours postprandial, respectively, compared to baseline in the untrained group. The trained group demonstrated significantly lower (5%) LDL-C values at four-hours postprandial compared to baseline with no change at two-hours postprandial. Our results are supported by those of Tsai et al. who demonstrated that LDL-C was significantly reduced in healthy, young men by approximately 9% at four-hours after a HFM¹⁰⁰. It has been suggested that oxidized LDL-C may decrease the bioavailability of NO and consequently impair endothelial function⁹⁵. The greater reduction in LDL-C levels in the untrained group compared to the trained group may explain why FMD remained reduced in the untrained group at four-hour post while the trained group only demonstrated an impairment of 21% in FMD at this same time period. However, due to lack of correlation between LDL and FMD it is recognized that other factors such as insulin, free radicals, or endothelin may be responsible for the decreases in FMD. Furthermore, it should be noted that recent studies have demonstrated that the level of apolipoprotein B (apoB), a measure of the number of LDL particles, was inversely related to attenuated endothelium-dependent vasodilation⁹⁶, and that apoB may be a more direct measure of LDL-C levels than the Friedewald formula⁵⁸. This suggests had we measured apoB to determine LDL-C, we may have determined different levels of LDL-C that might be related to changes in FMD.

High density lipoprotein-cholesterol levels were also found to be significantly decreased by approximately 2 and 5% at two-hours postprandial in trained and untrained subjects, respectively. Interestingly, HDL-C levels significantly improved in both trained and untrained ($\approx 3\%$) groups from two- to four-hours postprandial such that the four-hour

postprandial HDL-C level was similar to the baseline level. HDL-C levels have been shown to be directly and independently related to peripheral endothelial function in older men and women with HDL-C levels ≥ 40 mg/dL ⁵⁴. In the present study, no correlation was found between FMD and HDL-C at any time point, possibly due to the age difference between the two studies. Another potential explanation for the lack of difference in FMD between our groups may be explained by the fact that both groups had HDL-C values above 40 mg/dL at all time points.

It has been shown previously that a single HFM lowers LDL-C and HDL-C levels ⁹⁰. The mechanism behind this reduction has been suggested to be due to the transfer of cholesteryl ester from LDL-C and HDL-C to triglyceride-rich particles through the action of cholesteryl ester transfer protein. This transfer is associated with the influx and lipolysis of triglyceride-rich lipoproteins following a HFM and results in a modest decrease in LDL-C and HDL-C ¹³. Levels of HDL-C have been found to be related to the clearance rate of plasma triglycerides in young men ⁸⁹. Reductions in HDL-C levels seen in this study might suggest impaired TG clearance, thereby causing impaired FMD through enhanced oxidation of TG-rich lipoproteins.

High density lipoprotein-cholesterol levels have been found to increase by 14% in young men following a six-month endurance training program ⁹². Berg et al. found that endurance-trained young men had lower LDL-C concentration compared to untrained subjects ⁷. We found no significant differences between our groups on LDL or HDL. The lack of difference may be attributed to age, training status, or low power.

Limitations

One limitation of this study was a small sample size to detect between group differences. Flow mediated dilation ($p = 0.119$) and serum TG levels ($p = 0.093$) approached statistical significance but did not have adequate power to detect a difference between groups. It can only be postulated that significant differences might have been found if the sample size was increased. The cross sectional study design is another potential limitation, especially in light of the low power on several of our measures. A longitudinal design would have increased the power of this study as it would have removed the between subjects variability. Another limitation of this study was that all subjects were healthy, young males thus the findings from this study cannot be generalized to other populations. No control meal was used in this study and thus it is not possible to exclude the possibility that the calories from the meal could not have been possible for some of our results rather than the fat intake. However, this is not likely as previous literature has found that an isocaloric low-fat meal has no effect on FMD when compared to a HFM¹⁰². Lastly, the lack of diet logs to report daily food intake by our subjects could be another possible limitation of this study, as chronic high-fat intake has also been shown to affect endothelial function⁵⁷.

Future Research

The findings from our study suggest that other research could be conducted to better understand how diet affects endothelial function. To better understand whether training influences endothelial function and TG levels a larger sample needs to be studied to increase the power of this study. Our results on TG and FMD were interesting compared to the results obtained from Vogel¹⁰² as they suggested that age may be a

factor to consider when investigating the effects of a HFM on FMD. Thus, future research may want to examine various age groups to determine if the FMD response to a HFM is the same across different age groups. We found that FMD did not recover within a four-hour postprandial period and thus data could be collected over a longer period of time to document the time course at which endothelial function recovers after a HFM. Markers of oxidative stress have been found to be influenced by the consumption of a HFM^{5,100} and future examination of these markers may help to better explain the mechanism responsible for the impairment of FMD following a HFM. Future research should also examine different diets and antioxidants to determine how they might affect endothelial function in varied populations. Lastly, it may also be important to consider the effects of chronic high fat diets instead of an acute HFM on FMD. The examination of long-term diets may provide insight into the progression of endothelial dysfunction in at-risk populations and may help to decrease the occurrence of adverse events associated with endothelial dysfunction.

Summary

In summary, this study demonstrated that FMD was significantly reduced in both endurance-trained and untrained young men for up to four-hours after consuming a commercially-available HFM. Differences in FMD were not detected based on training status, most likely due to low power. Thus, the primary hypothesis that training could provide a protective effect on the endothelium after a HFM must be rejected. However, the research warrants future studies with an increase in sample size to investigate this hypothesis. A HFM was found to significantly increase TG and reduce LDL-C and HDL-C in both groups for four hours postprandial. No correlations were found between

any blood lipid measures and FMD. Thus, these results suggest that a meal high in dietary fat has an acute effect of up to four-hours at reducing endothelial function, independent of training status and blood lipids measured. Reduced endothelial function places individuals at increased risk for disease and adverse events. These findings are significant as many Americans regularly consume high-fat meals that may place them at increased risk for adverse events or disease due to reductions in endothelial function.

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APPENDIX A

Phone Screening for HFM Study

Subject's Name _____ Contact # _____
Screener's Name _____ Date and Time Contacted _____

Sex _____ (must be MALE) Age _____ (must be between 20-29 yrs.)

Height _____ **BMI = (lbs/inches²) * 703**
Weight _____ Calculated BMI =
(BMI needs to be ≤ 30)

Do you smoke? YES NO

Do you train aerobically (circle one)? YES NO

How long? _____ (must be > 1 year for trained)

Duration and frequency? _____

(Trained = 4-8 hrs/wk, > 1 year of aerobic training)

(Untrained = < 20 min/day, 3 days/wk)

Are you taking any medications including vitamins or supplements? YES NO

Does subject have: (If YES to ANY of these they are NOT eligible)

- | | | |
|-----------------|-----|----|
| 1. Diabetes | YES | NO |
| 2. Hypertension | YES | NO |
| 3. High lipids | YES | NO |
| 4. Asthma | YES | NO |

For study personnel

Does the subject qualify on age? YES NO

Does the subject qualify on BMI? YES NO

Does the subject qualify for one of two groups
based on exercise levels? YES NO

** If subject qualifies for study, notify them that they do and describe the requirements of the study to them in detail. If they want to participate assign them to a group and schedule a time for testing. Remind them to bring a pair of shorts, a short sleeve shirt, and reading material.

Group Assignment _____

Scheduled appointment date and time _____

Study personnel to be present _____ and _____

APPENDIX B

Medical History Form

Participant Name _____ Date _____

1. Do you have or have you ever had any of the following medical conditions?

- | | | |
|------------------------------------|-----|----|
| a. Diabetes | yes | no |
| b. Asthma | yes | no |
| c. Angina (chest pain on exertion) | yes | no |
| d. Any Heart Problems | yes | no |
| e. Stroke | yes | no |
| f. Fainting Spells | yes | no |
| g. High Blood Pressure | yes | no |
| h. High Cholesterol | yes | no |
| i. Thyroid Problems | yes | no |
| j. Cancer | yes | no |
| k. Kidney Problems | yes | no |
| l. Liver Problems | yes | no |
| m. Gout | yes | no |
| n. Heart Attack | yes | no |

2. Do you smoke? yes no

3. Have you participated in a regular exercise program over the past six months which consisted of at least 20minutes of activity, 3 days per week?

Yes No

If yes please describe type of exercise, duration of activity, and how long you have been participating in the exercise.

4. Please list all medications that you are currently taking on a regular basis:

MEDICATION

REASON FOR TAKING

Verification of information

I hereby declare the information provided by me in this medical history form is true, correct and complete to the best of my knowledge

Participant Signature _____

APPENDIX C

Exercise Physiology Laboratory

Crawford Gymnasium Room 002B
University of Louisville
Louisville, KY 40292
502-852-5000

INFORMED CONSENT

EFFECTS OF TRAINING ON POSTPRANDIAL FLOW MEDIATED DILATION

Introduction and Background Information

You are invited to participate in a research study. The study is being conducted by Jennifer Olive, Ph.D. and Kevin Ballard. The study is sponsored by the University of Louisville, Department of Health and Sport Sciences in Crawford Gym room 118 in the Vascular and Metabolic laboratory. Approximately 20 subjects will be invited to participate. Your participation in this study will last for approximately 5 hours.

Purpose

The purpose of this research study is to determine how training affects your vascular function following the consumption of a high fat meal.

Procedures

If you agree to participate in the study you will be asked to provide a medical history about yourself. If there is a question on the medical history form that you are uncomfortable with you may decline to answer the question. You will be asked to come to the laboratory for two sessions. The first will last 30 minutes and we will measure your overall fitness by walking/running on a treadmill. The second session will last approximately 5 hours starting at 8 AM after a 12-hour fast. During this time you will have the following items measured: your blood vessel health and your body composition. We will also remove from your vein a small sample of blood for the experimental purpose of determining your triglycerides and cholesterol. A tourniquet will be placed on your arm, a needle placed in your vein, and 10 mL of blood (approximately 2 teaspoons) will be removed. Once you have undergone the baseline testing you will be given a breakfast meal from a local fast food restaurant to eat. You will then have your vascular function and your blood drawn twice more at 2- and 4-hours after your meal. Each blood draw will remove approximately 2 teaspoons of blood for a total of approximately 6 teaspoons over the entire day.

Determination of your body composition will occur by using a machine that will pass a very small and safe amount of alternating current through your body. This procedure allows for the determination of the amount of fat and muscle within your body. Your blood vessel health will be determined by using Doppler ultrasound to look at your blood vessels in your arm and leg. In order to determine the health of your blood vessels we

will need to apply a blood pressure cuff to your arm (5 minutes) and leg (4 minutes) to occlude blood flow. We will then release the cuff and measure your blood flow and the size of your vessel.

Potential Risks

There are no known risks associated with any of the devices that we are using. However, there may be unforeseeable risks associated with this study.

- You should understand that there are some risks associated with determining your overall fitness. These risks are minimal but may include muscle discomfort, light headedness, nausea, fatigue, possible arrhythmias, and in an extreme case death (less than 1/10,000).
- You should understand that there might be some pain or discomfort during the blood draw, but that the risks are minimal. It is possible that there might be some bleeding from or bruising around the puncture site and there is a possibility of infection of the puncture site.
- You may experience some slight discomfort in your arm or leg during the vascular portion of the testing. The cuff occlusion is similar to having your blood pressure taken. The discomfort associated with the cuff occlusion is similar to have your arm or leg fall asleep. These feelings will disappear as soon as the cuff is released. At any point if the pain is unbearable you may ask to have the cuff released immediately.

Benefits

The possible benefits of this study include aiding in the understanding of the effects of training and high fat meals on vascular function. The information collected may not benefit you directly. The information learned in this study may be helpful to others.

Alternatives

You may choose not to participate in the research study if you are uncomfortable with any of the procedures.

Research Related Injury

If you are injured as a result of your participation in this study, treatment for the injury will be provided at University Hospital. You or your insurance company will be billed for the costs of such medical treatment. It is your responsibility to find out what costs your insurance will cover. Additional compensation, for things such as lost wages, inconvenience or discomfort, will not be provided. If you feel you have suffered a research related injury, please contact Jennifer Olive, Ph.D. at 502-852-5000.

Costs/Compensation

You will be reimbursed \$25 for completion of all of the testing procedures. There will be no additional costs to you for participating.

Confidentiality

Although absolute confidentiality cannot be guaranteed, confidentiality will be protected to the extent permitted by law. Should the data collected from this study be published, your identity will not be revealed. The sponsor, the Institutional Review Board (IRB), the Human Subjects Protection Program Office (HSPPPO) and other appropriate regulatory government agencies may inspect your research/medical records. The Investigator will supply your information to those responsible for regulatory and financial oversight of research subjects. Those responsible for financial oversight of research participants at this institution may review your research records. This is necessary so that any claim(s) for benefits arising from services rendered to you either as an inpatient or outpatient can be completed and submitted appropriately.

Voluntary Participation

Your participation in this research study is voluntary. You are free to refuse consent or withdraw your consent at any time without penalty or losing benefit to which you are otherwise entitled.

Participation in Other Research Studies

You may participate in this study if you are currently in another research study. You will let the researchers know if you are in another research study.

Termination

The investigator reserves the right to terminate or end this study at any point or withdraw you from this study with or without your consent if he/she believes it is in your best interest.

Research Subject's Rights and Contact Persons

You acknowledge that all your present questions have been answered in language you can understand and all future questions will be treated in the same manner. If you have any questions about the study, please contact Jennifer Olive, Ph.D. at (502) 852-5000.

If you have any questions about your rights as a research subject, concerns or complaints about the research or research staff, you may call the HSPPPO (502) 852-5188. You will be given the opportunity to discuss any questions about your rights as a research subject, in confidence, with a member of the IRB. The IRB is an independent committee composed of members of the University community, staff of the institutions, as well as lay members of the community not connected with these institutions. The IRB has reviewed this study.

You will be given a signed copy of this consent form to keep for your records.

Signature of Subject/Legal Representative

Date Signed

Signature of Person Explaining the Consent Form
(if other than the Investigator)

Date Signed

Signature of Investigator

Date Signed

LIST OF INVESTIGATORS	PHONE NUMBERS
Jennifer Olive, Ph.D.	852-5000
Kevin Ballard	852-3138

Consent version date 7/28/05

APPENDIX D

Ramp Protocol

TIME (minutes)	SPEED (mph)	GRADE (%)
2	6.0	0.0
4	7.0	0.0
6	8.0	0.0
8	8.0	5.0
10	8.0	7.5
12	8.0	10.0
14	8.0	12.5

Representative example of a ramp protocol for individual who reports running 8 mile/hour or 7.5 minute per mile pace.

CURRICULUM VITAE

Kevin David Ballard

1230 Weller Avenue
Louisville, KY 40208

Phone: (502) 681-7779
E-mail: kdball01@gwise.louisville.edu

EDUCATION

University of Louisville, Louisville, KY, August 2004-present
Degree: Currently pursuing M.S. in Exercise Physiology, GPA 3.93
Anticipated graduation date May 2006

University of Louisville, Louisville, KY, May 2004
Major: Health and Human Performance
Concentration: Exercise Science
Degree: B.S., Cumulative GPA 3.48

RESEARCH EXPERIENCE

Graduate Research Assistant

Department of Health and Sport Sciences, University of Louisville, 2004-present
Mentor: Jennifer Olive, PhD

Investigate metabolic rate and vascular function in individuals with a family history of type 2 diabetes
Perform venipunctures to measure blood markers
Assist and instruct undergraduate and graduate students in testing procedures
Manage subject database
Recruit subjects for research studies
Analyze collected data

Master's Thesis, University of Louisville, 2005-2006
Effects of Training on Postprandial Flow Mediated Dilation

WORK EXPERIENCE

Academic Tutor, Olga S. Peers Academic Center for Student-Athletes, Louisville, KY, 2005-present
Provide academic support to student-athletes participating in the University of Louisville's intercollegiate athletic programs by promoting self-responsibility, personal growth and academic development.

Certified Personal Trainer, Premier Health and Fitness Clubs, Louisville, KY, 2001-2004
Designed and conducted individualized exercise programs for clients
Conducted health assessments for clients
Instructed clients and members in aerobic and resistance training
Remained educated with current fitness research

Internship, Heuser Clinic (formerly Louisville Youth Training Center), Louisville, KY, 2003-2004
Designed and conducted individualized exercise programs for overweight children
Assisted in implementing group exercise programs for high school athletes
Performed health assessments for new members

ACADEMIC HONORS AND AWARDS

National Who's Who Among American College Students (2004)
UofL School of Education Dean's List (2001-2004)
UofL Dean's Scholar (2003)
Academic Commonwealth Scholarship (2003-2004)
National Dean's List (2003-2004)
Kentucky Educational Excellence Scholarship (2002-2004)

ABSTRACT PUBLICATIONS

Ballard, K.D., Olive, J.L. *Metabolic Rate in Individuals with a Predisposition for Type 2 Diabetes*. American College of Sports Medicine 53rd Annual Meeting, May 2006, Denver, CO.

Olive, J.L., **Ballard, K.D.**, Robinson, J. *Vascular Function in Individuals with a Predisposition for Type 2 Diabetes*. American College of Sports Medicine 53rd Annual Meeting, May 2006, Denver, CO.

Moreillon, J., Burgard, M.A., **Ballard, K.D.**, Mercer, S.L., Felker, J.M., Hare, J.L., Adams, K.J., FACSM (2005). Metabolic Costs of Two Types of Barbell Lunges. *Medicine and Science in Sports and Exercise* 37(5): S191.

CONFERENCE PRESENTATIONS

Milliner, B., **Ballard, K.**, Robinson, J., and Olive, J.L. *Metabolic Rate in Individuals with a Predisposition for Type 2 Diabetes*. Poster Presentation, Research Louisville, November 2005, Louisville, KY.

Ballard, K.D., Olive, J.L. *Metabolic Rate in Individuals with a Predisposition for Type 2 Diabetes*. Slide Presentation, University of Louisville College of Education and Human Development Spring Research Conference, April 2006, Louisville, KY.

Ballard, K.D., Olive, J.L. *Metabolic Rate in Individuals with a Predisposition for Type 2 Diabetes*. Slide Presentation (pending), American College of Sports Medicine 53rd Annual Meeting, May 2006, Denver, CO.

Olive, J.L., **Ballard, K.D.**, Robinson, J. *Vascular Function in Individuals with a Predisposition for Type 2 Diabetes*. Poster Presentation (pending), American College of Sports Medicine 53rd Annual Meeting, May 2006, Denver, CO.

AFFILIATIONS/ MEMBERSHIPS

National Strength and Conditioning Association-Certified Personal Trainer, 2002-present
UofL Health Sciences Club, Vice President, 2005-2006
American Heart Association CPR Certified, 2000-present
American College of Sports Medicine, 2006-present

COMPUTER SKILLS:

Proficient in Microsoft Windows, Microsoft Office, SPSS, Eat Right Analysis Nutrition software, LabVIEW and EndNote software