POSTPRANDIAL METABOLISM AND VASCULAR

FUNCTION: IMPACT OF AGING AND PHYSICAL

ACTIVITY LEVEL

By

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Bachelor of Science in Nutritional Sciences

Oklahoma State University

Stillwater, Oklahoma

2018

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2019

POSTPRANDIAL METABOLISM AND VASCULAR

FUNCTION: IMPACT OF AGING AND PHYSICAL

ACTIVITY LEVEL

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ACKNOWLEDGEMENTS

I would like to thank my primary advisor Dr. Sam Emerson for always providing constructive feedback throughout this project. He was the backbone for completing this study and made it a fun and enjoyable learning experience. I am also thankful for all the members of the LANES laboratory who have helped me in the completion of this project and its related endeavors.

Name: NICHOLAS ANDREW KOEMEL

Date of Degree: DECEMBER, 2019

Title of Study: POSTPRANDIAL METABOLISM AND VASCULAR FUNCTION: IMPACT OF AGING AND PHYSICAL ACTIVITY LEVEL

Major Field: NUTRITIONAL SCIENCES

Abstract: The consumption of a high-fat meal can induce acute postprandial lipemia (PPL) and endothelial dysfunction. We assessed the impact of age and physical activity on metabolic and vascular outcomes following meal consumption in healthy men and women. We recruited 4 groups of individuals: younger active (YA; age 22.1 ± 1.4 y; n = 9), younger inactive (YI; age 22.6 ± 3.7 y; n = 8), older active (OA; age 68.4 ± 7.7 y; n = 8), and older inactive (OI; age 67.7 ± 7.2 y; n = 7). Following a 10-hour overnight fast and 2 days of exercise avoidance, metabolic outcomes were measured at baseline and hourly for 6 hours after consumption of a high-fat meal (12 kcal/kg; 63% fat, 34% carbohydrate). Flow-mediated dilation (FMD) was measured at baseline, 2-hours, and 4-hours post-meal. Total area under the curve (tAUC) for TG was significantly lower in the more active individuals but did not differ based on age (YA = 578.3 ± 120.5 mg/dL x 6 hour, YI = 1032.0 ± 424.1, OA = 599.9 ± 235.3, OI = 1071.0 ± 149.5; p = 0.0004). FMD differed (p = 0.002) between groups at baseline (YA = 6.36 ± 1.70%; YI = 3.98 ± 1.67%, OA = 4.82 ± 1.27%, OI = 3.27 ± 1.33%), and decreased significantly in all groups 4 hours after the meal (Mean diff: 1.19%; 95% CI: 0.27 to 2.11%). These findings highlight the beneficial effect of regular physical activity on PPL, independent of age.

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CHAPTER I

INTRODUCTION

Nutrition plays an essential role in determining the health condition of an individual.

Health outcomes most commonly associated with poor nutrition are cardiovascular disease

(CVD), type 2 diabetes, obesity, and certain forms of cancer (Kimokoti and Millen 2016). CVD

accounts for 31% of all deaths globally (Stewart *et al.* 2017) and exhibits one of the strongest and

most comprehensive connections with dietary behavior. A growing avenue of assessment with

regard to nutrition and CVD risk is the postprandial metabolic response. Consumption of a single

high-fat meal (HFM) results in a deleterious postprandial metabolic state that can include

increased oxidative stress and inflammation (Jackson *et al*. 2012; Devaraj *et al*. 2008). The

adverse metabolic response to a HFM is characterized by significantly elevated plasma

triglycerides (TG), known as postprandial lipemia (PPL). The magnitude of this TG response is

directly associated with CVD risk (Hyson *et al*. 2003) and is more strongly associated with CVD

risk than fasting TG (Bansal *et al*. 2007). Given that most individuals likely spend the majority of

their day in a postprandial state, PPL represents a more externally valid determinant of CVD risk.

Age and physical activity are critical modifiers of PPL (Petitt and Cureton 2003; Gill and

Hardman 2000), although most previous studies have investigated their effects separately. In a

recent cross-sectional study by Emerson *et al.* (2018), the impact of both physical activity level

and age on PPL were examined in younger active, older active, and older inactive individuals.

This study found strong evidence that both aging and physical activity level modify the

postprandial TG response, with older inactive individuals exhibiting the greatest PPL, followed

by older active individuals, and then younger active individuals. However, this study was limited in that it did not include a younger inactive group, therefore precluding determination of whether age or physical activity was a more important modifier of PPL. Nevertheless, findings from this study suggest that aging and physical activity level are important independent factors in determining the postprandial TG response, although the mechanisms through which age and physical activity modify PPL are not clear.

Vascular function is another CVD risk indicator that is modified by nutrition, physical activity, and aging (Vogel 1997). Vascular function is primarily characterized by blood vessels' ability to dilate and constrict appropriately in response to local and systemic stimuli. Vasodilation refers to the expansion of a vessel by the relaxation of smooth muscle cells that surround the endothelial lining. This is a natural homeostatic response to a lack of oxygen and allows for increased blood flow to peripheral tissues (Huang and Vita 2006). Vascular function is a logical diagnostic tool, given its vital role in maintaining physiological homeostasis and its association with CVD risk (Halcox *et al.* 2002). A prominent noninvasive strategy for measuring vascular function includes a technique known as flow-mediated dilation (FMD), determined via Doppler ultrasound (Green *et al.* 2011). FMD has found to be a strong predictor of CVD risk (Inaba *et al.* 2010; Moens *et al.* 2005).

Younger and more active individuals exhibit greater FMD in the fasted state compared to their older and less active counterparts (Dawson *et al.* 2013; de Groot *et al.* 2006; Parker *et al.* 2006). However, little is known about how age and physical activity modify FMD after the consumption of a HFM. Most individuals in Western society spend the majority of the day in a continuous postprandial state; therefore, it is arguably more pragmatic to study vascular function after the consumption of a meal. To our knowledge, no previous studies have investigated the postprandial FMD response to a HFM in younger versus older individuals with different physical activity levels.

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The purpose of this study was to assess the impact of age and physical activity on postprandial metabolism and vascular function. We hypothesized that there would be differences in postprandial TG and FMD responses based on age and physical activity level and that group differences in postprandial lipemia and vascular function would be associated with differences in body composition.

CHAPTER II

LITERATURE REVIEW

Nutrition and Cardiovascular Disease

Nutrition has been comprehensively studied for the role it plays in the development of CVD. Specifically, increased consumption of processed foods, saturated fat (SF), refined sugars, and sodium (Poti *et al.* 2015) have all been demonstrated to significantly increase CVD risk. Conversely, a reduction in nutrient-dense food consumption such as fruits and vegetables also increases the risk for CVD (Aune *et al.* 2017; Blanck *et al.* 2008). These changes in lifestyle choices have been coined as "westernization" and are rapidly expanding to other regions of the world undergoing similar changes (Pappachan 2011). Certain sources of SF (e.g. animal products) have been directly linked to increased incidence of CVD and are commonly incorporated into processed foods to increase shelf life (Siri-Turino *et al*. 2015). In a recent meta-analysis reviewing 15 randomized controlled trials, Hooper *et al.* reported a 17% decrease in the risk of a cardiovascular event after incorporating a reduction of saturated fat. Additionally, sodium is liberally distributed throughout a vast array of foods to improve food longevity and palatability. The NHANES 2009-2012 analysis reported that nine out of ten Americans consume sodium in excess of the recommended daily upper limit (>2300 mg/day). Sodium has been linked to higher rates of hypertension and ultimately an increase in CVD risk (Jackson *et al.* 2016; Celermajer and

Neal 2013). Similarly, commonly consumed processed foods contain high amounts of refined

sugar, which on average comprises over 20% of total calories in processed foods, and is closely

linked with metabolic syndrome. Over 80% of Americans consume above the recommended 10%

of daily calories from refined sugars (Martinez *et al.* 2016). This link between disease and refined

sugars is grounded in numerous meta-analyses and has become increasingly evident with the 40-

fold increase in sugar consumption since 1750 in the United States (Dinicolantonio *et al.* 2014;

Bray *et al*. 2012). Intake of fruits and vegetables is markedly low, with 87% of Americans not

meeting the daily intake requirements for vegetables, and 76% failing to fulfill the daily fruit

consumption recommendations (Moore and Thompson 2015).

Atherosclerosis is the product of poor dietary consumption and represents a major route

to the development of CVD. Atherosclerosis is generally described as the progressive buildup of

fat and cholesterol within arterial vessels and can lead to diminished vascular function or a

cardiovascular event (Rafieian-Kopaei *et al.* 2014). Atherosclerosis causes nearly 50% of all

deaths in the United States (Pahwa and Jialil 2018). Lipids are packaged and transported in the

form of lipoproteins, such as low-density lipoprotein (LDL), that can contain high concentrations

of cholesterol. These lipoproteins, particularly LDL, can penetrate the endothelium and become

trapped and oxidized in the subendothelial space. Oxidized LDLs promote increased recruitment

of macrophages, pro-inflammatory cytokines, and other immune cells, ultimately furthering

intravascular plaque development (Mitra *et al.* 2011).

Glucose can also have a negative effect on the endothelium and continuously high levels

of blood glucose can lead to decreased insulin sensitivity. Insulin sensitivity allows cells to

adequately utilize glucose and remove it from the circulation. Prolong elevated glucose levels and

reduced insulin sensitivity are used as markers of disease risk. Hyperglycemia can disrupt normal

cell signaling, disrupt hormonal regulation, and cause systemic inflammation promoting the

development of chronic disease (Park *et al.* 2012).

Triglycerides (TG) are the primary form of lipid, both in the diet and in the human body. TG can be characterized into several different categories depending on their molecular morphology (e.g. saturated, unsaturated, *trans*). SF is defined by its hydrogenated nature, meaning it contains the maximum number of hydrogen atoms on each carbon. Foods high in SF include red meat, cheese, and butter. Unsaturated fats (e.g. polyunsaturated and monounsaturated) lack a hydrogen atom in the lipid molecule due to the presence of a double bond. *Trans* fats are naturally occurring lipids, but they are most commonly found in processed foods after undergoing partial hydrogenation. During the hydrogenation process, one or more double bond in a lipid molecule is replaced with the addition of hydrogen and re-configured in a *trans* formation. Saturated and *trans* fats have been found to be strongly associated with CVD (Nicholls *et al.* 2006), while unsaturated lipids possess properties that reduce the risk of CVD (Keogh *et al.* 2005). These dietary lipids differ with regard to their effects on CVD risk. High levels of serum LDL, TG, and cholesterol are strongly associated with an increased risk of CVD (Nelson 2013). SF reduces the LDL-receptor activity limiting LDL clearance capacity (Chiu *et al*. 2017). Unsaturated lipids have anti-inflammatory properties, which can potentially blunt the atherosclerotic process. An inverse association has been made with polyunsaturated fats and CVD, which can increase LDL receptor activity and attenuate LDL production (Siri-Tarino *et al.* 2015). Most notably are polyunsaturated omega-3 fatty acids, eliciting some of the most pronounced health benefits. Poly and mono-unsaturated lipids are involved in anti-atherogenic, anti-inflammatory, inhibited platelet aggregation, anti-thrombotic, and arrhythmia prevention (Ander *et al.* 2003).

Overall, poor nutrition is a major contributor to CVD, with nearly 70% of CVD attributed to poor lifestyle habits (Chieuve *et al*. 2008). Nutrition is a modifiable risk factor that can greatly reduce the risk of major chronic diseases. Dietary choices serve as an opportunity for individuals to improve health status and quality of life.

Physical Activity and Disease

The American Heart Association recommends adults conduct at least 150 minutes of moderate aerobic exercise or 75 minutes of vigorous exercise, or an equivalent combination (Lobelo *et al*. 2018). Adults are also recommended to conduct full-body resistance training at least twice per week (Piercy *et al.* 2018). Westernized areas are characterized by low levels of physical activity, which is associated with excess body fat mass and numerous chronic diseases, including CVD (Zeng *et al.* 2012). Physical activity is considered one of the most accessible preventative measures of CVD, with nearly 12% of all myocardial infarctions associated with a lack of physical activity (Carnethon 2009). Exercise can also improve insulin sensitivity, helping to maintain glucose homeostasis and lower risk for cardiometabolic disease. Simply walking for 30 minutes per day can reduce the risk of type II diabetes by 50% (Hamasaki 2016). Physical activity is also highly preventative for many types of cancer, with nearly 35% of cancer deaths in the United States attributed to a lack of physical activity. The reduction in cancer risk is commonly thought to be a result of decreased adiposity, reduced inflammatory markers, and increased regulation of growth factors that often accompanies higher levels of physical activity (Clague and Bernstein 2012). Increasing physical activity also improves cardiorespiratory fitness (CRF) and endothelial function, which are inversely associated with CVD, diabetes and all-cause mortality (Al-Mallah *et al*. 2018). Physical activity can beneficially modify cardiometabolic risk, and increasing physical activity is an accessible means of reducing risk disease (Vincent *et al.* 2017).

Postprandial Period and Cardiovascular Disease

A growing avenue of assessment with regard to nutrition and CVD risk is the postprandial metabolic response. Individuals consuming large volumes of low-quality foods on a frequent basis are consistently evoking a deleterious postprandial metabolic state (Heden *et al.* 2013; Klop *et al.* 2011; Waaler *et al.* 1991). This adverse metabolic state is characterized by excessive lipemia (high TG) and hyperglycemia (high glucose). Elevated circulating TG for

several hours following high-fat feeding is characteristic of postprandial lipemia and the

magnitude of this TG response is directly associated with CVD risk (Nelson 2013). In

postprandial lipemia, there is enzymatic competition for serum lipids, resulting in saturation of

clearance pathways, which leads to the deposit of the FFA within the endothelium (Nakamura *et

al.* 2016). All the major organ systems, such as hepatic, renal, pancreatic, and digestive tract, are

over time disrupted to some degree with large postprandial loads (Jacome-Sosa *et al.* 2016).

Postprandial states also upregulate the expression of adhesion molecules (e.g. selectins and

integrins), and increase the risk of cholesterol deposits in the vascular system which promotes

atherosclerosis (Karpe 1999). High-density lipoproteins (HDL) are a primary component in the

metabolic process of reverse cholesterol transport. Further, HDLs circulate in the bloodstream,

attaching to extra-hepatic tissues in order to transport cholesterol back to hepatic systems for re-

utilization and excretion (Spady 1999). High levels of HDL are inversely related to

atherosclerosis, whereas serum levels of LDL, TG, cholesterol, and glucose are positively

correlated with disease risk (Shimokawa and Satoh 2014). Interestingly HDL levels have been

found to decrease in the postprandial period, another example of the detrimental metabolic impact

of large-volume, high-fat feeding (Alssema *et al*. 2010).

These mechanisms through which a large postprandial metabolic response increases CVD

risk are supported by the findings of cross-sectional and prospective cohort studies. Postprandial

TG loads are the major driving force in the development of atherosclerosis and are positively

associated with the progression of atherosclerosis (Teno *et al*. 2000). A prospective cohort study

conducted by Bansal *et al*. (2007) assessed the association between fasting and non-fasting TG

values in 26,509 healthy women. In this study, a blood draw was taken either fasted or non-fasted

and the overall incidence of CVD was followed over 11 years. There was a stronger association

with 2-4 hours postprandial TG and CVD risk (HR: 4.48 [1.98-10.15]) compared to the fasted (8-

12 hr) TG and CVD risk (HR: 1.31 [0.73-2.36]). Notably, after fully adjusting for confounding

variables, non-fasting TG was still associated with CVD risk, although fasting TG was not. A

case-control study by Patsch *et al*. (1992) examined a postprandial TG in individuals with

coronary artery disease (CAD; n = 61) and healthy controls (n = 40). A predictive model was

created using fasted vs. postprandial metabolic outcomes in these participants to assess the

absence or presence of CAD. Not only were postprandial TG significantly greater in the CAD

patients, but the study revealed a greater ability to predict CAD with postprandial TG (83%)

compared to using fasted TG values (68%). Thus, several lines of evidence support the notion

that postprandial lipemia is highly influential with regard to CVD risk.

Physical activity improves the uptake and utilization of serum TG and glucose, which

attenuates endothelial damage caused by a meal (Emerson *et al.* 2018). In a cross-sectional study

by Braxton and colleagues (2018), the postprandial TG responses of 671 healthy individuals were

assessed following a high-fat meal and compared to their overall physical activity level. Activity

was measured continuously using accelerometers worn by participants for 7 days. Total area

under the curve (tAUC) TG responses were significantly lower in individuals with high levels of

activity (p = 0.003) after controlling for age, sex, BMI, and fasting LDL. In this study, individuals

in the highest quartile of habitual physical activity exhibited a 17% lower postprandial TG

response compared to individuals in the lowest quartile of physical activity. Acute and habitual

physical activity are both shown to increase the uptake and utilization of the deleterious

byproducts of meal intake in numerous prospective cohort and controlled trials (Homer *et al*.

2017; Freese *et al*. 2013).

It is likely that age is also an important modifying factor in postprandial lipemia. In a

recent cross-sectional study by Emerson *et al.* (2018), the impact of physical activity and age on

postprandial responses was examined. Healthy individuals were separated into 3 groups differing

in physical activity and age, including eight young active adults (YA: age 18-35 years; 4M/4W),

eight older active adults (OA: age 60+ years; 4M/4W), and six older inactive adults (OI: 3M/3W).

Metabolic outcomes (e.g. Glucose, HDL, LDL, TG) were compared after the consumption of a

HFM. The YA group had a significantly lower TG tAUC compared to the OA (p = 0.02) and OI

(p = 0.0002). Additionally, the OA TG tAUC was significantly lower compared to the OI TG

tAUC (p = 0.02). Thus, this study found evidence that both aging and physical activity level

modify the postprandial TG response, with younger and more active individuals generally

exhibiting a lesser response to a high-fat meal. However, this study was limited in that it did not

include a younger inactive group and did not address mechanisms of difference between groups.

Nevertheless, data to this point suggest that aging and physical activity level are important factors

in determining the postprandial metabolic response, which is associated with CVD risk.

Vascular Function

Vascular function is the plexus of physiological systems that allows an adequate supply

of blood throughout the body to maintain physiological homeostasis (Sandoo *et al.* 2010). The

heart is the primary mechanical driver in the circulatory system. Vessels are comprised of three

layers: the tunica intima, tunica media, and tunica adventitia. The tunica intima is the innermost

lining of the vessel and is also known as the endothelium, which mediates changing vessel

conditions (Pearson 2000). The blood that is distributed within this system acts as the carrier for

blood gases, enzymes, hormones, and nutrients. The vascular system is most commonly

delineated into two major systems: the arterial and venous systems. The arterial system is

comprised of vessels (arteries and arterioles) that carry oxygenated blood from the heart toward

peripheral tissues. Conversely, the venous pathway directs deoxygenated blood back to the heart

where it can become re-oxygenated via the pulmonary tract (Dela Paz and D'Amore 2009). Both

of these circulatory systems work concertedly to provide the tissues of the body with a sustained

supply of oxygen and facilitate normal cellular function (Pugsley and Tabrizchi 2000).

Blood vessels are not only important with regard to blood flow distribution and oxygen

delivery, but they also play a critical role in the development, prevention, and recovery of

diseases. Physical and cognitive development requires constant replenishment of nutrients that are

transported from the gastrointestinal system via vascular networks (Geboes *et al.* 2001).

Additionally, the endothelial lining acts as the surface where nutrient exchange occurs. Individuals who develop negative structural adaptations such as the development of plaque and increased vessel wall thickness exhibit diminished nutrient uptake potentially hindering cell growth and repair (Kusters and Barrett 2016). From a disease standpoint, deterioration of vascular health is a critical element in the development of CVD, diabetes, and cancer. Individuals who engage in unhealthy lifestyle behavior (e.g. alcohol abuse, obesity, smoking, poor diet, exercise) often first experience an impairment in vascular function which further promotes the cascade of disease (Odegaard *et al.* 2011).

Vascular function is largely characterized by the vessels' ability to vasodilate and constrict properly in response to local and systemic stimuli. Vasodilation is a critical element in providing adequate blood flow to specific regions throughout the body and is a natural homeostatic response to a lack of oxygen. This response propagates the relaxation of smooth muscle cells that compose the endothelial lining (Huang and Vita 2006). A lack of blood flow or excess constriction increases the frictional drag within a vessel – this biomechanical metric is known as shear stress. Shear stress is a potent stimulus for the cellular release of nitric oxide, which is the primary agent in relaxing smooth muscle cells to allow vessel dilation (Lu and Kassab 2011). Conversely, vasoconstriction is a physiological response that is characterized by vessels narrowing. Vasoconstriction is also mediated by vasoactive compounds (e.g. endothelin, angiotensin II, and epinephrine) to help regulate items such as heat loss, stress, and blood pressure (Thompson-Torgerson *et al.* 2008). Over time, vessels naturally lose elasticity to a certain degree, promoting arterial stiffness. A lack of arterial distensibility is directly related to the ability of the body to accommodate to physiological pressure changes, thus prolonged diminished function can elicit damage throughout the entire arterial tree (Cecelja and Chowienczyk 2012). An increase in arterial stiffness is an independent predictor of CVD risk (Yutaka *et al.* 2007). Arterial stiffness is caused in part because of the decreased ability of smooth

muscle cells to accommodate changing environmental conditions. Impaired dilation or constriction can exacerbate underlying disease conditions that require vascular adaptability. In the case of atherosclerosis, hardening of the arterial vessels from lipid accumulation also decreases vessel distensibility (Haluska *et al.* 2010). Arteries eventually can accumulate sufficient plaque to impair blood flow and vasodilation. Plaque can lead to a cardiovascular event such as a stroke, myocardial infarction, or cardiac arrest. This outcome remains difficult to prevent due to the highly asymptomatic nature of the vascular disease progression (Lauer 2010).

Vascular function is a logical diagnostic tool, given its vital role in maintaining homeostasis and its association with various health conditions (Halcox *et al.* 2002). There are several clinical methods for measuring the functional ability of the vascular system. Categorically, these can be thought of as invasive or non-invasive strategies (Al-Qaisi *et al.* 2008). Invasive strategies include biopsy operations, coronary angiography, or the infusion of vasoactive compounds (e.g. Acetylcholine, Nitroglycerine, or Adenosine) to elicit a vascular response (Petersen *et al.* 2014; Kuvin *et al.* 2001). Noninvasive strategies include magnetic resonance imaging (MRI) and flow-mediated dilation (FMD) techniques via Doppler ultrasound. MRI and Doppler ultrasound techniques are often used to provide an external image of how vascular dimensions compare to healthy individuals and a vessel's capacity to adequately supply blood flow. Lastly, FMD via Doppler ultrasound provides a stress stimulus by occluding a portion of the body or by voluntary muscular contraction (i.e. handgrip), and determining the dilatory response to increased blood flow (Flammer *et al.* 2012).

Indirect measurements of vascular health are more frequently used to evaluate an individual's risk for disease or a disease state. Metabolic markers such as blood pressure, glucose, HDL, LDL, TG, and cholesterol are highly correlated markers of vascular health (Montgomery and Brown 2013). These biomarkers are indicative of the physiological conditions that the vascular system is functioning within and provide insight into an individual's overall health

status. Other biomarkers, such as reactive oxygen species (ROS) represent levels of oxidative stress, which can promote atherosclerotic cascades (He and Zuo 2015). ROS can actively promote endothelial damage leading to low-grade inflammation and the release of cytokines. Inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and vascular cell adhesion molecule-1 (VCAM-1) are standard makers used for gauging overall levels of inflammation (Hall *et al.* 2013). Additionally, deteriorating vascular health has been linked to chronically increased levels of angiogenic substances (Shibuya 2006). These growth factors play an integral role in the production of new red blood cells to supply more oxygen in surrounding tissues. Markers such as vascular endothelial growth factor (VEGF), Angiopoietin, bone morphogenic protein 9 (BMP-9), epidermal growth factor (EGF), Endoglin, Endothelin-1, and fibroblast growth factor 1 (FGF-1) are regularly recognized agents involved in angiogenesis and indirect indicators of vascular health (Nashida *et al.* 2006). Elevated levels of these substances are common in disease states, wound healing, or tumor development. However, prolonged elevated levels can cause pathogenic hyperpermeability of vessels and potentially impair the ability of the vascular system to drain waste, nutrients, and water properly (Nagy *et al.* 2008). Lastly, high levels of these angiogenic molecules can exacerbate the development and growth of certain cancerous tissues (Carmeliet *et al.* 2000).

Flow-Mediated Dilation

Flow-mediated dilation is the natural dilatory response of vessels to an increase in local blood flow. This dilatory response is produced from the release of nitric oxide (NO) in endothelial cells (Bian *et al.* 2008). Items such as platelet aggregation, cellular proliferation, and the development of adhesion molecules can be blunted by the release of NO, which allows relaxation of smooth muscle cells (Bruno *et al.* 2014). The inability to dilate vessels (i.e. a lower FMD response) can, over time, lead to detrimental vascular remodeling and increase damage to the endothelial lining (Rudic *et al.* 1998). Moreover, understanding how well an individual can

stimulate a NO-dependent dilatory response is considered an indicator of vascular health (Cannon 1998). The first FMD assessments were explored in 1992 by Celermajer and colleagues as a non-invasive procedure to detect endothelial dysfunction in children and adults (Celermajer *et al.* 1992). Individuals with familial hypercholesterolemia, smokers, and those with coronary artery disease expressed a greatly reduced or absent dilatory response. Many studies have built on this seminal work, and over time there has been widespread adoption of FMD as an assessment tool for vascular function. In a recent meta-analysis (Ras *et al.* 2013) comprised of 14,753 subjects, FMD was inversely associated with the incidence of CVD with a relative risk of 0.92 (95% CI: 0.88; 0.95) per 1% higher FMD. Another meta-analysis in 2005 by Witte *et al.*, reviewed 211 FMD studies and found a 1.42% decrease in FMD for every 1% increase in Framingham risk, which is a gender-specific 10-year risk scale for developing cardiovascular disease. These findings support the conclusion that FMD is proportional to individual CVD risk. Due to the close relationship between vascular function and CVD development, examining vascular biology via FMD is an underutilized metric for the development, progression, and diagnosis of cardiometabolic diseases (Shechter *et al.* 2009; Yeboah *et al.* 2009).

Overview of Flow-mediated Dilation Protocol

FMD has been used in a variety of studies and follows a fairly consistent protocol (for reviews see Alley *et al.* 2014; Harris *et al.* 2010; and Corretti *et al.* 2002). An FMD assessment begins with an individual laying in a supine position for 5-20 minutes in a dark space to acclimate to the environment (Thijssen *et al.* 2011). FMD trials then typically incorporate occlusion of an extremity that falls in close proximity to a large conduit artery. For example, occlusion of the forearm/wrist and analyzing the proximal brachial artery is common. Before beginning occlusion, baseline measurements of the artery diameter are measured for roughly 1-2 minutes. Occlusion is induced by placing a blood pressure cuff at a point distal to the given artery to deprive tissues of oxygen and simulate a stress stimulus. After occluding for 3-5 minutes, the blood pressure cuff is

then deflated, allowing a hyperemic response to occur. This large amount of blood volume increases the shear stress in the vessel, eliciting the release of NO, and causing dilation of the artery. During the entire procedure, the brachial artery is being imaged via duplex ultrasound to capture a grayscale image of the chosen vessel, through which the dilatory changes can then be assessed. The vessel diameter change that occurs during this acute period of hyperemia (after cuff deflation diameter) is known as the FMD% and is the primary outcome of interest. Shear stress is thought of as the amount of frictional drag caused on the walls of the vessel and is directly proportional to the viscosity and velocity of blood within the vessel (Pyke and Tschakovsky 2005). Transiently increased levels of shear stress are a major driver of FMD responses (Padilla *et al.* 2008; O'Rourke and Nichols 2004). A larger FMD response is considered reflective of more optimal vascular health. Healthy individuals with no ongoing chronic disease will often exhibit an FMD between 8-15% (Weissgerber 2014). Unhealthy individuals experience diminished FMD%, and those with existing CVD can exhibit an FMD% < 5% (Maruhashi *et al*. 2013).

Factors that Influence Flow-mediated Dilation

Many factors can influence the FMD response, including individual-level factors such as gender, disease states, physical activity, age, and medications, as well as variability associated with the technique itself. To help control for some of these sources of variability, subjects are typically fasted and have abstained from caffeine and alcohol for at least 8-24 hours prior to the assessment (Noguchi *et al.* 2015). Exercise also has an acute negative effect on the FMD response, so research subjects are typically instructed to refrain from strenuous exercise for 24-48 hours prior to data collection (Currie *et al.* 2012). FMD responses have been previously shown to be influenced by the time of data collection as well (i.e. increases from 2:00 PM until peaking at 2:00 AM). Physiological conditions related to FMD have a tendency to remain elevated in the morning hours (e.g. blood pressure, cortisol, and epinephrine) (Ringqvist *et al.* 2000). Moreover,

most trials are executed during a consistent time window in the morning hours to limit these effects (Padilla *et al.* 2007).

Gender is often an important factor to consider when studying FMD because of the well-known physiological differences between men and women. FMD for premenopausal women is typically adjusted to account for potential hormonal variation (Hashimoto *et al.* 1995). Females exhibit cardiovascular protective hormones such as estrogen and progesterone to a greater extent than males, which is suggested to result in a greater FMD response in females (Pérez-López *et al.* 2010). Males generally have more muscle mass and increased arterial size. The size of the artery has been suggested to influence the FMD response, where larger arteries exhibit a lesser dilatory response (Mizia-Stec *et al.* 2007; Paradossi *et al.* 2004; Celermajer *et al.* 1992).

Pharmacological influences are some of the most potent regulators of FMD. Medicine is often prescribed to enhance the dilatory response in individuals at risk for a CVD event to prevent the detrimental effects of vascular remodeling. Hypertensive medications such as diuretics, beta-blockers, ace inhibitors, and vasodilators are common within westernized society to help mitigate vascular resistance, albeit through different mechanisms (Ghiadoni *et al.* 2003). Cholesterol-lowering drugs are also prescribed from a preventative standpoint due to the prevalent nature of hypercholesterolemia in the United States. As previously mentioned, cholesterol plays a crucial role in the atherosclerotic process. Therefore these medications have a direct relationship with FMD (Takenaka *et al.* 2013). Lastly, over-the-counter medications, such as aspirin, can modify blood viscosity, thus potentially altering FMD (Magen *et al.* 2005). In a 2011 study by Furuno *et al.*, brachial artery FMD exhibited a positive dose-dependent response with the intake of aspirin with intake between 80-660 mg; however, FMD begins to worsen with intake above 660 mg/day. Complete abstinence from medication may not be permissible with all populations, but the confounding properties of medication should be considered in FMD studies.

Other factors to consider in FMD data collection are inter- and intra-experimenter variability. In this context, intra-experimenter variability is designated for variability within an individual researcher, whereas inter-experimenter variability refers to the variability between different researchers when analyzing the same data (Marietta *et al.* 2010). Each form of variability is quantified by calculating the inter- and intra-collection coefficient (ICC). Overall, variability is minimized by maintaining the consistency of experimenters and methods throughout data collection. ICC variability for intra- and inter-experimenter variability has been reported to be 0.84-0.99 and 0.82-0.87, respectively, with trained sonographers (Charakida *et al.* 2013). Additionally, data should be void of names or specific labels and analyzed randomly to minimize bias introduced from the experimenter's perspective. Variations in cuff placement can also prompt different mechanistic dilatory responses. Placement of the cuff distal to the artery is found to create the most prominent NO response and have less physical influence on the tissues being analyzed (Ghiadoni *et al.* 2015; Betik *et al.* 2004). Further, when comparing different cuff placements, wrist cuff occlusion has been associated more closely with CVD risk (Doshi *et al.* 2001). Magnitude and duration of occlusion also independently modulates FMD outcomes. Occlusion is found to be effective at roughly 25-50 mmHg above an individual's systolic blood pressure for a duration of 5-10 minutes (Uehata *et al.* 1997). Other mechanisms outside of NO have been noted to become more active beyond the 5-minute occlusion window (Harris *et al.* 2010). Studies have demonstrated that, when meticulously executed according to protocol, FMD assessments can produce valid and reproducible data, despite the risk of experimenter error (Simova *et al.* 2008).

Nutritional Impacts on Flow-Mediated Dilation

Nutritional factors such as sodium, antioxidants, alcohol, protein, glucose, and lipids have been shown to alter FMD or be associated with certain FMD patterns. Sodium is an important mineral because of its role in maintaining normal fluid balance and cellular homeostasis

(Farquhar *et al.* 2015). However, the amount needed in the diet to sustain adequate function is vastly different from the mean average sodium intake in the US diet (500mg vs. 3200mg). Sodium in excess has a well-known relationship with the development of hypertension (Ha 2014 and Aronow 2017). Irregular blood pressure and fluid dynamics due to excess sodium can consequently affect FMD in an adverse manner. Specifically, sodium elicits a decrease in FMD independently of blood pressure. In 2015, Mathews *et al.* measured FMD in a controlled feeding study where individuals (n = 20) consumed a 7-day high-salt diet (HS) or a 7-day low salt diet (LS). In this study, 10 individuals were determined to be salt-sensitive (SS; age 39 ± 5 yr; 5 men, 5 women), and 10 individuals were salt-resistant (SR; age 42 ± 5 yr; 5 men, 5 women). FMD was significantly lower in the HS diet compared to the LS diet (FMD: SR LS $10.6 \pm 1.3\%$, SR HS $7.2 \pm 1.5\%$, SS LS $12.5 \pm 1.7\%$, SS HS $7.8 \pm 1.4\%$). Interestingly, there was no significant difference between SR and SS, thus supporting the adverse effect of sodium on FMD independent of blood pressure. A decreased FMD from chronic salt consumption is proposed because dietary sodium causes a concomitant superoxide response (ROS), and suppression of angiotensin II, which decreases SOD expression (Edwards and Farquhar 2015). Extracellular levels of sodium can also induce increased secretion of aldosterone which can lead to endothelial stiffness and decreases NO secretion. Thus, long-term excess sodium consumption plays an integral role in diminished vascular function and decreases FMD ability in individuals (Dickinson *et al.* 2009).

Dietary protein also has the potential to modify FMD. Inadequate protein intake is associated with a decrease in serum arginine availability, which is used in the synthesis of NO. Thus, individuals who acquire a protein deficiency may have an impaired ability to produce a NO-mediated FMD response (Luiking *et al.* 2010). Protein sources such as red meat, processed meat, and dairy are positively associated with CVD. Conversely, plant sources of protein, such as soy, nuts, and beans, have an inverse relationship with CVD (Micha *et al.* 2012). Many of these specific food sources have not been studied in an FMD context. Protein as a whole has not been

extensively studied with regard to FMD, and consumption of soley protein sources is not common in a typical western diet. Protein constitutes a small portion of the overall diet, and the impact of chronic high protein consumption on vascular function has yet to be studied.

Some variation in the FMD response can be explained by the intake of vitamins and minerals (Plantiga *et al*. 2007). With respect to vitamins, the most common mediators of endothelial function include vitamins C, E, and A. Vitamins C, E, and A actually increase the release of NO within vessels. Additionally, these act as potent antioxidant agents which can mitigate cellular ROS production. Antioxidants remain one of the primary agents in fruits and vegetables that improve the diet. It has been demonstrated that supplementation of fruit, vegetables, or an oral antioxidant vitamin supplement can improve FMD responses (Varadharaj *et al.* 2017; Khan *et al.* 2014; Esposito *et al.* 2003).

The important dietary minerals concerning FMD include calcium, iron, and magnesium. Normal cellular levels of calcium can improve the synthesis and release of NO within the endothelium. However, calcium supplementation in excess has been associated with an increase in cardiovascular morbidity and arterial calcification (Anderson *et al.* 2016). Iron plays a critical role in the production of NO and can ultimately affect the expression of NOS. Once ingested, iron improves the mRNA expression of NOS2 which is the primary producer of NO (Nairz *et al.* 2013). Magnesium is a potent vasorelaxation mineral and has a positive dose-dependent NO response. Magnesium is a cofactor for acetylcholine-induced vessel relaxation, and even subtle alterations in extracellular magnesium can elicit a dilatory response. Further, magnesium supplementation has been associated with improved FMD responses in healthy individuals and those with CVD-related conditions (Rosique-Esteban *et al.* 2018). Barbagallo *et al.* (2010) conducted a magnesium supplementation intervention in which the elderly (>65 years) individuals with pre-existing diabetes and hypertension consumed 368mg/day for one month. Interestingly, the experimental group saw an increase in FMD% (from $3.3 \pm 3.6\%$ to $8.4 \pm 3.9\%$;

$p < 0.05$), whereas no significant difference was found in the control group. Overall, intake levels of certain vitamins and minerals can influence vascular function and FMD.

Another nutritional perspective not commonly considered for FMD is alcohol consumption. Alcohol has acute benefits with regard to dilatory responses in healthy individuals (Vlachopoulos *et al.* 2003). The primary cardioprotective mechanism behind alcohol is thought to be prompted by the decrease in plasma fibrinogen levels, platelet aggregation, and an increase in NOS stimulation (Vlachopoulos *et al.* 2003). Certain beverages, such as wines, contain active phenolic compounds as a derivative of plant products used in the fermentation process. Drinks containing polyphenol substances are more likely to exhibit a beneficial FMD response due to their antioxidative properties (Barona *et al.* 2012). However, these beneficial responses likely do not outweigh the other detrimental effects of consuming alcoholic beverages. Alcohol consumption induces the production of superoxide anions (ROS) that can ultimately cause endothelial inflammation. Intake beyond the recommended daily allowance (>2 drinks/day in men and 1 drink/day in women) has been associated with a decrease in endothelial function (Suzuki *et al.* 2009).

Postprandial Flow-Mediated Dilation

Meal-induced elevations in glucose and TG can impair vascular function. Frequent meal consumption increases the duration that the circulatory system remains in this deleterious state. Elevated levels of lipids in circulation inhibit normal cell signaling, promotes adhesion molecule production, and can exacerbate atherogenesis (Jagla and Schrezenmeir 2001), ultimately leading to an impaired FMD response. In a study done by Vogel *et al*. in 1997, FMD was examined in the postprandial period each hour over a 6-hour period after a high- and low-fat isocaloric meal (900kcal). Interestingly, the high-fat meal decreased FMD from $21 \pm 5\%$ at baseline to $11 \pm 4\%$, $11 \pm 6\%$, and $10 \pm 3\%$ at 2, 3, and 4 hours after the high-fat meal, respectively. However, no changes in FMD were observed when the low-fat meal was consumed. Serum TG levels were

significantly correlated with FMD responses (p = 0.02), suggesting a strong connection between postprandial lipemia and FMD.

From a postprandial perspective, protein can be beneficial because of its ability to delay gastric emptying. This concept was recently demonstrated in a study by Phillips *et al.* 2014, where participants consumed either a high-fat meal (HF; fat content: 0.35g/kg, no protein), high fat + low protein meal (HFLP; fat content: 0.35g/kg, protein content: 0.18g/kg), or a high fat + high protein meal (HFHP; fat content: 0.35g/kg, protein content: 0.36g/kg). FMD measurements were taken at baseline and 60 minutes after completion of the meal. They found that the HF (-3.8% ± 1.9; p = 0.01) FMD was significantly lower than the HFLP (+1.5 ± 0.8%; p = 0.98) and HFHP (+1.7% ± 1.3; p = 0.79) There were no significant differences between HFLP and HFHP (p = 0.79). Thus, protein can play a role in blunting some of the negative FMD effects of a HFM meal.

Glucose provides the sustained fuel source for cells throughout the body but can be detrimental to vascular function at high levels. Most pronounced in individuals with type II diabetes, hyperglycemia is characterized as a major suppressor of FMD. The majority of clinical glucose measurements occur in a fasted state, while most negative hyperglycemic effects occur in the postprandial period (Kawano *et al.* 1999). These high concentrations of glucose after the consumption of a meal provoke ROS production within the endothelium, which can prevent or counteract vasodilation (Bonnefont-Rousselot 2002). As serum glucose levels rise following the consumption of a meal, there is also a consequential rise in insulin in a healthy individual. Subjects who have impaired insulin sensitivity can experience prolonged levels of serum insulin. High levels of insulin prevent insulin-dependent vasorelaxation, and acetylcholine-induced vasorelaxation (Hwang and Lee 2016). Acutely, high levels of serum glucose promote vessel shear stress leading to an increased vasodilatory response necessary for cellular uptake of glucose (Carlo *et al.* 2003). However, chronic endothelial exposure to glucose can lead to impaired

dilatory action. In addition to the desensitization of vasodilatory substances, chronic

hyperglycemia can induce the production of vasoconstrictive biomarkers such as thromboxane,

thus decreasing FMD (Xiang *et al.* 2007). Hyperglycemia overloads the glycolytic pathway

which can obstruct NO synthase activity from occurring properly and increases the expression of

pro-inflammatory cytokines. Hyperglycemia has also been found to reduce FMD via increases in

lipid peroxidation, further supporting the role of fat in vascular dysfunction (Mah *et al.* 2011).

Excessive lipid breakdown fuels superoxide radical production, which compromises NO

bioavailability. In a recent review by Wallace and colleagues in 2010, 20 studies were analyzed

for postprandial FMD interactions, reporting a strong association between decreased FMD in

postprandial states. However, very few studies have assessed components such as age and

physical activity in the experimentation of postprandial FMD.

 Physical Activity and Flow-Mediated Dilation

Physical activity is an important determinant of overall health. Exercise can transiently

reduce free radical damage by engaging protective myocardium mnSOD activity (Golbidi 2012).

Similarly, exercise also can cause a short-term pro-inflammatory state, with the upregulation of

items such as IL-6, TNF-α, and CRP. However, this is met with a more pronounced long-term

anti-inflammatory response that reduces detrimental markers (Kasapis *et al.* 2005). Physical

activity also increases the expression of eNOS, which stimulates NO production. Further, a bout

of exercise can also help maintain an optimal balance between angiogenic and angiostatic

phenotypes (Golbidi *et al.* 2011). Thus, physical activity can modify vascular function and FMD

outcomes, although several factors influence this relationship, such as the type of exercise,

frequency, and intensity.

Exercise is commonly referenced into two large categories: resistance training and aerobic

training. Resistance training is the process of providing progressive overload to skeletal muscles

(e.g. weight lifting). Resistance training typically produces enhancement of skeletal muscle

strength and mass. Skeletal muscle mass is largely involved in the process of glucose and TG

metabolism (Jensen *et al.* 2011). Thus, resistance exercise can influence both body composition

and macronutrient metabolism (Bea *et al.* 2010). In relation to endothelial function, resistance

exercise can improve FMD responses. In a recent randomized controlled trial, a 10-week

resistance training intervention with healthy individuals improved FMD from 8.5% to 9.8%.

Individuals in this study conducted resistance training twice a week for approximately 70 minutes

(Yu *et al.* 2016). The improvements in vascular function provided from resistance training make

it a potential component in the prevention of CVD.

Aerobic exercise refers to an activity that requires oxygen and typically involves

rhythmic activities such as running, cycling, and swimming. Aerobic training is associated with

large improvements in FMD responses. A study in 2011 by Kwon *et al.* reviewed the differences

in FMD improvements in overweight women with T2DM (n = 40) after 12 weeks of being in one

of three groups: aerobic exercise group (AEG), resistance exercise group (REG), or a control

group (CG). The AEG experienced significantly greater FMD improvements after the exercise

intervention (2.2% ± 1.9%; p < 0.05) compared to the REG (0.7% ± 3.6%; p = 0.535) and CG (-

0.7% ± 2.8%; p = 0.365). This has been reproduced in other studies as well as supporting the

notion that aerobic training may produce more beneficial effects on endothelial health than

resistance training (Black *et al.* 2008; Desouza *et al.* 2000).

Physical activity is greatly involved in the reduction of risk for many diseases. The

impact of frequency, intensity, duration, and exercise type all remain influential modalities of

disease risk. Although each type of exercise (i.e. anaerobic or aerobic) contains independent

benefits, the most beneficial program contains a combination of the two (Ho *et al.* 2012). The

benefits of physical activity include reduced inflammation, and improved body composition and

oxygen utilization. However, the mechanism of exercise type is a relatively understudied topic in

relation to FMD responses. Overall, physical activity and exercise remain excellent avenues through which to improve vascular function and FMD.

Age and Flow-Mediated Dilation

Vascular function generally decreases with age due to many different biological processes. Decreased NO bioavailability is thought to be a result of reduced production of tetrahydrobiopterin (BH4) synthesis, which is a cofactor for NO production (Pierce and LaRocca 2008). As individuals age, they are exposed to detrimental vascular markers for a longer period of time. In addition to postprandial metabolic markers, items such as inflammatory markers, ROS, and angiogenic compounds have had more time to inflict damage upon the endothelium. Smooth cell function is also thought to play a role in the decrement of aging vascular function (Al-Shaer *et al.* 2006). Pro-constrictors such as endothelin-1 become upregulated with aging, thus causing unnecessary arterial constriction (Thijssen *et al.* 2006). These impairments stand in clear contrast with the vascular function of younger individuals. Younger individuals rarely experience impaired nitric oxide production and dilatory responses. Age-related decline in FMD generally does not begin until age 40 years in men and 50 years in women (Celermajer *et al.* 1994). This age-related decrement in FMD is thought to partially be a derivative of arterial stiffening, enlargement of arterial diameter, and the thickening of the intima-media in older individuals (Nagai *et al.* 2013). Additionally, postmenopausal women experience a sharp decrease in FMD due to the reduction of estrogen production (Moreau *et al.* 2012). Nonetheless, FMD is a strong independent predictor of CVD within older individuals (Yeboah *et al.* 2008). In a recent meta-analysis, Montero *et al.* 2014 reviewed 530 long-term athletes (452 endurance-trained, 49 strength-trained, and 29 endurance- and strength-trained) to cross-sectionally establish the different FMD responses in young athletes compared to older athletes. Interestingly, the younger athletic individuals exhibited an FMD similar to their control (p = 0.22), while older athletic individuals had a significantly greater FMD response than the control (p = 0.0005). This finding

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suggests that the benefits of exercise with regard to FMD may be age-dependent. However, this finding could also be due to an already adequate FMD response in the younger population. Aging individuals also generally experience a decreased ability to conduct physical activity along with an increased risk of skeletal muscle injury, cardiovascular events, and an increased risk of falling (Bird *et al.* 2013). Overall, it is important to understand the relationship between aging, physical activity, and FMD. Moreover, a balance between exercise, diet, and vascular function improvement must be identified to explore new avenues of CVD prevention.

Purpose and Hypotheses

Despite a large amount of literature investigating FMD, there is limited data focusing on aging individuals. Age-related decline in FMD could be explained by several mechanisms, including increased systemic inflammation, oxidative stress, and impaired metabolic clearance (e.g. Glucose, TG). However, these outcomes can also be modified by lifestyle factors, such as diet and physical activity. Additionally, many previous studies analyzed FMD in a fasted context. However, given that most individuals in Western society spend the majority of the day in a continuous postprandial state, it is arguably more pragmatic to study vascular function after the consumption of a meal. No previous studies have investigated the postprandial FMD response to a high-fat meal in older adults. In addition, the influence of regular physical activity in modifying the relationship between aging and postprandial vascular function has not been studied.

Therefore, the purpose of this study is to determine the impact of age and physical activity level on postprandial metabolism and vascular function. This study will assess fasting and postprandial metabolic (Glucose, TG) and vascular (FMD) responses in groups that differ by age and physical activity level. Specifically, this will involve four groups of individuals: younger active (YA), younger inactive (YI), older active (OA), and older inactive (OI) adults. A secondary purpose is to elucidate potential mechanisms explaining postprandial differences between groups. The two primary hypotheses are that 1) There will be differences in postprandial TG and FMD

responses based on age and physical activity level, with younger and more active individuals exhibited better metabolic and vascular function outcomes 2) Group differences in postprandial lipemia and vascular function will be associated with differences in body composition.

CHAPTER III

METHODOLOGY

Participants

Participants were recruited based on age (younger: 18-35 years; older: 60+ years) and physical activity level (i.e. active and inactive). Participants were classified as active if they met physical activity guidelines (Piercy *et al*. 2018), which was defined as ≥150 minutes per week of moderate-intensity physical activity or ≥75 minutes per week of vigorous-intensity physical activity, or some equivalent combination. Individuals were classified as inactive if they did not meet physical activity guidelines and engaged in <30 minutes of planned exercise each week. Participants were also excluded from the study if their physical activity habits had changed significantly within the past 5 years. A medical history questionnaire was also administered to ensure individuals did not have any ongoing chronic disease, were not currently taking lipid-lowering or hypertensive medication, and were not consuming tobacco products. This study was approved by and conducted in accordance with the guidelines and regulations of the Oklahoma State University's Institutional Review Board.

Initial Assessment

Individuals recruited for this study completed an initial assessment to record anthropometrics, complete questionnaires, and receive experimental instruction. Height was collected via stadiometer (Seca 213 portable stadiometer; Seca GmbH; Hamburg, Germany).

Weight and body composition were assessed using a combination of digital scale and bioelectrical impedance analyzer (Seca mBCA 514; Seca GmbH; Hamburg, Germany). Blood pressure was measured using an automatic blood pressure cuff (Omron 5 Series BP742N; Omron; Kyoto, Japan). We then conducted an extensive interview with each participant to determine their regular physical activity level. Afterward, each participant wore a wrist-based accelerometer (wGT3X-BT; Actigraph LLC; Pensacola, FL) to confirm appropriate physical activity group placement. Activity trackers were placed on the non-dominant wrist and recorded activity data for 5 days prior to the main assessment.

Meal Tolerance Test

Based on their scheduling availability, participants selected a time to begin the meal tolerance test between 0500-0900 hours. Participants were instructed to refrain from exercise for 48 hours prior to their test and caffeine consumption on the day of the experiment. Participants were given a small pre-packaged snack (Peanut butter crackers 230 kcal; Snyder's-Lance, Inc.; Charlotte, NC) to consume prior to initiating a 10-hour fast, in order to ensure that all participants were similarly fasted prior to the HFM.

Upon arriving in the laboratory, an indwelling 24-gauge safelet catheter (Exel International; Redondo Beach, CA) was inserted into a forearm vein and a slow infusion of 0.9% NaCl was initiated. Tegaderm film (3M Healthcare; Neuss, Germany) was placed over the insertion region to prevent dislodgement of the catheter. A baseline blood sample was collected, after which the HFM was given to the participant with a completion time limit of 20 minutes. We chose to use chocolate pie as our HFM to reflect a realistic food item (Marie Callender's Chocolate Satin Pie; Conagra Brands; Omaha, NE, USA; 12 kcal/kg body weight; 63% fat, 34% carbohydrate). We have utilized this HFM to elicit a PPL response in a previous similar study (Emerson *et al*. 2018). Water was available *ad libitum* with the meal and during the post-meal period. All participants consumed the entire HFM without difficulty. Serial blood draws were

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taken each hour to measure postprandial TG, glucose, total cholesterol (Total-C), high-density

lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) via Cholestech

LDX analyzer (Alere Cholestech; San Diego, CA). For each blood draw, a 3 mL syringe (BD;

Franklin Lakes, NJ, USA) was used to remove saline from the line, after which the actual blood

sample was drawn into a 5 mL syringe (BD; Franklin Lakes, NJ, USA). The 5 mL syringe was

then emptied into a 6 mL vacutainer test tube (BD; Franklin Lakes, NJ, USA) coated with EDTA.

For lipid analysis, approximately 40 µL of whole blood was drawn into a capillary tube and

placed in a Cholestech cassette and then inserted into the Cholestech LDX analyzer. In the

baseline/fasting blood sample only, hemoglobin A1C (HbA1c) was measured in whole blood via

DCA Vantage analyzer (DCA Vantage Analyzer; Boston, MA, USA).

Vascular Function

Vascular function was assessed using FMD. This technique incorporates temporary distal

occlusion of a limb to elicit a dilatory response. This experiment used the brachial artery for the

dilatory examination and the wrist as the distal occlusion site. Participants were instructed to lie

in a supine position for 15 minutes in a darkened area to normalize to the room conditions.

Afterward, the individual extended their arm into a cradle to gain a longitudinal view of the

brachial artery with a Doppler ultrasound and a 12 MHz probe (Mindray Z5 Ultrasound System;

Mindray North America). Video feedback was transposed to a local computer via HDMI video

converter (AV.io HD; Epiphan). A blood pressure cuff was placed distally on the wrist, and initial

baseline video of the brachial artery was recorded for 2 minutes. After the baseline recording, an

occlusion pressure of 50 mmHg greater than the participant's systolic blood pressure was applied

for 4 minutes. The pressure was then released, and video of the brachial artery hyperemic

response was recorded for an additional 4 minutes. A total of 10 minutes of video recording was

collected for each FMD analysis (i.e. 2 minutes baselines, 4 minutes occlusion, 4 minutes post-

occlusion). We used continuous wall-tracking, edge detection software to analyze the vessel

diameter continuously during recorded videos (CardioSuite, Quipu; Pisa, IT). The FMD was reported as the percentage change (%) in brachial artery diameter from baseline to the maximum dilation after cuff pressure is released. FMD was conducted at baseline (fasting), 2 hour, and 4 hour post-HFM (Fig. 1).

Statistical Analyses

Using mean differences and standard deviations from a previous cross-sectional study from our laboratory (Emerson *et al*. 2018), we estimated that at least 6 individuals per group would be necessary to detect differences in TG total area under the curve (tAUC). In this prior study, we detected significant group differences with n = 7-8 per group. With regard to vascular function, we selected a minimum necessary difference (between groups or in response to the meal) of 4% for FMD with an estimated standard deviation of 2% based on previous studies (Pierce *et al*. 2011; Marchesi *et al*. 2000). This calculation estimated a minimum necessary sample size of n = 7 per group. To be conservative, we aimed to recruit n = 7-10 participants per group. For the metabolic outcomes, we calculated tAUC, incremental area under the curve (iAUC) responses, and the metabolic load index (MLI). The tAUC represents the total response for the entire 6-hour postprandial period, while the iAUC response represents the total relative change from baseline/fasting during the postprandial period. The MLI represents a summative glycemic and lipemic value and was calculated by adding together the TG and glucose together. We also determined the peak concentration for each metabolic outcome, as well as the time in the postprandial period at which the peak occurred. A one-way analysis of variance (ANOVA) to determine group differences in participant characteristics, fasting metabolic values peak, time to peak, tAUC, and iAUC. For metabolic outcomes and FMD, a two-way (group × time) repeated-measures ANOVA was used to test for time-course interactions between groups in the postprandial period. Tukey's post hoc adjustment was utilized if main group effects were found. The ANOVA analyses were conducted in GraphPad Prism (Version 7; GraphPad Software, Inc.;

La Jolla, CA). We also conducted a partial correlation analysis of body composition and glucose control (HbA1c) with metabolic and vascular outcomes while controlling for age and physical activity (steps/day) in SPSS (IBM Corp, SPSS Statistics for Windows, Version 24.0; Armonk, NY). Values were considered statistically significant at $p < 0.05$ for all of the analyses conducted in this study.

CHAPTER IV

RESULTS

Participant characteristics

Participant characteristics are shown in Table 1. Significant group effects were observed for age, BMI, body fat percentage, visceral adipose tissue, and HbA1c. YA and YI were significantly younger than OA and OI (YA vs OA: $p < 0.0001$; YA vs OI: $p < 0.0001$; YI vs OA: $p < 0.0001$; YI vs OA: $p < 0.0001$). There was no significant difference in age between YA and YI ($p = 0.99$) or OA and OI ($p = 0.99$). YA had significantly lower BMI compared to OI ($p = 0.007$). There were no other significant differences between groups with regard to BMI (YA vs YI: $p = 0.73$; YA vs OA: $p = 0.10$; YI vs OA: $p = 0.55$; YI vs OI: $p = 0.09$; OA vs OI: $p = 0.65$). Body fat percentage was significantly different between groups, wherein YA had a lower body fat percentage compared to OA (Mean diff: -12.70; 95% CI: -19.75 to -5.64; $p = 0.0002$) and OI (Mean diff: -17.78; 95% CI: -25.09 to -10.47; $p < 0.0001$); YI had a lower body fat percentage than OA (Mean diff: -8.91; 95% CI: -16.42 to -1.40; $p = 0.02$) and OI (Mean diff: -17.78; 95% CI: -25.09 to -10.47; $p = 0.0002$). There were no significant differences in body fat percentage between YA vs YI ($p = 0.50$) or OA vs OI ($p = 0.27$). These findings were similar when comparing the differences in visceral adipose tissue between groups. YA was lower than OA (Mean diff: -0.96; 95% CI: -1.91 to -0.01; $p = 0.04$) and OI (Mean diff: -1.68; 95% CI: -0.69 to 3.53; $p = 0.0004$); YI was lower than OI (Mean diff: -1.55; 95% CI: -2.56 to -0.54; $p = 0.0013$).

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There were no significant differences in visceral adipose tissue between YA vs YI (p = 0.98), YI vs OA (p = 0.11), or OA vs OI (p = 0.23). Skeletal muscle mass did not differ between groups (p = 0.85). There was a significant group effect for HbA1c, where YI was lower than OA (Mean diff: -0.54; 95% CI: -0.97 to -0.12; p = 0.009) and OI (Mean diff: -0.44; 95% CI: -0.88 to 0.0; p = 0.048). There were no other significant differences between groups in HbA1c (YA vs YI: p = 0.51; YA vs OA: p = 0.13; YA vs OI: p = 0.44; OA vs OI: p = 0.91). The test meal energy (kcal) did not significantly differ between groups (p = 0.11).

Physical activity level, measured in steps per day, was significantly different between groups, where the active groups had significantly higher steps per day than the inactive groups. The YA was more active than YI (p = 0.03); YA was more active than OI (p = 0.03); and OA was more active than OI (p = 0.0495). There were no other significant group differences in steps/day (YI vs OA: p = 0.06; YA vs OA: p = 0.87; YI vs OI: p = 0.87).

Fasting metabolic outcomes

Fasting metabolic outcomes are shown in Table 2. There were no significant differences in fasting TG between YA vs YI (p = 0.09), YA vs OA (p = 0.97), YA vs OI (p = 0.21), YI vs OI (p = 0.99), or OA vs OI (p = 0.11). However, YI had significantly higher fasting TG than OA (p = 0.04). Two participants were found to have fasting TG >150 mg/dL fasting (1 YI and 1 OI). There were no significant differences between groups for fasting glucose (p = 0.32). There was a significant group effect with fasting MLI (p = 0.02), but no significant differences between groups were found with post hoc comparisons. A significant group effect was observed for fasting Total-C (p = 0.03). Fasting Total-C was significantly lower in YA compared to OI (p = 0.02); however, no significant differences were found in fasting Total-C when comparing YA vs YI (p = 0.70), YA vs OA (p = 0.44), YI vs OA (p = 0.98), YI vs OI (p = 0.17), or OA vs OI (p = 0.33). There were 11 individuals that had fasting Total-C >200 mg/dL (1 YA, 2 YI, 3 OA, and 5 OI). A significant group effect was also observed for LDL-C (p = 0.01). YA had a significantly

lower fasting LDL-C compared to OI (p = 0.01). Fasting LDL-C was not significantly different in YA vs YI (p = 0.81), YA vs OA (p = 0.61), YI vs OI (p = 0.07), YI vs OA (p = 0.99), or OA vs OI (p = 0.14). There were no differences between groups in fasting HDL-C (p = 0.99). Despite this, there were 5 individuals (3 YA, 1 YI, and 1 OA) that presented with HDL-C <40 mg/dL.

Postprandial metabolic responses

Postprandial metabolic outcomes are presented in Table 3. There was a significant group × time interaction for the postprandial TG response (p < 0.0001). YA had a significantly lower TG peak compared to YI (p = 0.02) and OI (p = 0.02). Similarly, OA had a lower TG peak compared to YI (p = 0.03) and OI (p = 0.02). There were no significant differences in peak TG for YA vs OA (p = 0.99) or YI vs OI (p = 0.99). The TG time to peak was significantly different between groups (p = 0.02), but there were no significant post hoc comparisons between groups. TG tAUC was significantly different in YA vs YI (p = 0.007), YA vs OI (p = 0.004), YA vs OA (p = 0.01), and OA vs OI (p = 0.008). However, there were no significant differences in TG tAUC comparing YA vs OA (p = 0.99) or YI vs OI (p = 0.99). TG iAUC was significantly lower in YA vs YI (p = 0.02), YA vs OI (p = 0.005), and OA vs OI (p = 0.03). TG iAUC was not statistically different comparing YA vs OA (p = 0.92), YI vs OA (p = 0.11), or YI vs OI (p = 0.89).

The postprandial glucose response did not exhibit a significant group × time interaction (p = 0.59) or time effect (p = 0.18) during the postprandial period. However, there was a significant group effect (p = 0.007). Specifically, YA was lower than OA (p = 0.04) and OI (p = 0.01) across the entire postprandial period. There were no significant differences in peak glucose responses (p = 0.05), time to peak (p = 0.71), or glucose iAUC (p = 0.09). YA had a significant lower glucose tAUC compared to OA (p = 0.04) and OI (p = 0.01). There were no other significant differences in glucose tAUC (YA vs YI: p = 0.69; YI vs OA: p = 0.37; YI vs OI: p = 0.13; OA vs OI: p = 0.91).

MLI exhibited a significant group × time interaction ($p < 0.0001$). YA had a significantly lower peak compared to YI ($p = 0.01$) and OI ($p = 0.005$). OA had a significantly lower peak response compared to OI ($p = 0.02$) and YI ($p = 0.049$). MLI time to peak did not differ between groups. There were no significant differences in peak MLI responses comparing YA vs OA ($p = 0.97$) or YI vs OI ($p = 0.95$). The MLI tAUC was significantly lower in YA vs YI ($p = 0.005$), YA vs OI ($p = 0.001$), and OA vs OI ($p = 0.008$). YI had a significantly higher MLI tAUC compared to OA ($p = 0.03$). The MLI tAUC responses did not differ comparing YA vs OA ($p = 0.90$) or YI vs OI ($p = 0.90$). The MLI iAUC was lower in YA vs YI ($p = 0.01$), YA vs OI ($p = 0.001$), and OA vs OI ($p = 0.02$). There were no significant differences in MLI iAUC for YA vs OA ($p = 0.64$), YI vs OI ($p = 0.74$), or YI vs OA ($p = 0.16$).

There was no significant group × time interaction for postprandial Total-C ($p = 0.07$). However, there were significant time ($p = 0.04$) and group ($p = 0.01$) effects. Peak Total-C was significantly lower in YA compared to OI ($p = 0.007$). There were no other significant differences in Total-C time to peak. There were no significant differences in peak Total-C comparing YA vs YI ($p = 0.51$), YA vs OA ($p = 0.57$), YI vs OA ($p = 0.99$), YI vs OI ($p = 0.16$), or OA vs OI ($p = 0.13$). Total-C tAUC was significantly lower in YA vs OI ($p = 0.006$). No significant comparisons were found between YA vs YI ($p = 0.49$), YA vs OA ($p = 0.53$), YI vs OA ($p = 0.99$), YI vs OI ($p = 0.16$), or OA vs OI ($p = 0.14$). The Total-C iAUC was not significantly different between groups. Total-C time to peak was lower in the OA compared to OI ($p = 0.005$).

There was no group × time interaction detected in postprandial LDL-C ($p = 0.74$), although there were significant time ($p = 0.002$) and group ($p = 0.009$) effects. YA had a significantly lower peak postprandial LDL-C compared to OI ($p = 0.008$). However, there were no significant group differences in LDL-C time to peak. Moreover, there were no significant differences between YA vs YI ($p = 0.57$), YA vs OA ($p = 0.21$), YI vs OA ($p = 0.90$), YI vs OI ($p = 0.15$), or OA vs OI ($p = 0.43$). LDL-C tAUC was lower in YA vs OI ($p = 0.006$). No significant

comparisons were found between YA vs YI (p = 0.27), YA vs OA (p = 0.27), YI vs OA (p = 0.84), YI vs OI (p = 0.07), or OA vs OI (p = 0.32). There were no significant group differences in LDL-C iAUC.

Regarding HDL-C, two-way repeated measures ANOVA revealed no significant group $\times$ interaction (p = 0.43), time effect (p = 0.21), or group effect (p = 0.98). There were no significant differences in HDL-C peak, time to peak, tAUC, or iAUC.

Vascular function

Postprandial vascular function is shown in Figure 4. A one-way ANOVA detected a significant difference between groups in BL (Baseline) FMD (p = 0.002). YA exhibited significantly greater FMD compared to YI (Mean Diff: 2.38; 95% CI: 0.37 to 4.40; p = 0.02) and OI (Mean Diff: 3.09; 95% CI: 1.00 to 5.17; p = 0.002). There were no significant differences in BL FMD comparing YA vs OA (Mean Diff: 1.54; 95% CI: -0.47 to 3.55; p = 0.18), YI vs OA (Mean Diff: -0.84; 95% CI: -2.92 to 1.23; p = 0.67), YI vs OI (Mean Diff: 0.70; 95% CI: -1.44 to 2.85; p = 0.81), or OA vs OI (Mean Diff: 1.55; 95% CI: -0.60 to 3.69; p = 0.22).

In the postprandial period, there was no significant group $\times$ time interaction (p = 0.08) or group effect (p = 0.06), although there was a significant time effect (p = 0.008). When analyzing all groups together, there was a significant decrease in FMD from BL to 4th hour (Mean Diff: 1.19; 95% CI: 0.27 to 2.11; p = 0.008). YA had a significantly decreased FMD from BL to 4th hour post-meal (Mean Diff: 2.54; 95% CI: 0.82 to 4.25; p = 0.02). However, there were no significant differences in YA FMD comparing BL vs 2nd hour FMD (Mean Diff: 1.69; 95% CI: -0.08 to 3.46; p = 0.06) or 2nd hour vs 4th hour (Mean Diff: 0.84; 95% CI: -0.93 to 2.61; p = 0.49). YI had no significant differences in FMD between any two-time points. OA FMD significantly decreased at 2nd hour compared to BL (Mean Diff: 1.89; 95% CI: 0.08 to 3.7; p = 0.04). There were no significant differences in FMD with OA at BL vs 4th hour (Mean Diff: 1.76; 95% CI: -

0.13 to 3.65; p = 0.07) or 2nd hour vs 4th hour (Mean Diff: -0.13; 95% CI: -2.03 to 1.76; p = 0.98).

The OI had no significant differences in FMD between any two-time points.

Associations with body composition and HbA1c

Associations between primary outcomes and body composition and HbA1C are found in Table 4. After controlling for physical activity (steps/day) and age, there was a significant negative correlation between body fat % (BF) and BL FMD (r = -0.50; p = 0.007). Visceral adipose tissue (VAT) was positively associated with peak TG (r = 0.43; p = 0.02), TG tAUC (r = 0.38; p = 0.04), TG iAUC (r = 0.50; p = 0.005), MLI peak (r = 0.44; p = 0.02), MLI tAUC (r = 0.39; p = 0.03), and MLI iAUC (r = 0.50; p = 0.006). Skeletal muscle mass (SMM) was positively associated with MLI iAUC (r = 0.43; p = 0.02). HbA1C was not correlated with any metabolic or vascular outcomes.

CHAPTER V

DISCUSSION

Main Findings

The primary aim of this study was to determine the independent effects of age and physical activity on postprandial metabolic markers and vascular function. The data partially supported our hypothesis that younger and more active individuals would experience an attenuated TG response compared to older less active individuals. The postprandial TG responses for the active groups (YA vs OA) were not different, nor were those of the inactive groups (YI vs OI). This finding suggests that physical activity may be a more influential modifier of PPL responses than age. With regard to vascular function, there were distinct differences in baseline vascular function, where YA individuals exhibited higher FMD compared to the inactive groups. We observed a significant reduction in FMD across groups 4 hours after the HFM; however, we did not find group differences in postprandial vascular function. Lastly, we found that PPL was correlated with some but not all components of body composition.

Postprandial Metabolic Markers

Previous literature has demonstrated the negative implications of elevated postprandial TG in relation to CVD risk (Nordestgaard and Varbo 2014; McBride 2007; Hokanson 1998). Specifically, postprandial TG have been shown to be more predictive of CVD risk than traditional fasting values (Bansal *et al*. 2008). After the consumption of a meal, the body enters an acute period where metabolic markers such as TG and glucose often remain elevated. Excessive and

prolonged concentrations of these markers can induce physiological damage. Namely, the period

of elevated TG following a meal known as PPL is associated with endothelial dysfunction,

inflammation, and oxidative damage. Individuals typically consume meals frequently throughout

the day, making PPL a physiologically relevant measurement for CVD risk.

Several studies have separately examined the impact of age and physical activity on

postprandial TG (Altena *et al*. 2004; Cassader *et al*. 1996). Acute exercise prior to a HFM (i.e.

24-48 hours prior) can blunt postprandial TG (Zhang and Nunez 2006). Exercise can acutely

increase lipoprotein lipase (LPL) activity, which removes TG from very-low-density lipoprotein

(VLDL) and chylomicron (CM) particles (Al-Shayji *et al*. 2011). After exercise, LPL activity

remains elevated in skeletal muscle, allowing for improved lipid clearance after a meal (Zderic

and Hamilton 2006; Zhang *et al*. 2002). However, LPL activity typically peaks between 4 and 18

hours following acute exercise (Peddie *et al*. 2012). In the present study, participants avoided

exercise for 48 hours prior to the HFM. Therefore, it is unlikely that enhanced LPL activity is the

explanation for group differences in PPL in our study. Habitual physical activity can also exert a

beneficial impact on PPL. In addition to increased LPL activity, habitual exercise has the

potential to induce body composition changes that are beneficial for the reduction in PPL. More

specifically, exercise can reduce body fat and increase levels of skeletal muscle mass, which can

reduce the magnitude and duration of PPL (Geer *et al*. 2009). Lastly, overtime physical activity

can help create a caloric deficit, which also can reduce PPL (Malkova *et al*. 2006).

There is clear evidence supporting the notion that older individuals exhibit an

exaggerated PPL compared to younger individuals (Cohn *et al*. 1988). There is some speculation

that age-related decline in gastric emptying results in an exaggerated PPL response. However,

one of the most well-studied contributors to increased PPL is the reduction in CM clearance

capacity. This decrease in CM clearance is thought to be a product of the age-related reduction of

LPL enzyme activity and negative hepatic morphological changes. As individuals age, physical

exercise can become more challenging, increasing the likelihood of negative body composition changes such as a loss of muscle mass, increase in body fat percentage, increase in visceral adiposity, and an increase in liver steatosis. Together, a reduction in skeletal muscle mass and increased fat deposits have been associated with elevated PPL (Herd *et al*. 2001). In the present study, there were no differences in skeletal muscle mass between groups, although there were large differences in postprandial TG responses.

Elevated visceral fat is associated with insulin resistance and dyslipidemia, causing exaggerated postprandial responses and, ultimately, an increased risk of CVD (Jensen *et al*. 2008; Banerji *et al*. 1999). In a study conducted by Couillard and colleagues (1998), the relationship between PPL and body composition was assessed in obese men after the consumption of a HFM (n = 43). Interestingly, they found a stronger positive relationship between VAT and tAUC TG (r = 0.45; p < 0.01) compared to BF (r = 0.40; p < 0.01). We assessed these associations in the present study by examining the relationship between body composition and postprandial responses. Our data partially supports this relationship, as we found a significant positive correlation between VAT and numerous PPL indices across our total sample. On the other hand, BF and VAT were greatly different between groups, where the older individuals had a significantly higher visceral fat content compared to the younger groups. Despite these stark differences, both the younger and older active groups sustained an attenuated PPL response. Also, BF was not associated with any PPL indices. Taken together, our findings suggest that body composition likely explains some, but not all, of the variation in PPL due to age and physical activity status.

Postprandial glucose was not different between groups, with the exception of a few significant post hoc comparisons. The lack of group differences in postprandial glycemia was not surprising, given that the composition of the meal (63% fat and 34% carbohydrate) was designed to elicit a robust TG response rather than glucose. However, we were more interested in whether

differences in PPL could be explained by variations in HbA1c, an indicator of long-term glucose control. Older and less active individuals tend to have worse insulin sensitivity than younger and more active counterparts (Amati *et al*. 2009). Regular physical activity has been shown to improve insulin sensitivity. Therefore, differences in insulin sensitivity due to aging and/or physical activity have been suggested as a determinant of PPL (Guerci *et al*. 2000). However, despite substantial differences in PPL, groups did not differ in HbA1c. Additionally, there was no correlation between HbA1C and any of the postprandial metabolic outcomes after controlling for age and physical activity level, suggesting that glucose control is not the sole driver of group differences in PPL. However, this data is limited in that it utilized HbA1c as a measure of glucose control rather than measuring insulin sensitivity directly.

MLI provides a summation of both glucose and TG responses and is intended to represent the total metabolic challenge induced by a mixed meal (Emerson *et al*. 2016). Both elevated levels of glucose and TG are associated with increased CVD risk making this metric relevant to the prediction of CVD (Ceriello *et al*. 2006; Lefebvre and Scheen 1998). MLI was lower in the more active individuals compared to the inactive, regardless of age. Despite the relatively little change in postprandial glucose, group differences in MLI were anticipated given the large differences in TG responses. This further demonstrates the differences in postprandial metabolism between groups and supports our expectation that there would be a reduced metabolic clearance capacity in older and less active individuals.

Many studies have found Total-C and LDL-C to increase with age and inactivity (Félix-Redondo *et al*. 2013; Mora *et al*. 2006). Supporting this notion, Total-C was highest in OI. However, due to the latency of this biomarker to change, large changes in postprandial Total-C are not commonly seen. This concept was supported by our results, where no time effect throughout the postprandial period was observed. Similarly, there were no significant postprandial changes in LDL-C. Although there were several group differences, postprandial

LDL-C remained relatively stable in the postprandial period across groups. Despite the lack of acute postprandial differences between groups, Total-C is largely dependent upon age and physical inactivity. Supporting this notion, Total-C was significantly lower in the younger and more active groups in this study. HDL-C exerts highly anti-atherogenic properties and has been reported to increase in individuals exhibiting beneficial lifestyle patterns, namely high physical activity (Barter *et al*. 2011; Skoumas *et al*. 2003). Most notably, HDL-C exerts its beneficial effects by playing an integral role in reverse cholesterol transport. HDL-C promotes the efflux of cholesterol from peripheral cells and then facilitates transportation to the liver for re-use or excretion. Given the role of peripheral cholesterol in plaque development, HDL-C plays an important role in CVD prevention (Filippatos *et al*. 2013). However, we found no differences in HDL-C between groups in this study, nor were there any changes or group differences in the postprandial period.

Vascular Function

Vascular function is highly regarded as a predictor for CVD (Verma *et al*. 2003). More specifically, vasodilation reflects the functional ability of the endothelium to supply peripheral tissues with nutrients and oxygen. As we age, the body undergoes a variety of changes that reduce the functional capacity of the endothelium to dilate and constrict. This includes changes such as an increase in oxidative stress, reduced nitric oxide bioavailability, and an increase in vasoconstrictive compounds, which impair adequate vessel dilation (Gerhard *et al*. 1996). Over time, these detrimental changes can elicit negative structural changes within the vasculature including vessel stiffening, promotion of plaque development, and a reduced endothelial repairing capacity (Cheng *et al*. 2017). According to Celermajer *et al*. (1994), age-related vascular decline begins affecting male individuals > 40 years of age at a rate of 0.21% reduction in FMD per year. Interestingly, this age-related decline was preserved in women and did not begin declining until 50 years of age at a rate of 0.49%/year. Exercise has an extremely pronounced ability to prevent

this age-related decline in endothelial function (Black et al. 2008). It has been proposed that exercise can improve vasodilation via the upregulation of NO-synthase, vascular endothelial growth factors (VEGF), and proangiogenic factors (Hoier *et al*. 2014). Lastly, exercise promotes physiological protective mechanisms against free radical damage by increasing the function of antioxidative cellular mechanisms (Radak *et al*. 2008).

Vascular function has also been observed to decrease in response to a HFM (Ceriello *et al*. 2002; Kawano *et al*. 1999). The PPL that acutely follows the consumption of a HFM evokes an enormous amount of free radical production. PPL initiates an influx of free fatty acids (FFA), ultimately producing large amounts of acetyl-coA. This rapid influx of acetyl-coA creates an overabundance of electron donors (e.g. $NADH/FADH_2$) promoting free radical production. NO acts as a prevalent electron acceptor leading to a reduction in bioavailability during the postprandial period (Wallace *et al*. 2010). NO plays a crucial role in vasodilation, thus during times of reduced bioavailability have a negative impact on vascular function. In the present study, serum TG levels were significantly correlated with FMD responses, suggesting a strong connection between PPL and FMD. This relationship between PPL and FMD was partially supported by our findings, as all groups experienced a decrease in vascular function after the HFM. Most notably, FMD reduction was more pronounced in the active groups. However, group differences in FMD during the postprandial period were not as large or distinct as those for PPL. The greater decreases in vascular function observed in the active individuals could be explained by a higher degree of function before the consumption of the HFM. Similarly, the lack of change in older and less active individuals might be due to pre-existing, lower vascular function prior to the HFM. This low baseline FMD may have also influenced the ability to detect group differences in the postprandial period.

Body composition also plays an important role in vascular function. This has been reproduced in numerous studies examining the influence of obesity on vascular function (Ne *et*

al. 2017; Parikh *et al*. 2009). As BF increases, concomitant increases can be found in inflammatory cytokines, insulin resistance, and reduction in adiponectin. Together, these changes in physiological function can exert negative effects on endothelial function. The primary mechanism is thought to be a product of insulin resistance that is often prevalent in individuals with greater degrees of adiposity. Insulin generally has a positive effect on vasodilation, improving blood flow in a healthy individual. However, in disrupted metabolic states such as insulin resistance, the endothelial insulin NO mechanism becomes ineffective ultimately leading to a lack of vasodilation in postprandial periods (Steinberg *et al*. 1996). Additionally, insulin resistance leads to increased serum glucose levels which can promote oxidative stress, further impairing vasodilation. Interestingly, there was a significant association between BF and FMD; however, unlike PPL there was no association between VAT and FMD. Body composition was able to explain some, but not all of the postprandial metabolic and vascular responses.

Strengths and Limitations

The present study contains several strengths and limitations that should be considered when interpreting findings. Notably, a strength of this study was the frequent measurement of metabolic outcomes. The TG response can essentially be captured with a single blood draw 4-hours post-HFM (Sciarrillo *et al*. 2019); however, we were able to more thoroughly capture this response in the present study via serial blood draws for 6 hours. Next, our prohibition of exercise for 48 hours prior to the HFM removed the potential confounding effects of recent exercise, thereby better allowing us to test the effects of regular physical activity. Lastly, some postprandial studies have used mixed meals that are unreflective of the caloric density common in a western diet meal. On the other hand, the present study used a realistic western meal with energy and fat content reflective of a true-to-life scenario to produce an adequate but reasonable PPL response.

In this study, individuals were excluded if they were taking blood pressure- and lipid-lowering medications, since our primary outcomes were postprandial lipids and vascular function. However, this exclusion criterion likely limits the applicability of our data, especially within the older population. Regarding vascular function, we only measured FMD at BL, 2 hours, and 4 hours post-HFM, which may have not fully captured postprandial vascular changes. Finally, our groups did not have identical distributions of men and women, possibly introducing error into group comparisons and precluding the opportunity to analyze possible sex differences within our sample.

CONCLUSION

This study aimed to determine differences in postprandial metabolic and vascular outcomes in groups that differed by age and physical activity level, as well as explore factors that may explain group differences. Our data speaks to the importance of physical activity in mediating TG responses to a HFM, which directly corresponds to the risk of CVD development. Moreover, the OA adults in our study exhibited a nearly identical PPL response to YA adults, suggesting that physical activity level is more important in modifying PPL than age *per se*. With regard to vascular function, we observed HFM-induced decrements in FMD that were primarily observed in active individuals. We found that younger active individuals had the highest degree of vascular function in the fasting state compared to inactive individuals. However, further elucidation of mechanisms involved in the reduced baseline function seen in the older and less active population is greatly needed for understanding the preservation of vascular function with aging. Additionally, the original design of the study controlled for sex differences; however, few studies have examined the differential impact of sex across the lifespan while also considering physical activity. Our data also suggest that body composition, specifically visceral fat and SMM, are associated with the PPL response regardless of age and physical activity level, while BF was the only predictor of vascular function. However, body composition did not fully explain group

differences in our study. Therefore, future studies should further investigate other physiological

determinants of PPL and vascular function, such as liver fat content, that are modified by age and

physical activity level.

Participant Characteristics					
	Younger Active n=9	Younger Inactive n=8	Older Active n=8	Older Inactive n=7	P-value
Sex	4F/5M	3F/5M	4F/4M	5F/2M	
Age (years)	22.1 ± 1.4^a	22.6 ± 3.7^a	68.4 ± 7.7^b	67.7 ± 7.2^b	<0.0001
BMI (kg/m^2)	23.8 ± 2.7^a	25.7 ± 3.7^{ab}	28.2 ± 3.4^{ab}	30.4 ± 4.9^b	0.008
Body fat (% total mass)	15.1 ± 4.6^a	18.9 ± 2.8^a	27.8 ± 7.1^b	32.87 ± 5.7^b	<0.0001
Visceral Adipose Tissue (kg)	0.48 ± 0.37^a	0.61 ± 0.55^a	1.44 ± 0.59^b	2.16 ± 1.20^b	0.0002
Skeletal Muscle Mass (kg)	26.9 ± 6.4	24.9 ± 11.3	24.0 ± 5.3	23.8 ± 8.6	0.85
HbA1c (%)	5.36 ± 0.25^{ab}	5.14 ± 0.36^a	5.69 ± 0.24^b	5.51 ± 0.39^{ab}	0.008
Steps/day	12935 ± 3216^a	8498 ± 2655^{bc}	12232 ± 3373^{ab}	8059 ± 2719^c	0.005
Test meal energy (kcal)	839.8 ± 11.1	904.2 ± 145.3	957.8 ± 133.6	1036.0 ± 235.6	0.11

Table 1. Participant characteristics. Normally distributed data are displayed as Mean ± SD and non-normally distributed data are displayed as Median (Interquartile Range). Within main effects (by row), values with shared superscript letters are not significantly different, determined by *post hoc* pairwise comparison. Rows with no superscript letters present contain no significant differences. See Results section for *post hoc* pairwise comparison p-values. n, number of participants; BMI, body mass index; HbA1c. Hemoglobin A1c.

Metabolic Values Measured in Fasting Participants						
Fasting Values (mg/dL)	Optimal Values (mg/dL)	Younger Active n=9	Younger Inactive n=8	Older Active n=8	Older Inactive n=7	P-value
TG	<150	65.1 ± 16.6^{ab}	96.4 ± 46.3^{a}	59.4 ± 15.6^{b}	91.7 ± 10.5^{ab}	0.02
GLU	<100	85.8 ± 3.8	85.3 ± 6.1	89.1 ± 9.7	90.4 ± 4.1	0.32
MLI	N/A	150.9 ± 17.3	181.6 ± 45.3	148.5 ± 21.1	182.1 ± 12.2	0.02
Total-C	<200	150.1 ± 28.1^{a}	169.1 ± 45.5^{ab}	176.5 ± 39.0^{ab}	208.7 ± 27.8^{b}	0.03
LDL-C	<100	85.1 ± 22.1^{a}	98.1 ± 30.2^{ab}	103.3 ± 36.9^{ab}	137.7 ± 31.0^{b}	0.01
HDL-C	40-60	53.0 ± 21.7	51.6 ± 15.7	51.6 ± 10.7	52.9 ± 132	0.99

Table 2. Fasting Metabolic Values. Normally distributed data are displayed as Mean ± SD, and non-normally distributed data are displayed as Median (Interquartile Range). A one-way ANOVA was conducted to test for differences between groups. Within main effects (by row), values with shared superscript letters are not significantly different, determined by *post hoc* pairwise comparison. Rows with no superscript letters present contain no significant differences. See Results section for *post hoc* pairwise comparison p-values; TG, triglycerides; GLU, glucose; MLI, metabolic load index; Total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Postprandial Metabolic Outcomes					
	Younger Active n=9	Younger Inactive n=8	Older Active n=8	Older Inactive n=7	P-value
Triglycerides					
Peak (mg/dL)	128.6 ± 36.1[a]	228.0 ± 95.9[b]	131.6 ± 61.1[a]	232.6 ± 45.8[b]	0.002
Time to peak (hours)	3.2 ± 1.5	3.1 ± 0.8	4.5 ± 0.9	4.3 ± 0.5	0.02
tAUC (mg/dL x 6 hr)	578.3 ± 120.5[a]	1032.0 ± 424.1[b]	599.9 ± 235.3[a]	1071.0 ± 149.5[b]	0.0004
iAUC (mg/dL x 6 hr)	187.6 ± 151.7[a]	453.8 ± 246.4[bc]	243.7 ± 156.6[ab]	520.9 ± 135.9[c]	0.002
Glucose					
Peak (mg/dL)	96.0 ± 11.0	96.5 ± 7.5	105.4 ± 10.5	110.1 ± 15.8	0.05
Time to peak (hours)	1.9 ± 1.9	2.9 ± 2.3	2.0 ± 1.5	2.4 ± 1.7	0.71
tAUC (mg/dL x 6 hr)	494.1 ± 54.5[a]	520.9 ± 27.2[ab]	561.9 ± 63.6[b]	579.1 ± 45.2[b]	0.008
iAUC (mg/dL x 6 hr)	-20.3 ± 59.9	9.4 ± 40.2	27.2 ± 41.5	36.5 ± 40.5	0.09
Metabolic Load Index					
Peak (mg/dL)	212.8 ± 38.7[a]	316.1 ± 96.1[b]	226.8 ± 63.6[a]	334.6 ± 47.3[b]	0.001
Time to peak (hours)	3.6 ± 1.1	3.3 ± 0.7	3.6 ± 1.1	4.4 ± 0.5	0.11
tAUC (mg/dL x 6 hr)	1073.0 ± 131.3[a]	1553.0 ± 418.8[b]	1162.0 ± 253.0[a]	1650.0 ± 177.8[b]	0.0002
iAUC (mg/dL x 6 hr)	167.1 ± 166.6[a]	463.1 ± 221.1[bc]	270.9 ± 168.1[ab]	557.4 ± 150.4[c]	0.0006
Total Cholesterol					
Peak (mg/dL)	160.7 ± 29.1[a]	186.1 ± 52.5[ab]	184.5 ± 40.1[ab]	228.3 ± 18.9[b]	0.01
Time to peak (hours)	3.2 ± 1.9[a]	4.1 ± 1.6[ab]	2.3 ± 2.0[a]	5.4 ± 0.8[b]	0.007
tAUC (mg/dL x 6 hr)	905.0 ± 176.1[a]	1057.0 ± 292.2[ab]	1050.0 ± 235.2[ab]	1302.0 ± 134.5[b]	0.01
iAUC (mg/dL x 6 hr)	4.3 ± 47.2	42.5 ± 78.1	-9.1 ± 39.8	49.2 ± 38.7	0.11
LDL-Cholesterol					
Peak (mg/dL)	88.2 ± 23.6[a]	108.5 ± 37.9[ab]	119.5 ± 39.9[ab]	144.9 ± 23.6[b]	0.01
Time to peak (hours)	1.0 (0.0-4.0)	1.0 (0.3-5.8)	0.5 (0.0-2.5)	2.0 (0.0-4.0)	0.57
tAUC (mg/dL x 6 hr)	463.8 ± 128.4[a]	547.2 ± 195.2[ab]	619.9 ± 206.3[ab]	777.8 ± 159.6[b]	0.01
iAUC (mg/dL x 6 hr)	-28.5 (-231.0-33.0)	-52.0 (-63.6-11.0)	16.0 (-87.5-351.5)	-37.6 (-74.5- -11.5)	0.78
HDL-Cholesterol					
Peak (mg/dL)	58.4 ± 23.5	54.1 ± 17.0	57.9 ± 8.7	56.7 ± 14.1	0.96
Time to peak (hours)	3.6 ± 1.9	2.9 ± 2.2	2.4 ± 1.4	2.3 ± 2.5	0.57
tAUC (mg/dL x 6 hr)	322.0 ± 124.8	302.7 ± 103.4	309.4 ± 59.6	307.2 ± 70.3	0.98
iAUC (mg/dL x 6 hr)	4.0 ± 36.5	-7.1 ± 19.9	-0.3 ± 24.6	-9.9 ± 22.0	0.73

Table 3. Postprandial Metabolic Outcomes. Normally distributed data are displayed as Mean ± SD and non-normally distributed data are displayed as Median (Interquartile Range). A one-way ANOVA for parametric data and a Kruskal-Wallis test for non-parametric data were conducted to test for differences between groups. The p-value column denotes main effects between groups assessed via one-way ANOVA or Kruskal-Wallis. Within main effects (by row), values with shared superscript letters are not significantly different, determined by *post hoc* pairwise comparisons. Rows with no superscript letters present contain no significant differences. See Results section for *post hoc* pairwise comparison p-values. tAUC, total area under the curve; iAUC, incremental area under the curve; incremental area under the curve; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ANOVA, analysis of variance

Partial Correlation of Body Composition and Glucose Control with Metabolic and Vascular Outcomes									
		TG fasted	TG peak	TG tAUC	TG iAUC	MLI fasted	MLI peak	MLI tAUC	MLI iAUC	FMD fasted
BF	r	0.31	-0.11	0.04	-0.20	0.34	-0.10	0.03	-0.24	-0.50
	P	0.11	0.58	0.83	0.30	0.07	0.60	0.86	0.21	0.007
VAT	r	0.12	0.43	0.38	0.50	0.161	0.44	0.39	0.50	0.13
	P	0.55	0.02	0.04	0.005	0.40	0.02	0.03	0.006	0.50
SMM	r	-0.19	0.26	0.16	0.41	-0.16	0.26	0.17	0.43	0.30
	P	0.32	0.18	0.42	0.03	0.40	0.17	0.37	0.02	0.11
HbA1C	r	0.08	0.18	0.17	0.20	0.11	0.18	0.16	0.17	0.21
	P	0.69	0.36	0.39	0.31	0.60	0.35	0.41	0.40	0.30

Table 4. Partial Correlation of Body Composition and Glucose Control with Metabolic and Vascular Outcomes. A partial correlation was utilized to determine the association between body composition variables and HbA1c (surrogate of glucose control) with postprandial TG, MLI, and baseline FMD while controlling for physical activity (steps per day) and age. The r row represents the partial correlation coefficient and the P row represents the P-value for the correlation. TG, triglycerides; tAUC, total area under the curve; iAUC, incremental area under the curve; MLI, metabolic load index; FMD, flow-mediated dilation; BF, body fat percentage; VAT, visceral adiposity tissue; SMM, skeletal muscle mass; HbA1C, hemoglobin A1C.
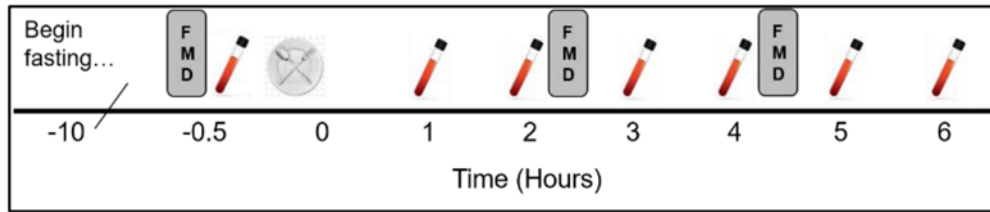

Figure 1. Hourly metabolic and vascular function data collection timeline.
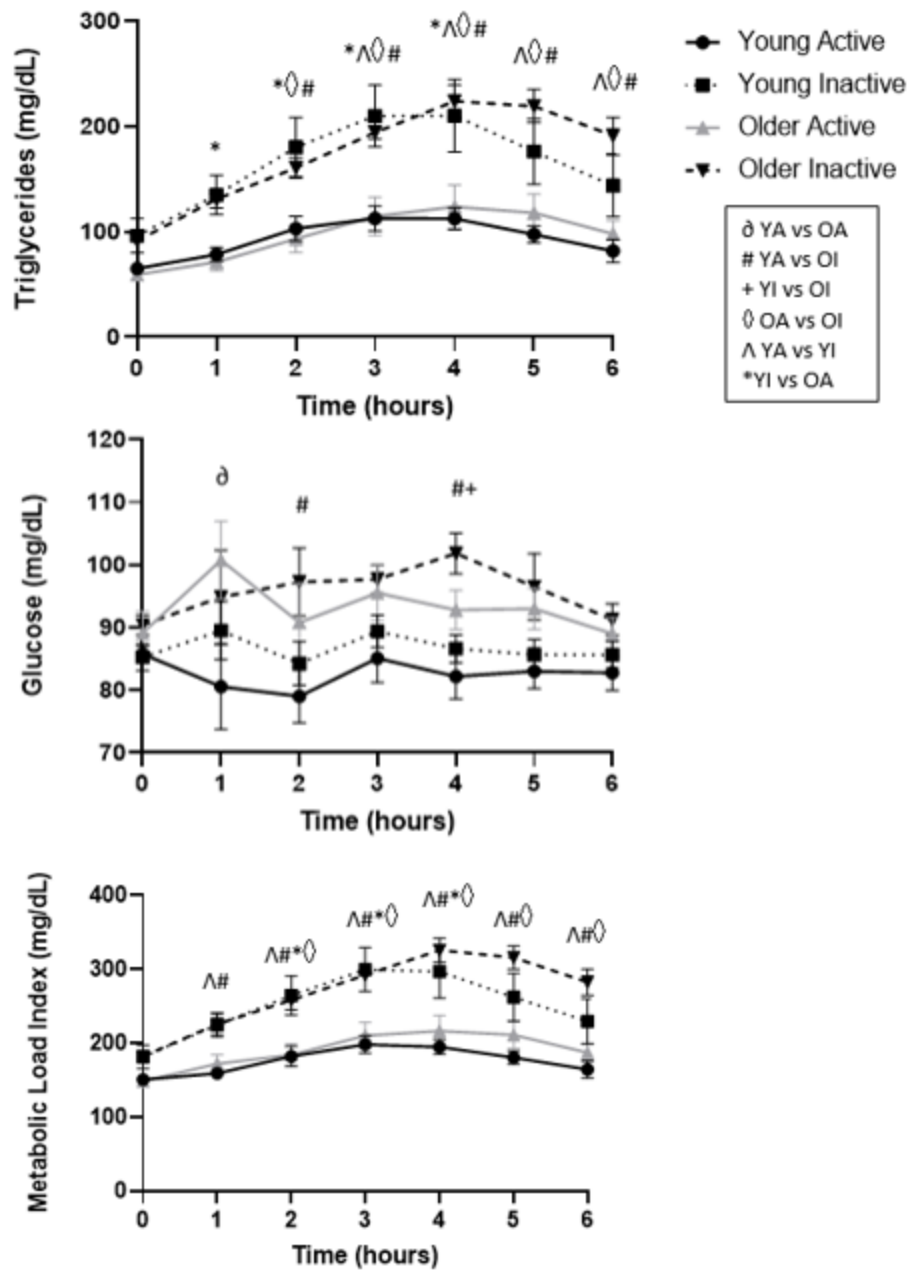
Figure 2. Postprandial Responses for Triglycerides, Glucose, and Metabolic Load Index.
Triglycerides, glucose, and the metabolic load index were assessed at baseline/fasting (time 0)
and serially for six hours after the high-fat meal. Significant differences ($p < 0.05$) at specific
time-points reflect the results of a two-way (group × time) repeated measures ANOVA. Error
bars reflect standard error of mean (SEM). ∂ YA vs OA, # YA vs OI, + YI vs OI, ◊ OA vs OI, Λ
YA vs YI, *YI vs OA. YA, younger active adults; OA, older active adults; OI, older inactive
adults.

Figure 3. Postprandial Cholesterol Responses. Total cholesterol, LDL-C, and HDL-C were assessed at baseline/fasting (time 0) and serially for six hours after the high-fat meal. Significant differences ($p < 0.05$) at specific time-points reflect the results of a two-way (group x time) repeated measures ANOVA. Error bars reflect standard error of mean (SEM). ∂ YA vs OA, # YA vs OI, + YI vs OI, ◊ OA vs OI, Λ YA vs YI, *YI vs OA. YA, younger active adults; OA, older active adults; OI, older inactive adults. Total-C, total cholesterol; LDL-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.
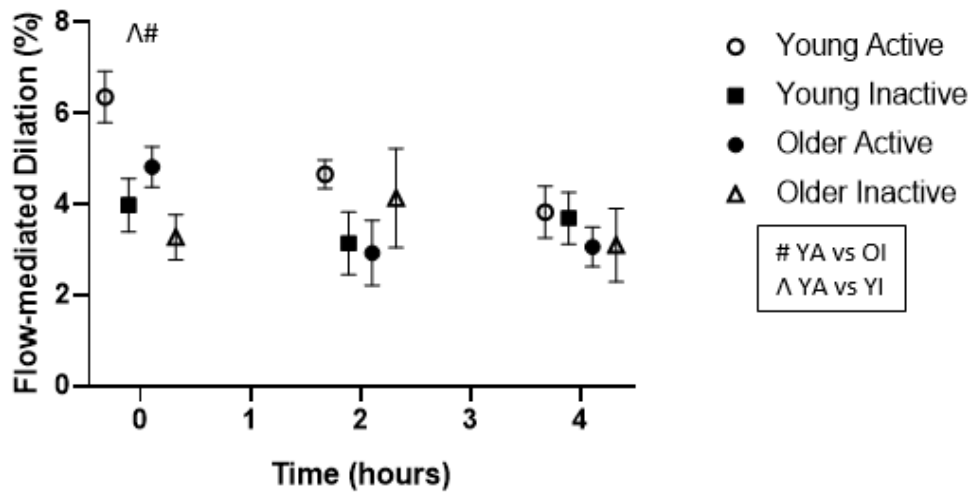
Figure 4. Postprandial Vascular Function. Flow-mediated dilation was measured at baseline/fasting, 2 hours, and 4 hours after the high-fat meal. Significant differences (p < 0.05) at specific time-points reflect the results of a two-way (group x time) repeated measures ANOVA. Error bars reflect standard error of mean (SEM). ∂ YA vs OA, # YA vs OI, + YI vs OI, ◊ OA vs OI, Λ YA vs YI, *YI vs OA. YA, younger active adults; OA, older active adults; OI, older inactive adults.

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Candidate for the Degree of

Master of Science

Thesis: POSTPRANDIAL METABOLISM AND VASCULAR FUNCTION: IMPACT OF AGING AND PHYSICAL ACTIVITY LEVEL

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in December 2019.

Completed the requirements for the Bachelor of Science in Nutritional Sciences Oklahoma State University, Stillwater, Oklahoma in May 2018.

Experience:

Graduate Research Assistant, Laboratory for Applied Nutrition and Exercise Science, Oklahoma State University

Graduate Teaching Assistant, Student and Faculty Career Readiness Program, College of Human Sciences, Oklahoma State University

Professional Memberships:

American Academy of Nutrition and Dietetics
Oklahoma Academy of Nutrition and Dietetics
American Society of Nutrition