The Effect of High-Intensity Interval Exercise on Postprandial Endothelial Function in

Youth

by

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ABSTRACT

In adults, consuming a high-fat meal can induce endothelial dysfunction while exercise may mitigate postprandial endothelial dysfunction. Whether exercise is protective against postprandial endothelial dysfunction in obese youth is unknown. The purpose of this study was to determine if high-intensity interval exercise (HIIE) performed the evening prior to a high-fat meal protects against postprandial endothelial dysfunction in obese adolescent males. Fourteen obese adolescent males (BMI%tile=98.5±0.6; 14.3±1.0yrs) completed the study. After initial screening, participants arrived, fasted at 9:00 in the morning where brachial artery flow-mediated dilation (FMD) was measured using duplex ultrasound after 20min of supine rest (7.0±3.0%) and completed a maximal exercise test on a cycle ergometer (VO$_2$peak=2.6±0.5 L/min). Participants were randomized and completed 2 conditions: a morning high-fat meal challenge with evening prior HIIE (EX+M) or a morning high-fat meal challenge without prior exercise (MO). The EX+M condition included a single HIIE session on a cycle ergometer (8 X 2min at ≥90%HRmax, with 2min active recovery between bouts, 42min total) which was performed at 17:00 the evening prior to the meal challenge. In both conditions, a mixed-meal was tailored to participants body weight consisting of 0.7g of fat/kg of body weight consumed (889±95kcal; 65% Fat, 30% CHO). FMD was measured at fasting (>10hrs) and subsequently measured at 2hr and 4hr after high-fat meal consumption. Exercise did not improve fasting FMD (7.5±3.0 vs. 7.4±2.8%, P=0.927; EX+M and MO, respectively). Despite consuming a high-fat meal, FMD was not reduced at 2hr (8.4±3.4 vs. 7.6±3.9%; EX+M and MO, respectively) or 4hr (8.8±3.9 vs. 8.6±4.0%; EX+M and MO, respectively) in either condition and no differences were observed between

condition ($p_{(condition*time)}$=0.928). These observations remained after adjusting for baseline artery diameter and shear rate. We observed that HIIE, the evening prior, had no effect on fasting or postprandial endothelial function when compared with a meal only condition. Future research should examine whether exercise training may be able to improve postprandial endothelial function rather than a single acute bout in obese youth.

DEDICATION

ad astra per aspera - to the stars through difficulties

I dedicate this dissertation to my wife, Patricia. She has provided vital support and
encouragement every step of the way through my doctoral process. Her words of
encouragement and love have been instrumental to my success. I certainly would not
have wanted to gone through this process without her.

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CHAPTER 1: GENERAL INTRODUCTION

Pediatric obesity represents one of the most significant public health challenges facing our society. Obese youth, particularly those at the higher end of the BMI spectrum (i.e. those above the 97th percentile) are at increased risk for developing cardiovascular disease (CVD) and type 2 diabetes (T2D) (Bacha F, 2006; Srinivasan SR, 2006). Up to 30% of obese youth > 97th percentile exhibit the metabolic syndrome which tracks into adulthood and predicts the development of CVD and T2D (Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003; Morrison, Friedman, & Gray-McGuire, 2007; Rodriguez et al., 2006). This is further exacerbated by the fact that adolescents > 97th percentile are the only growing population of obese youth (Ogden, Carroll, Kit, & Flegal, 2012), making this population of youth an optimal target for intervention. In addition to obesity, declines in physical activity levels are thought to contribute independently to increased chronic disease risk (Trigona et al., 2010; Trost et al., 2002). The Exercise is Medicine™ campaign, supported by the American College of Sports Medicine (ACSM), necessitates that more effective exercise prescriptions are necessary to improve the health and reduce long-term disease risk among obese youth. Moreover, exercise prescriptions for obese youth should lead to clinically measurable improvements in health indicators.

A major challenge to addressing cardiovascular health in youth is the long latent period between elevated cardiovascular risk and future cardiovascular outcomes. Although the atherosclerotic process begins in childhood, it generally remains subclinical for a number of years (Newman, Wattigney, & Berenson, 1991). Impaired endothelial function (i.e. endothelial dysfunction) is one of the earliest manifestations of atherosclerosis, (Jean Davignon & Peter Ganz, 2004) and non-invasive imaging

techniques provide an avenue to assess this subclinical process in younger populations (Urbina et al., 2009). Several studies suggest that obesity is associated with endothelial dysfunction in youth, but very few studies have evaluated whether endothelial dysfunction can be reversed via exercise in this population (Aggoun et al., 2008; Mahmud, Hill, Cuerden, & Clarson, 2009; Woo et al., 2004). The few studies to date which have utilized exercise as medicine for endothelial dysfunction have focused exclusively on endothelial function under fasting conditions (Kelly et al., 2004; Watts, Beye, Siafarikas, O'Driscoll, et al., 2004). Most recommendations for assessment of cardiovascular risk factors, including endothelial function, call for testing to occur after a prolonged fast; (Kavey et al., 2006; Thijssen et al., 2011) However, this state may not reflect the true physiologic nature of the vascular system in relation to health and disease (F. Kaufman, 2005). Humans spend most of their waking time in the postprandial state, and postprandial dysregulation of carbohydrate and fat are critical pathophysiologic processes underlying CVD and T2D (Ebenbichler, Kirchmair, Egger, & Patsch, 1995; Tushuizen, Diamant, & Heine, 2005). Moreover, it is clear that regular physical activity is cardioprotective, but the greatest benefit of exercise on cardiometabolic health may be observed during the postprandial state due to endothelial adaptation (Graham, 2004; Haram et al., 2006).

Evidence in adults suggests that exercise attenuates postprandial lipemia (Katsanos, 2006) and improves endothelial function (Gill et al, 2004). However, the majority of these studies use continuous exercise at a moderate intensity (i.e. traditional exercise) as the exercise prescription. Recent evidence suggests that high-intensity

exercise is more beneficial in improving markers of CVD among adults, including

endothelial function, than a traditional clinical approach (Gibala, Little, Macdonald, &

Hawley, 2012). These data are accompanied by evidence that high-intensity exercise

completely ameliorates high-fat meal induced postprandial endothelial dysfunction

(Tyldum et al., 2009). Nevertheless, despite the increasing literature to support intensity-

dependent benefits of exercise on cardiovascular outcomes,(Tanasescu et al., 2002) very

few studies incorporate high-intensity exercise in youth (Ingul, Tjonna, Stolen, Stoylen,

& Wisloff, 2010). In order to respond to the growing epidemic of obesity in youth,

translational research on the effects of exercise on cardiovascular health is needed.

Compiling this evidence using defined protocols and proximal indicators of vascular

health is an essential step towards developing exercise prescriptions for disease

prevention approaches. Therefore, the overall goal of this dissertation is to examine the

potential for high-intensity exercise to improve postprandial endothelial function in obese

youth.

CHAPTER 2: REVIEW OF LITERATURE

Origins of Cardiovascular Disease

Seminal data using autopsy studies provided conclusive evidence that the earliest visual manifestations of the cardiovascular disease (CVD) process begin to appear at an early age. Strong and McGill dissected and removed 548 coronary arteries via necropsy in persons aged 1-69.(J. Strong & McGill, 1962) The coronary arteries were then blindly evaluated for lesion presence (fatty streaks) and progression as well as fibrous plaque formation through visual and manual inspection. They observed that by age 10-19, 22 out of 44 (50%) individuals showed some lesion formation with the extent of lesion formation increasing with age. Additionally, they observed that while few persons age 10-19 exhibited fibrous plaque formation (2 out of 44), this formation progressed with age in which 46 out of 86 (54%) showed plaque formation by age 30-39. Additionally, the cause of death from myocardial infarction increased with age and was associated with lesion and plaque development. Importantly, these early works provided evidence that while some youth show the presence of CVD morphology these formations do not lead to overt CVD outcomes until later in-life.

Follow up investigations by Strong and McGill showed earlier development of fatty streak lesion development. They examined autopsies of over 1600 persons and found that aortic fatty streaks are often present in children under age 3 and consistently present once children reach age 3.(J. Strong & McGill, 1963) While they did not observe coronary fatty streaks until age 10, the presence of fatty streaks was almost always observed by age 20. These observations were independent of race, ethnicity or geographic origin.(J. P. Strong & McGill Jr, 1969) This suggests that early

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atherosclerotic development, particularly in the aorta, can be observed in just a few short years after birth, and its progression to other vessels is observed in a linear manner later in life.

These studies laid the foundation for later studies to show that fatty streaks can develop *in utero*, wherein the fetuses of mothers with normal cholesterol levels show signs of fatty streak formation in 63.3% of aortic structures and up to 80% in mothers with hypercholesterolemia.(Napoli et al., 1997) These data are also supported by associative evidence that the fetal environment may be a key mediator in the development of atherosclerosis.(Barker, 1995) Therefore, since CVD has its antecedents early in life and is likely to be modulated by a number of early life factors. Therefore, early life is the most applicable population in which to investigate CVD and develop strategies to reduce disease risk. Particularly, between the ages of 10-20 years old where there is a progression from aortic lesion presence to more distal coronary artery lesion development, suggesting that this period may offer a unique window to reduce CVD development.

The Endothelium

In a landmark paper published in 1980, Furchgott and Zawadski first described the obligatory role that the endothelial cells play in vasorelaxation (Furchgott & Zawadzki, 1980). They observed that the endothelium is required in order for acetylcholine to induce the release of a substance(s) that produces relaxation of the vascular smooth muscle. This suggests that vasorelaxation was dependent upon production of these substances from the endothelium, and without the endothelium,

acetylcholine could not cause the smooth muscle to relax. This breakthrough finding awarded Furchgott the Nobel Prize in 1998 and set the stage for the hunt for the elusive substance that the endothelium was producing, in response to acetylcholine, which resulted in vascular smooth muscle relaxation.

In subsequent studies, Furchgott's group identified a number of substances (i.e. substance P, bradykinin, adenosine diphosphate, etc.) that produced endothelium-dependent vasorelaxation in isolated arteries. However, it was unknown which of these substances was primarily responsible for vasodilation *in vivo*; thus, the elusive substance was termed endothelium-derived relaxing factor (EDRF)(Cherry, Furchgott, Zawadzki, & Jothianandan, 1982). It was evident that EDRF, whatever it was, likely stimulated guanylyl cyclase in vascular smooth muscle, which would result in an increased production of cyclic monophosphate (cGMP), and induce relaxation of the smooth muscle (Rapoport & Murad, 1983). Subsequent investigation found that a number of substances (e.g. norepinephrine, serotonin, vasopressin) were able to induce endothelium-dependent vasorelaxtion through the same mechanism, providing further credence to the pivotal role EDRF played in mediating relaxation of blood vessels.(Furchgott & Vanhoutte, 1989) Importantly, during this time, increased amounts of EDRF were shown to be released by the endothelium due to increased amounts of shear stress resulting from increased blood flow rate both *in vitro* and *in vivo* (Griffith, Edwards, Lewis, Newby, & Henderson, 1984; Kaiser & Sparks, 1986; Rubanyi, Romero, & Vanhoutte, 1986). These findings provided the foundation for the development of an *in vivo* bioassay for the assessment of endothelial-dependent dilation using shear stress as a stimulus.

In 1986, two separate labs, headed by Furchgott and Louis Ignarro, presented evidence that EDRF was in-fact nitric oxide (NO).(L. Ignarro, Buga, Wood, Byrns, & Chaudhuri, 1987) Through a series of cell culture investigations, both labs concluded that NO was the substance produced by endothelial cells, either by a pharmacological agent (acetylcholine) or mediated via shear stress. Shortly thereafter, it was found that the source of endothelial-derived NO was the byproduct of L-arginine converted into L-citrulline via an enzyme-mediated reaction. Mediating this reaction was the enzyme called endothelial nitric oxide synthase (eNOS), which is a calcium/calmodulin dependent oxygenase (Palmer, Ashton, & Moncada, 1988). The use of a catalyst, in this case eNOS, is essential for NO production, because the heat of formation is endothermic, and in order for molecular nitrogen and oxygen to combine to produce NO, temperatures in excess of 1000°C are required. These important steps were necessary for determining the mechanism of endothelial-dependent relaxation.

Mechanism of Shear Stress Mediated Vasorelxation

In 1981, Dewley and colleagues described an in-vitro assay for modulating fluid shear stress in cultured endothelial cells.(Dewey, Gimbrone, Davies, & Bussolari, 1981) This work was based upon previous research which suggested that the endothelium was not just a simple barrier but a dynamic system that regulated blood flow and was permeable to environmental changes. In their experiment, they found conclusive evidence that modulating the hemodynamic environment and exposing isolated endothelial cells to fluid shear stress results in change to the physiological function of endothelial cells, in contrast to a static medium. They suggested, correctly, that this fluid shear stress system

was more akin to normal physiological conditions and could potentially be a more robust

environment for studying the endothelium as it related to disease. In subsequent

investigations, the same group noted that both the frequency and amplitude of shear stress

effected the response of basic endothelial function. Interestingly, they also noted that

under certain conditions the endothelium showed an adaptive response that shed light on

how the endothelium may be affected by acute and chronic conditions (P. F. Davies,

Dewey, Bussolari, Gordon, & Gimbrone, 1984).

The mechanism by which fluid shear stress mediated vasorelaxtion is due to a

complex cascade of events (P. F. Davies, 2009). Shear stress induced

mechanotransduction (the conversion of mechanical stresses into biological response) is

central in the regulation of vascular tone. This occurs due to a mechanically stimulated

release of EDRF's from the endothelial cells and is mediated by the amount of shear

forces produced by blood flow. Thus, shear stress is the primary mechanism by which

endothelial cells produce a number of vasorelaxing substances, including NO. The

amount of shear is dictated by Poiseuille flow (law), which is a mathematical model that

dictates that shear stress (τ) is directly proportional to the velocity of blood flow (Q) and

inversely proportional to the cube of the arterial radius (R). In addition, the viscosity of

blood (μ) is also taken into account, resulting in the following equation:

$$\tau = 4\mu Q/R^3$$

Therefore, the primary determinant of shear stress is the diameter of the blood vessel in

which blood flow and viscosity of blood are contributory factors. However, acute

changes in either blood flow or viscosity of the blood can substantially alter shear stress.

The endothelial cells are lined with mechanoreceptors, which are sensitive to changes in shear stress occurring due to blood flow within the vessel. These mechanoreceptors, under the presence of increased shear stress, act within the endothelial cell to convert mechanical energy into biochemical processes. The mechanoreceptors lining the endothelial cells include ion channels, G proteins, tyrosine kinase receptors, adhesion proteins, caveolae, cytoskeleton, glycoalyx, and the primary cilia on the cell membrane. However, the primary mechanisms for shear induced mechano-signal transduction are not fully understood (Ando & Yamamoto, 2009). For example, some endothelial cells mechanoreceptors, such as an adhesion molecule platelet endothelial cell adhesion molecule-1 (PECAM-1), play a key role in shear-stress mediated mechanotranduction in a Akt/PI3K dependent pathway but not a MAPK pathway. This suggests that once a mechanotransduction pathway is activated by shear-stress it may or may not result in signal transduction by the endothelial cell (Fleming, Fisslthaler, Dixit, & Busse, 2005).

However, to simplify, it can be stated that shear-stress, based on its action in the endothelial cell wall, activates a mechanotransduction pathway, whichever it happens to be, that activates a cell signaling cascade within the endothelila cell. In turn, this results in the eventual activation of eNOS to stimulate the production of NO, which is likely due to an integrated response of multiple signaling networks at different subcellular locations (Boo et al., 2002; Dimmeler et al., 1999). Once shear-stress mediated mechanotransduction has been stimulated, the primary result is the phosphorylation of eNOS at Ser^{1177} which activates the enzymes activity (Fisslthaler, Dimmeler, Hermann,

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Busse, & Fleming, 2000). This allows for the conversion of L-arginine to L-citrulline, in which NO is the byproduct of this reaction. Once NO is produced, it can diffuse from the endothelial cell into the interstitial space and then into the vascular smooth muscle cell. In the vascular smooth muscle NO stimulates its receptor, soluble guanylyl-cylcase (sGC), which stimulates the conversion of GMP to cGMP, which induces relaxation of the vascular smooth muscle (L. J. Ignarro & Kadowitz, 1985).Therefore, the ability of the vascular smooth muscle to relax, allowing for vasodilation, is directly dependent upon the effect of shear-stress on the endothelial cell, which plays a key role in vascular tone regulation.

Endothelial Dysfunction in Atherosclerosis

Generally, high amounts of shear-stress are beneficial for arterial health, as it promotes adaptive structural remodeling within the artery and promotes adaptive dilation through endothelium-dependent mechanisms (D. J. Green, Maiorana, O'Driscoll, & Taylor, 2004; Mattsson, Kohler, Vergel, & Clowes, 1997). When this process becomes deregulated and the endothelium can no longer induce acute or chronic changes in vascular tone, vasodilation, and remodeling, an endothelial dysfunctional state has occurred. The primary mechanism by which chronic, systemic endothelial dysfunction can be defined is the impaired ability of eNOS to either be expressed or activated in order to produce NO (Corson et al., 1996; P. F. Davies, 2009). Given that eNOS is primarily stimulated *in vivo* by shear stress, it is likely that endothelial dysfunction results from a multifactorial mechanism by which the endothelial cell no longer responds to shear stress. Endothelial dysfunction is considered one of the earliest manifestation of the

atherosclerotic process, as it occurs even before morphological changes (fatty streaks and plaque formation) occur (J. Davignon & P. Ganz, 2004). Thus, endothelial dysfunction can be considered a pre-clinical manifestation of the atherosclerotic process. Furthermore, endothelial dysfunction declines with increased presence of traditional risk factors such as age, smoking, family history, hypercholesterolemia, and high blood pressure (D. S. Celermajer, Sorensen, Bull, Robinson, & Deanfield, 1994). Therefore, assessment of endothelial function may allow researchers to better define those at higher risk for later development of CVD.

Non-invasive Assessment of Endothelial Function

Given that the endothelium is only assessable via invasive means, the only practical way for endothelial function to become a tool for physiological or clinical assessment is through non-invasive means. The development of non-invasive assessment of endothelial dysfunction was established in an innovative paper by Celermajer et al., which determined endothelial function in conduit arteries (brachial and femoral) using non-invasive high-resolution ultrasound after a period of reactive hyperemia (D. S. Celermajer et al., 1992). The underlying principle of the technique was to measure the diameter of a conduit vessel under resting conditions and then to apply a stimulus that would cause the endothelium to respond by inducing vasodilation. As a stimulus, Celermajer et al. utilized evidence that laminar and orbital shear stress induced endothelial cells to produce NO and developed a means of inducing shear stress *in vivo* by creating reactive hyperemia. Reactive hyperemia is a transient increase in blood flow generally due to a physiological response expressing the need to increase oxygen supply

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to downstream tissues. Therefore, a corresponding increase in reactive hyperemia will result in an increase in shear stress. To induce reactive hyperemia in the conduit vessel, Celemajer and colleagues induced ischemia in the distal limb by using a blood pressure cuff to occlude blood flow for a period of about 5min. After this time period, the cuff was released, reactive hyperemia was induced, a corresponding increase in shear stress was observed, and a change in arterial diameter could be measured with ultrasound in order to determine the amount of vasodilation. After cuff release, they measured conduit vessel diameter at 90s and 15s thereafter in order to determine peak response of individuals. Using the following equation, they could determine the amount of flow-mediated dilation (FMD):

$$FMD(\%) = \frac{peak\ diameter\ \bullet\ baseline\ diameter}{baseline\ diameter}\ X\ 100\%$$

Using this technique, they observed that children with familial hypercholesterolemia, adult smokers, and those with CVD exhibited significantly less FMD compared with "health controls". The evidence in children was the most important finding from the clinical aspect of this study, suggesting that asymptomatic youth with risk factors for CVD present reduced FMD despite the lack of clinical manifestation. In addition, this study, despite technical flaws that were later corrected in subsequent publication (Corretti et al., 2002; Thijssen et al., 2011), provided the foundation for a repeatable *in vivo* assay of endothelial function that could be widely applied to many populations.

Currently, the recommended guidelines for FMD requires an external stimulus (blood pressure cuff inflation >200mmHG below the antecubital fossa) which is used to

create ischemia (after 5min of prolonged exposure). Upon cuff release, there is a reactive

hyperemia response which results in an increase in blood flow through the conduit

vessels (brachial or femoral), thus, creating an increase in shear stress.

A full description of the measurement technique used in our lab appears in

Appendix K with our lab's reliability in Appendix O. Briefly, measurement of conduit

vessels is done continuously via high-resolution ultrasound in B-mode. Ultrasound video

is captured of the lumen of the arterial walls and is measured at both baseline (prior to

cuff occlusion) and for 3 minutes post-cuff release. Videos are then analyzed using semi-

automated edge detection software which captures the artery diameter and measures it

continuously for the entire video length, providing an accurate and less-biased approach

of measurement.(Woodman et al., 2001) Additionally, B-mode ultrasound needs to be

coupled in duplex with Doppler in order to acquire blood velocity measurements. Duplex

allows for simultaneous acquisition of vessel diameter and blood flow, which allows for a

more robust means of assessment.

In theory, the degree of dilation that occurs can be used as a surrogate for both

NO bioavailable and reflective of the function of the conduit arteries function.(Pyke &

Tschakovsky, 2005) A meta-analysis comparing FMD of the same participants using

either saline or L-NMMA (a NO blocker) infusion showed that FMD was at least

partially NO-dependent, and with current automated techniques an estimated 67% of

dilation was accounted for by NO.(D. J. Green, Dawson, Groenewoud, Jones, & Thijssen,

2014) While the majority of dilation due to shear stress can be accounted for by NO,

there remains a significant portion of the FMD that is likely due to other EDRF's that are

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stimulated. These may include endothelial derived hyperpolarizing factor (EDHF) and prostacyclin, which are both shown to be induced by shear stress.(Bellien et al., 2006; Osanai et al., 2000) Additionally, it also supports the hypothesis that vasoconstricting agents may be stimulated by shear stress which may hinder the FMD response in the presence of a shear mediated response. Both endothelian-1 and angiotensin II, two potent vasoconstrictors, are stimulated by shear stress, which may explain the lack of dilation under shear stimulated conditions compared with L-NMMA.(Thijssen, Rongen, Smits, & Hopman, 2008)

Risk Factors and Development of Cardiovascular Disease in Youth

The Bogalusa Heart Study has provided a wealth of epidemiological evidence examining the relationship between antemortem CVD risk factors and observed morphological atherosclerotic (i.e. fatty streak and plaque formations) development in children and adolescents.(Berenson, 2002) In autopsies of 66 persons from birth to age 26, they observed that traditional risk factors for CVD, particularly TC, LDL, VLDL, and blood pressure, were associated ($r = 0.36 - 0.62$ (age adjusted)) with the earliest stages of visible atherosclerotic lesions in both aorta and coronary arteries. (Newman et al., 1991) Furthermore, these associations were independent immutable risk factors of age, gender, and race. Subsequent studies using the Bogalusa cohort have revealed that not only are risk factors associated with the development of fatty streaks and fibrous plaques but as risk factors aggregate or cluster so too does the severity of asymptomatic coronary and aortic atherosclerosis.(Berenson et al., 1998)

While autopsy studies have provided the background to establish that fatty streak and plaque development begins in early life and is increased in association with a greater number of risk factors, autopsy studies are difficult and provide a low number of subjects. Non-invasive techniques for imaging the carotid artery (e.g. common carotid, carotid bulb, and internal carotid) have been developed using high-resolution ultrasound imaging. The thickness of the regions in the common carotid, carotid bulb, and internal carotid are all related to autopsy derived increases in fatty streak and plaque formation. (Zarins et al., 1983) Therefore, carotid intima medial thickness (cIMT) provides a means of determining arterial wall thickness without cumbersome autopsy studies and gives a means of determining the extensiveness of asymptomatic diseases progression. In the Bogalusa cohort, cIMT was shown to increase with a greater number of risk factors (family history, blood pressure, obesity, lipoproteins, insulin, glucose, and smoking), similar to that of autopsy studies.(Berenson, 2002) Interestingly, the increase in cIMT appeared to be in a dose-dependent manner, highlighting the effect of clustering of risk factors. Together, these data suggest that risk factors are associated with presence and progression of the morphological manifestations for CVD. Furthermore, the use of risk factors may likely provide an opportunity to identify youth at the highest risk for CVD development.

Evidence provided by the Cardiovascular Risk in Young Finns Study has extended the vast work of the Bogalusa heart study and added the assessment of endothelial function (FMD) to their cohort. The Cardiovascular Risk in Young Finns Study is a large cohort ($n > 3000$) longitudinal study in Finland that has measured CVD

risk factors in youth (aged 3-18yr old) and followed them with subsequent measurement later in early adulthood (aged 24-39yr old) in order to investigate the early childhood factors that might be associated with increased presence of CVD risk later in senescence. This study has been instrumental in providing epidemiological data on the relationship between endothelial function and childhood/early adulthood characteristics.

The Young Finns Study observed that endothelial function is associated with increased cIMT and that association is enhanced with increased presence of traditional CVD risk factors (M. Juonala et al., 2004). This suggests that endothelial dysfunction may compound the effect of traditional risk factors by enhancing or speeding up the CVD process as observed by increased levels of cIMT. Additionally, this study showed that increases in systolic blood pressure in adolescent (12-18 yr old) were associated with reduced endothelial function in early adulthood (M. Juonala et al., 2006).This is likely the effect of systemic shear stress increases over an extended duration, which is likely to result in a decrement in endothelial function through multiple mechanisms of action (Vanhoutte, 2009). Furthermore, they observed that gender effects on endothelial function are likely the result of males presenting increased artery diameter and that the effect of gender is lost when traditional risk factors are taken into account (M Juonala et al., 2008).Therefore, these data suggest that in order to preserve or enhance endothelial function from youth into adulthood prevention of increased traditional CVD risk factors may be essential.

Cardiovascular Disease Risk and Obesity

Obesity has increased in prevalence in the United States as well as other countries around the world since the 1970s. Current estimates suggest that in the United States ~32-35% of adults and ~17% of youth are obese (Flegal, Carroll, Kit, & Ogden, 2012; Ogden et al., 2012). These trends have leveled off in recent years and there have been no increases in overall obesity. However, whether obesity is associated with an increase in mortality remains a controversial issue. Flegal and colleagues suggest that obesity only at the highest ends of the BMI spectrum ($\geq$35 kg/m^2) are associated with an increase in all-cause mortality. With class 1 obesity (BMI= 30-35 kg/m^2) may not be associated with an increase in mortality and overweight adults (BMI= 25-30 kg/m^2) exhibiting the lowest risk of all-cause mortality. These data suggest that overweight and obesity at its lowest ends (25-35 kg/m^2) are not associated with increased risk of mortality; however, those at the highest end of the BMI spectrum are at increased risk. It should be noted that this association between obesity and mortality is not without controversy (Flegal, Kit, & Graubard, 2013).

Obesity has been associated with a number of chronic disease conditions including CVD. The Framingham Heart study was one of the first to show that increases in obesity (as measured by body weight) was associated with an increased number of CVD events (Hubert, Feinleib, McNamara, & Castelli, 1983). However, important confound variables, such as lifestyle factors, were not taken into account in this very homogenous cohort. Additionally, it is important to note that this relationship is not linear. In fact there is a J or U shaped relationship which shows that person at both the

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low and the high end of the BMI-spectrum exhibit increased disease risk, CVD, and all-cause mortality. Perhaps the best meta-analysis on the topic was published by Romero-Corral et al, they combined the results of 40 large cohorts, with 250,152 patients with a mean follow up of 3-8 yrs (Romero-Corral et al., 2006). This study demonstrated a U-shaped relationship for total mortality and cardiovascular mortality, both adjusted and unadjusted for confounding variables. They demonstrated, in their adjusted model, that overweight persons had the lowest risk of all-cause mortality (RR: 0·87 [0·81–0·94]), whereas obese and severely obese people had no increase in risk (RR: 0·93 [0·85–1·03] and 1·10 [0·87–1·41], respectively). However, they did note that severely obese persons had significantly elevated risk of CVD mortality (RR=1·88 [1·05–3·34]). These findings suggesting that those at the highest end of the BMI-spectrum should be the primary focus of studies which aim to reduce the burden of CVD risk associated with obesity.

There is substantial evidence to support that reasonable levels of physical activity or cardiorespiratory fitness ameliorate chronic disease conditions associated with obesity (Lee, Sui, & Blair, 2009). Prospective data from the Aerobics Center Longitudinal Study (ACLS) first showed that low levels of physical fitness were associated with increased risk of all-cause mortality and CVD events (Blair et al., 1995; Blair et al., 1989). Interestingly, studies from the ALCS have also shown that changes in fitness were the best predictor of mortality risk, regardless of baseline fitness levels. This suggests that improving or maintaining fitness levels is the best way to reduce mortality risk. Further investigation from the ALCS and others have shown that fitness may counteract the effects of obesity on mortality risk wherein obese fit persons show no elevated risk for

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all-cause mortality (Barry et al., 2014). Together these data suggest that, obesity at the highest end of the BMI spectrum is associated with an elevated risk for CVD and all-cause mortality. However, when important confounding variables are taken into account, particularly fitness, obesity appears to be relatively benign until you reach higher ends of the BMI spectrum.

Obesity and Cardiovascular Disease Risk in Youth

Due to the long-latent period between elevated CVD risk and development of overt CVD disease it is difficult to determine whether obesity in childhood will result in a corresponding increase in CVD events and/or mortality in adulthood. The current body of literature would suggest that there are associations between overweight and obesity and childhood and adolescence and premature mortality and morbidity. A systematic review by Reily and Kelly found that 4 out of 5 studies, which examined overweight/obesity in childhood, showed an increased risk of premature mortality. In studies (n=11) where cardiometabolic morbidity (diabetes, hypertension, stroke, CHD) were available, they found that obesity in youth was associated with an increased risk of cardiometabolic morbidity ranging from 1.1-5.1 fold risk (Reilly & Kelly, 2011). The findings were consistent between studies despite differences in nationality and sample sizes. It should be noted that none of the studies included in the review were able to account for lifestyle factors, which may have impacted the results. Nevertheless, these data suggest that obesity in youth may lead to an increased risk of morbidity and may reduce life expectancy.

Due to the difficulty in examining the longitudinal association between childhood issues and their corresponding effect in later life, risk factors for disease are often used. Obesity in youth is associated with a number of risk factors for CVD and these risk factors tend to increase in accordance with increases in BMI regardless of age or gender. Data from the Bogalusa heart study shows that as obesity increases there is a dose-response relationship between the degree of obesity and number of CVD risk factors (Freedman, Mei, Srinivasan, Berenson, & Dietz, 2007). With those above the 97th percentile being at the highest risk, and showing an exponential increase once obesity reaches the 99th percentile. These data suggest that targeting intervention in those at the extreme end could yield the greatest benefits and make the largest impact on future disease burden.

Obesity and Endothelial Function in Youth

Most studies have observed that obese youth have significantly lower endothelial function in comparison with lean, healthy counterparts (Farpour-Lambert et al., 2009; C. L. Kaufman, Kaiser, Steinberger, & Dengel, 2007; Meyer, Kundt, Lenschow, Schuff-Werner, & Kienast, 2006). However, it should be noted that several studies used sub-standard methodology. While their findings were published in peer-reviewed journals, substantial technical/standardization progress has been made since Celermajer et al. published their landmark paper (D. S. Celermajer et al., 1992).Therefore, in an effort to review the most current and up-to-date data regarding the relationship between obesity and endothelial function in youth, only studies which measured FMD using the current

guidelines and utilizing edge-detection software (Harris, Nishiyama, Wray, &

Richardson, 2010; Thijssen et al., 2011).

Prior to puberty, obesity in children has been shown to reduce endothelial

function. Farpour-Lambert observed that obese children had significantly lower FMD

(n=22, FMD = 4.1%, 95%CI 3.7-4.8%) than lean controls (n=22, FMD = 7.8%, 95%CI

7.1-9.5%) (Farpour-Lambert et al., 2009). In conflict with these findings, a population

based study by Charakida and colleagues which found no significant difference between

normal-weight and obese children. Examining a cohort of 6,576 children aged 10-11

years old (10.6±0.1%), they found no difference in FMD between normal weight

(8.1±3.3%), overweight (8.1±3.4%), and obese youth (8.2±3.2%). The authors suggested

that perhaps obesity, prior to puberty, may not impact endothelial function despite

observed increases in many other CVD risk factors with obesity (Charakida et al., 2012).

While this study is very strong in methodology and in terms of sample size, it is limited

by the small amount (4%) of obese youth in the cohort. However, these findings suggest

that obesity, at least prior to age 11, may not impact endothelial function when observed

at a population level versus that of a clinical setting, as in the Farpoup-Lamert et al.

findings.

In adolescents, more studies have examined the effects of obesity on endothelial

function. Meyer et al. observed significantly reduced FMD in obese adolescents

(4.79±1.76%) versus their lean counterparts (10.65±2.0%, p<0.001). In accordance with

these findings, Watts and colleagues showed that FMD was significantly impaired in

obese adolescents relative to lean controls (5.3 ± 0.9% to 8.9 ± 1.5%, p < 0.05). These

data suggest that obesity during adolescence may contribute to reduced endothelial

function. However, these sample sizes are relatively small, n=34 and n=20 respectively.

One study in obese adolescents observed no significant difference between lean and

obese adolescents with respect to FMD (Naylor et al., 2011). This study by Naylor et al.

did find that obese adolescents with type 2 diabetes exhibited reduced FMD (p<0.05).

This suggests that insulin resistance may play a vital role in endothelial dysfunction in

youth, which is not accounted for in any studies to date. More population based samples

are needed to define the role of obesity on FMD. It is likely that obesity per se may not be

the only contributing factor to the reduced endothelial function observed, findings which

cannot be examined in small cohorts. Furthermore, additional work needs to be done in

order to define the roles of extraneous factors that may impact both obesity and

endothelial function.

Significant associations have been observed between adiposity and FMD.

Kaufman et al showed that FMD was significantly and inversely associated with body

fatness as measured by DXA (r = -0.34, p<0.05) in children (11.5±0.1 yr old) (C. L.

Kaufman et al., 2007). Suggesting that body composition may play an important role in

endothelial function; whether this is independent of obesity status is unknown.

Heritability of FMD has been examined in cohorts of twins with an estimated 44% of the

variability in FMD explained by genetic factors (Hopkins et al., 2010). This study

suggests the potential roles of pre- and peri-natal influences on endothelial function

which have yet to be examined. While aging is associated with increased arterial diameter

(Sarkola et al., 2012) and pubertal onset insulin resistance is a common phenomenon,

puberty does not appear to impact endothelial function in youth (Marlatt et al., 2013).

However, the role of insulin resistance and endothelial function in youth has yet to be

defined, despite the substantial mechanistic evidence of its role (Muniyappa, Montagnani,

Koh, & Quon, 2007). Thus, while obesity may play a role in reducing endothelial

function, many other factors need to be considered and further research is needed in order

to define the specific mechanism by which obesity alone inhibits endothelial cell

function.

Exercise Training and Endothelial Function

Lifelong physical activity is associated with decreased mortality and reduced

CVD events, in adults.(Blair et al., 1989; Paffenbarger, Hyde, Wing, & Hsieh, 1986)

Exercise training has been shown to reduce risk for CVD with improvements in

endothelial function being a potential mediating mechanism. Perhaps the best evidence to

support the efficacy of exercise as a means of improving endothelial function comes from

Hambrecht and colleagues, they examined the effects of a 4-wk moderate intensity

exercise program on patients with stable coronary artery disease (Hambrecht et al., 2003).

Training improved *in vivo* and *in vitro* acetylcholine (ACh) responses and adenosine-

mediated blood flows, indicating that both conduit and resistance artery endothelium

dependent vasodilator function were enhanced. Training also resulted in increased eNOS

mRNA, eNOS protein expression, and shear stress related eNOS phosphorylation,

supporting that a shear stress dependent mechanism may be responsible for the enhanced

NO bioactivity with training. While the intensity of exercise was moderate ~70% of HR

max, this suggests that with training even moderate intensity exercise can result in major

improvements in vasodilator capacity. This is even true in short periods of time (7 days; 60 min/d; 60% VO2max) as exercise training has been shown to improve femoral blood flow in type 2 diabetics (Mikus et al., 2011). Thus, exercise training may improve vascular function in various populations and of various time-lengths.

Improvements in vascular function have been shown in obese children undergoing an 8-week exercise training. The exercise consisted of a circuit training program which combined moderate-vigorous aerobic and moderate resistance training. After 8-weeks, the endothelial dysfunction associated with obesity in children was ameliorated, in which the vascular function of the obese children was the same as the lean control (Watts, Beye, Siafarikas, Davis, et al., 2004). In addition to the functional changes of the vasculature that occur with exercise, structural changes also take place. Arterial remodeling occurs in large conduit vessels due to exercise training. This has been observed in cross sectional studies where trained endurance athletes had larger conduit arteries than sedentary controls.(D. J. Green et al., 2013)

While the specific mechanisms of arterial remodeling are not elicited yet, there is growing evidence to support that chronic changes in shear stress induce arterial remodeling that is endothelium and NO dependent. Therefore, it is plausible that arterial remodeling may occur in a shear-stress dependent manner. Furthermore, exercise modes which promote higher shear rates may elicit greater vascular adaptations. It is important to note that the specific mechanisms and exercise intensities which contribute to arterial remodeling are not yet understood. However, the time course of remodeling is thought to take place between 4-8weeks after starting an exercise training program (D. Green,

2009). This results in a research predicament as remodeled arteries often have a reduced FMD due to increased vessel diameter. Whether improvements are achieve through remodeling or increases in function, it should be recommended that adoption of any exercise training program may elicit positive effects on vascular function in high-risk populations.

Postprandial Metabolism and Endothelial function

Although most recommendations for assessment of cardiovascular risk factors, including endothelial function, suggest that testing occur after a prolonged fast,(Kavey et al., 2006; Thijssen et al., 2011) this state may not reflect the true physiologic nature of the vascular system in relation to health and disease (F. Kaufman, 2005). Humans spend most of their waking time in the postprandial state, and postprandial dysregulation of carbohydrate and fat are critical pathophysiologic processes underlying CVD (Ebenbichler et al., 1995; Tushuizen et al., 2005).

Several studies have examined the effects of various meal compositions on postprandial endothelial function in adults with mixed results. Ayers et al., gave 7 health normal weight and 7 healthy obese young adults (32±6 yrs) a standardized high-fat meal consisting of 60g of fat and 1000kcal (Ayer, Harmer, Steinbeck, & Celermajer, 2010). They measured FMD along with other vascular reactivity measures at 1 hour and 3 hours post-meal ingestion. Despite consuming the high-fat meal, they observed no change in FMD from baseline to 3hr post-meal in either the normal-weight (4.7±4.1 to 4.3±3.9%) or obese group (6.2±1.7 to 5.8±4.3%, $p(_{group*time}) = 0.975$). They additionally observed no difference in any other measures of vascular reactivity. Similarly, de Roos and

colleagues, observed no change in FMD at 3 hours post-meal (2.62 to 3.00%, p=NS) in healthy men (>35 yrs old, BMI = 25.4 ± 2.6 kg/m^2) after consuming a high fat meal (1178 kcal, 79g fat) (de Roos et al., 2002). The de Roos study should be taken with a cautionary note, that the FMD's observed were considerably lower than that of other investigation, and current standardized methods of FMD assessment were not able to be adhered to.

The mechanism of the lack of impairment in vasodilation due to a mixed-meal observed in these studies in not well understood. Raitakari and colleagues, examined the effect of two high-fat meals (1030kcal, 61g fat) with differing fatty acid compositions in 12 normal-weight/overweight men and women (33 ± 7yr, BMI = 24.3 ± 3.1 kg/m^2)(Raitakari et al., 2000). The observed no change in FMD at 3 hours or 6 hours post-meal in either meal. However, they did observe that with either meal, participants had an increase in forearm blood flow, increased triglycerides and increased insulin at 3 hours, in response to the meal. The increase in forearm blood flow was highly associated with the insulin response observed (r = 0.80, p<0.002). The authors suggested that the hyperinsulinemia response observed in the postprandial state mediated changes in forearm blood flow which may counter-act any detrimental effects of that meal on endothelial function.

Despite these studies, several studies have shown the ability of high-fat meal to induce endothelial dysfunction in adults (Jackson, Armah, & Minihane, 2007). Cortes et al., examined the effect of 2 high-fat meals (~1200kcal, 63% fat; 1 with walnuts (40g), 1 with olive oil (25g)) on endothelial function 4hrs post meal in healthy normal-weight controls (n=12, 32 ± 8yr old, BMI=24.7 ± 3.0) and those with hypercholesterolemia (n=12,

45±13yr old, BMI=26.3±3.5). (Cortes et al., 2006) They observed that neither meal reduced endothelial function in the controls, while the meal which was high-fat with olive oil significantly reduced FMD in the hypercholesterolemia group (3.6±1.3 to 2.3±2.2, P=0.006). They did however observed no reduction in the hypercholesterolemia group with walnuts in the meal. These data suggest that healthy adults may not be as prone to the effects of a high-fat meal on postprandial endothelial function, while persons a high risk for CVD may be more effected.

In support of this concept, Johnson and colleagues examined the effects of a high-fat meal on postprandial endothelial function in physically inactive and activity normal-weight, healthy young adults. FMD was measured at fasting and 4hrs post-meal (940kcal, 46%fat). They observed that while the active individuals were protected against endothelial dysfunction at 4hrs post-meal, the inactive persons exhibited significant endothelial dysfunction (p=0.02) (Johnson, Padilla, Harris, & Wallace, 2011).

Studies, in a non-mixed meal setting, which utilize oral and intravenous fat challenges have been successful at inducing endothelial function. In a study of 13 obese, healthy adults (32.2±9.8yr, BMI= 36.7±5.1) Gosmanov et al., provided evidence that fat loads, whether received intravenously or orally, impair endothelial function at 4hr and 8hr after being given (Gosmanov, Smiley, Robalino, Siqueira, et al., 2010). They gave participants 2 different oral fat loads as an emulsion (low fat = 32g, high fat = 64g; 100% fat load) at equal doses every 2hours over 6 hours. They observed significant decreases in FMD from baseline in both meals at 4hr and 8hrs, with a relative decrease in FMD of 38% for low fat and 51% for high fat (P_{both}<0.001). They had similar findings with the

intravenous lipid infusions and there data suggest that a pure oral fat load impacts

endothelial function. However, mixed-meals appear to be more physiologically relevant

and therefore need to be further evaluated in their efficacy. Moreover, postprandial

studies offer a unique opportunity to examine whether endothelial function can be

improved in a more relevant period of the day.

Acute Exercise and Postprandial Endothelial Function in Adults

Acute exercise, especially high-intensity exercise, has been shown to increase

markers of inflammation and oxidative stress (K. J. Davies, Quintanilha, Brooks, &

Packer, 1982). Thus, there may be an acute impairment following exercise on vascular

function, as inflammation and oxidative stress have been shown to acutely impair NO-

mediated vasodilator function. However, this acute effect of exercise is biphasic, wherein

a transient negative period of vascular stress occurs immediately after exercise, followed

by a prolonged positive phase (Haram et al., 2006). Therefore, the time course of acute

exercise must be examined in order to fully understand the impact of exercise on vascular

function. To investigate the effects of a prolonged intense bout of exercise, Dawson et al.

examined the vascular function of fifteen men pre- and post- a marathon (42.2

km).(Dawson et al., 2008) The researchers examined conduit artery function of the

brachial artery and femoral artery within 1 hour post-race. They found that while brachial

conduit artery function remained the same, there was a significant reduction in femoral

artery function post-exercise. From this study, we can gather that acute exercise of

prolonged duration >3hrs, which will be largely vigorous throughout, may impair

vascular function acutely in local site of maximal flow. Data from Haram et al. confirms

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these findings. However, they found that while this effect exists for the 1st hour, at 6 hours post-exercise this impairment no longer exists and that an improvement in vascular function is seen for up to 192 hours post-exercise. Further, they showed that this relationship exists for both exercise trainers and sedentary controls, wherein trained individuals show the greatest benefits, but both groups showing improvement. Thus, it can be taken that acutely (less than 6hours) there is an impairment of vascular function, but this is followed by a substantially prolonged period by which vascular function is improved.

Gill and colleagues were the first to observe that acute exercise may protect against endothelial dysfunction induced after a high fat meal (J. M. Gill et al., 2004). They examined the effects of a high-fat meal challenge on endothelial function as measured by Peripheral micro-vascular function using acetylcholine infusion. Twenty participants (10 lean (waist <90cm), 10 centrally obese (waist >100cm)) completed 2 visits, in which one was with prior exercise (14-16hrs, 90min walking at 50% VO2peak) and one was without prior exercise. Participants were given a fixed high-fat meal (80g Fat, 70g CHO, 12g PRO, 1027kcal) and endothelial function was measured every 2 hrs post-meal for 8hrs. They observed no difference between lean and obese adults. When groups were combined, they observed that exercise improved fasting endothelial function (+25%, p=0.02). They also observed that endothelial function was significantly elevated at each time point (p<0.01), in which an overall response was 15% greater than the control condition (p=0.048). It should be noted that while peripheral micro-vascular function is a validated technique, it does not measure dilatory capacity; rather, it assesses

skin profusion. Whether this is applicable to conduit artery function cannot be determined by this study. Despite this limitation, this study provided the framework for exercise to be examined as a potential means of improving fasting and postprandial endothelial function.

In a subsequent study, Padilla et al. using FMD examined whether exercise after consumption of a high-fat meal could protect against endothelial dysfunction induced by a high-fat meal. Eight participants (5 males, 3 females; 25.5±0.8 yrs old, BMI = 22.8±0.6 kg/m^2) completed 3 challenges. One low-fat meal (0g Fat, 209g CHO, 23g PRO, 945kcal), one high-fat meal (48g Fat, 91g CHO, 33g PRO, 940kcal), and one high-fat meal with exercise 2hrs post ingestion (45min walking at 60% VO$_2$peak). They measured FMD at fasting and at 4 hours and observed that the high-fat meal induced endothelial dysfunction while the low-fat meal did not. Importantly, the exercise protected against the postprandial endothelial dysfunction (p<0.01) from the high-fat meal. These findings suggest that exercise has the ability to improve endothelial function despite the presence of a high-fat meal and that a low-fat meal not inhibit endothelial function. However, whether exercise prior to a high-fat meal can protect against postprandial endothelial dysfunction needed to be further investigated.

Tyldum et al. examined the effect of high-intensity exercise versus continuous exercise prior to a high-fat meal on postprandial endothelial function. Eight healthy men (42±4 yr old, BMI = 28.8±0.9 kg/m^2, VO$_2$peak 52.6±2.6) completed 3 visits. On each visit, participants consumed a high-fat meal (48.3 g fat, 80.4 g CHO, 38.5 g PRO, 911.2 kcal). In addition to a no exercise prior control, 2 visits were preceded by treadmill

exercise 16 hours prior, including a high-intensity interval exercise bout (4 X 4 min at 85-95% HRmax with 3 min of active recovery between bouts, 47min total) and a continuous moderate exercise (60-70% HRmax for 47min). They observed that continuous exercise improved fasting endothelial function and sustained endothelial function in comparison to a high-fat meal control, which showed dysfunction at 2hrs and 4 hrs post-meal. The high-intensity interval exercise improved fasting endothelial function beyond that of either control or continuous exercise. In addition, the high-intensity interval exercise exhibited significantly higher endothelial function throughout the entire meal challenge. This study was the first to demonstrate the protective effects of exercise prior to a high-fat meal on postprandial endothelial function using FMD. Taken together, these studies suggest that exercise whether given prior or after a high-fat meal, protects against postprandial endothelial function.

Acute Exercise and Postprandial Endothelial Function in Youth

To date there have only been limited investigation into whether exercise can protect against postprandial endothelial dysfunction in youth. Sedgwick and colleagues were the first to examine whether exercise is able to protect against postprandial endothelial function in youth.(MJ Sedgwick et al., 2013) They provided youth with 2 meals (breakfast and lunch) and measured FMD at fasting, 3 hours, and 6 hours after consumption of the first meal. For breakfast, youth consumed a chocolate milkshake of 92.9 kJ/kg body weight (22.2 kcal/ kg body weight), which was 60% Fat, 33% CHO, and 7%PRO. After the 3 hour FMD was taken, a cheese sandwich 84.6 kJ/kg body weight (20.2 kcal/kg body weight) 50% Fat, 37% CHO, and 13% PRO was consumed. Thirteen

healthy, adolescent males (13.6±0.6 yrs old, BMI=22.3±4.2 kg/m^2, VO$_2$peak = 48.1±7.5 ml/kg/min) completed 2 visits, including an prior exercise bout (~14hr) and another without prior exercise. The exercise consisted of four, 15 min walking periods at 60% of VO$_2$peak, which included 3 min of rest between bouts. They observed no differences in fasting FMD was observed (p=0.449). However, the researchers observed that exercise was able to protect against postprandial endothelial function at both 3 hours and 6 hours post meals (p<0.05).(MJ Sedgwick et al., 2013)

In a subsequent investigation by the same group, Sedgwich et al.used an identical meal design with differing exercise stimulus and confirmed their findings in a similar population. (MJ. Sedgwick, Morris, Nevill, & Barrett, 2014) They split 60min of exercise into 6 x 10 min of bouts throughout the day with 50min of rest between each bout. Each 10min bout was at 70% of VO$_2$peak on a treadmill. Fourteen adolescent males (12.9±0.7 yrs old, BMI=20.3±3.5 kg/m^2, VO2peak=45.4±7.9) completed 2 study visits, measuring FMD at fasting, 3 hours, and 6.5 hours post-meals. At fasting, they observed no difference in FMD; however, at 3 hours and 6.5 hours post-meal, the exercise was able to protect against postprandial endothelial dysfunction observed in the meal only condition (p<0.001). Combined, the Sedgwick et al. studies suggest that exercise, whether given as a single dose or spread throughout the day, can protect against postprandial endothelial dysfunction in healthy, normal-weight adolescent males.

Definition of Terms

Pediatric Obesity: We will define pediatric obesity using criterion established by the Center for Disease Control, which uses body-mass index percentile based on age, gender, height, and weight. These criterions state that individuals who are greater than or equal to the 95th percentile for their respective age and gender category are defined as Obese.

High-Intensity Interval Exercise: A single exercise bout characterized by short (6 seconds to 6 minute) periods of exercise at an intensity level just below maximal exertion and separated by low-intensity recovery or active rest periods.

Shear Stress: The frictional force created by flowing blood across the endothelial cell surface.

Endothelial Function: Ability of the endothelial cell, a single monolayer which lines arteries, to produce endothelial-derived relaxing factors, primarily nitric oxide, to stimulate smooth muscle relaxation and vasodilation.

Brachial Artery Flow-mediated Dilation: Ability of an artery to dilate after reactive hyperemia, induced by ischemia, produces shear stress, which creates NO and results in an endothelium-dependent vasodilation that can be measured using high-resolution ultrasound.

Postprandial Endothelial Dysfunction: The inability of the endothelium to function properly after the ingestion of a meal, resulting in a dilation that is less than baseline fasting values.

CHAPTER 3: STUDY INTRODUCTION

Rationale

    Pediatric obesity represents one of the most significant public health challenges facing our society. Obesity in youth is associated with increased cardiometabolic morbidity and premature mortality (Reilly & Kelly, 2010), and most obese youth will become obese adults (Freedman et al., 2005). Obese youth, particularly those at the higher end of the BMI spectrum (i.e. those above the 97th percentile), are at increased risk for developing cardiovascular disease (CVD) and type 2 diabetes (T2D) (Bacha F, 2006; Srinivasan SR, 2006). Up to 30% of youth $> 97^{th}$ percentile exhibit the metabolic syndrome, which tracks into adulthood and predicts the development of CVD and T2D (Cook et al., 2003; Morrison et al., 2007; Rodriguez et al., 2006). This is further exacerbated by the fact that adolescents $> 97^{th}$ percentile are the only growing population of obese youth (Ogden et al., 2012), making this population of youth an optimal target for intervention.

Cardiovascular Disease Risk: Role of Endothelial function

    A major challenge to addressing cardiovascular health in youth is the long latent period between elevated cardiovascular risk and future cardiovascular outcomes. Although the atherosclerotic process begins in childhood, for the most part, it remains subclinical for a number of years (Newman et al., 1991). Impaired endothelial function (i.e. endothelial dysfunction) is one of the earliest manifestations of atherosclerosis, (Jean Davignon & Peter Ganz, 2004). When non-invasive techniques to assess subclinical atherosclerosis are applied to the pediatric population, they clearly illustrate that early atherosclerotic processes are compounded by adiposity, insulin resistance, and

hyperglycemia in youth (Urbina et al., 2009). Several studies suggest that obesity is independently associated with endothelial dysfunction in youth, but very few studies have evaluated whether endothelial dysfunction can be reversed via exercise in this population (Aggoun et al., 2008; Mahmud et al., 2009; Woo et al., 2004).

Exercise and Cardiometabolic Disease Risk in Youth

In addition to obesity, declines in physical activity levels which are observed throughout childhood, are thought to contribute independently to increased chronic disease risk, including endothelial dysfunction (Trigona et al., 2010; Trost et al., 2002). Although lifestyle modifications represent the cornerstone of weight management in youth, very few successful pediatric obesity treatments are currently available (Whitlock, O'Connor, Williams, Beil, & Lutz, 2010). Unfortunately, most studies in youth focus on adiposity as the outcome of interest (Dietz, 2005), and only a small number include measures of subclinical atherosclerosis (e.g endothelial function) as endpoints (Fernhall & Agiovlasitis, 2008). Therefore, there is a pressing need for better programs that address the increases in cardiometabolic disease risk observed with obesity and inactivity which utilize proximal indicators of vascular health.

Intensity-dependent Recommendations

Current American College of Sports Medicine (ACSM) recommendations for physical activity in adults acknowledge the benefits of vigorous-intensity physical activity for promoting and maintaining health (Haskell et al., 2007). Additionally, these recommendations acknowledge recent advances in science and suggest that vigorous-intensity exercise may have a more robust benefit for reducing CVD and premature

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mortality than moderate-intensity exercise. In contrast to the adult literature, current physical activity guidelines for youth (W. B. Strong et al., 2005) are limited by insufficient science and a lack of evidence concerning the effects of physical activity on cardiovascular health, as most studies focus on adiposity as the outcome of interest (Dietz, 2005). Therefore, more evidence is needed in order to determine what intensities of exercise are most beneficial in youth, and more effective exercise prescriptions are needed in order to optimize reductions in long-term disease risk among youth. Moreover, these exercise prescriptions should lead to clinically measurable improvements in health indicators beyond adiposity.

Exercise as Vascular Medicine

To date, the few studies that have utilized exercise as medicine for endothelial dysfunction in youth have focused exclusively on endothelial function under fasting conditions (Kelly et al., 2004; Watts, Beye, Siafarikas, O'Driscoll, et al., 2004). Although most recommendations for assessment of cardiovascular risk factors, including endothelial function, suggest that testing occur after a prolonged fast (Kavey et al., 2006; Thijssen et al., 2011), this state may not reflect the true physiologic nature of the vascular system in relation to health and disease (F. Kaufman, 2005). Humans spend most of their waking time in the postprandial state, and postprandial dysregulation of carbohydrate and fat are critical pathophysiologic processes underlying CVD and T2D (Ebenbichler et al., 1995; Tushuizen et al., 2005). Furthermore, it is clear that regular physical activity is cardioprotective, but the greatest benefit of exercise on cardiometabolic health may be

observed during the postprandial state due to endothelial adaptation (Graham, 2004; Haram et al., 2006).

Exercise and the Postprandial State

Evidence in adults suggests that exercise attenuates postprandial lipemia (Katsanos, 2006) and improves endothelial function (J. M. R. Gill et al., 2004). However, the majority of these studies use continuous exercise at a moderate intensity (i.e. traditional exercise) as the exercise prescription. Recent evidence suggests that, in adults, high-intensity exercise is more beneficial in improving markers of CVD, including endothelial function, than a traditional clinical approach (Gaesser & Angadi, 2011; Gibala et al., 2012). These data are accompanied by evidence that high-intensity exercise prior to a high-fat meal completely ameliorates high-fat meal induced postprandial endothelial dysfunction (Tyldum et al., 2009). Nevertheless, despite the increasing literature to support intensity-dependent benefits of exercise on cardiovascular outcomes, (Tanasescu et al., 2002) very few studies incorporate high-intensity exercise in youth (Ingul et al., 2010).

Youth and High-intensity Exercise

Interestingly, younger populations may be better suited for high-intensity exercise than adults, because children and adolescents exhibit greater resistance to fatigue in response to repeated bouts of high-intensity exercise (Ratel, Lazaar, Williams, Bedu, & Duché, 2003). Additionally, during high-intensity exercise, children exhibit an ability to reach a steady state of oxygen consumption faster than adults, and children experience

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less oxygen drift during high-intensity exercise than adults (Armon, Cooper, Flores, Zanconato, & Barstow, 1991). This lack of oxygen drift may explain why children experience lower lactate levels at similar workloads than adults (Eriksson, 1980), which makes children a prime population to utilize high-intensity type exercise.

Despite this evidence, no studies have examined whether high-intensity interval exercise can improve measures of endothelial function in high-risk youth in either a fasted or postprandial state. As more obese youth are increasingly referred for treatment (Force), high-intensity interval exercise may offer clinicians an exercise prescription that is well-tolerated and more efficacious at improving cardiovascular health in this patient population.

In order to respond to the growing epidemic of obesity in youth, translational research on the effects of exercise on cardiovascular health is needed. Compiling this evidence using defined protocols and proximal indicators of vascular health is an essential step towards developing exercise prescriptions for disease prevention approaches.

Significance

Obesity in youth is associated with increased cardiometabolic morbidity and premature mortality (Reilly & Kelly, 2010), and most obese youth will become obese adults.(Freedman et al., 2005) Recent projections suggest that by the time today's youth enter the workforce, obesity will account for as much as $956 billion dollars or 16-18% of the total US health-care costs (Wang, Beydoun, Liang, Caballero, & Kumanyika, 2008). Much of the increased costs are due to obesity-related diseases such as CVD and

T2D, which have antecedents early in life (Freedman, Dietz, Srinivasan, & Berenson, 1999). Approximately 90% of obese youth exhibit at least 1 CVD risk factor (Cook et al., 2003), and this is exacerbated by incremental increases in obesity (Freedman et al., 2007). When non-invasive techniques to assess subclinical atherosclerosis are applied to the pediatric population, they clearly illustrate that early atherosclerotic processes are compounded by adiposity, insulin resistance, and hyperglycemia in youth (Urbina et al., 2009). These findings underscore the urgency for addressing cardiometabolic health in targeted populations of obese youth and highlight the importance of proximal measures of vascular health and subclinical atherosclerosis.

Lifestyle modifications represent the cornerstone of weight management in youth; however, very few successful pediatric obesity treatments are currently available (Whitlock et al., 2010), and only a small number include measures of subclinical atherosclerosis as endpoints (Fernhall & Agiovlasitis, 2008). Most interventions that focus on improving cardiovascular health in obese youth evaluate traditional CVD risk factors. Even in adults, traditional CVD risk factors do not fully explain cardiovascular health,(Naghavi et al., 2003) and evidence suggests that postprandial dysregulation may be a better predictor of future cardiovascular events (Bansal et al., 2007). The postprandial state may be more reflective of a physiological regulation and dysregulation, as most individuals eat throughout the day. Therefore, it is critical to examine if and how interventions impact cardiovascular health during the critical postprandial period. Given the potential prognostic value of endothelial function on cardiovascular events in adults(Vita & Keaney, 2002), the degree to which acute perturbations are associated with

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endothelial dysfunction across different populations of youth is of interest. Moreover, the ability of exercise to attenuate postprandial endothelial dysfunction has important implications for developing future CVD prevention programs in high-risk youth. This dissertation addresses one of the most significant public health problems facing our society in terms of the numbers of youth impacted by obesity and the current and future healthcare costs of the target population. The approach is significant in that we employed direct measures of cardiovascular health under physiologically meaningful conditions using a clinically relevant translational design. The overall goal of this study was to examine the potential for high-intensity exercise to improve postprandial endothelial function in obese youth.

Specific Aims and Hypotheses

Aim #1: To determine the effect of high-intensity interval exercise on fasting and postprandial endothelial function in obese (BMI-percentile $\geq 97^{th}$) adolescents.

Hypothesis #1.1: A single bout of exercise prior to consumption of a high-fat meal will protect against postprandial endothelial dysfunction as measured by sustained brachial artery flow mediated dilation.

Hypothesis #1.2: A single bout of exercise will improve fasting endothelial function.

Delimitations

The delimitations for this study include: Males who are between the ages of 13 and 17. They must possess a BMI $\geq 97^{th}$ percentile for their given age. They must be non-smokers with no history of Type 1 or Type 2 diabetes and no current medication use. In addition, since the study required exercise, participants must have no physical

impairments that might limit exercise ability (e.g. Asthma, orthopedic limitations etc.). Also, participants must not meet the current evidence based guidelines for physical activity for youth (<60min/day).

Limitation

There may be alternative approaches to examining how exercise improves CVD risk in youth. The challenge with assessing cardiovascular health in younger populations is that no one measure best predicts future risk. Several risk factors/biomarkers of CVD have been proposed in youth (Balagopal et al., 2011; Kavey et al., 2006), but we opted to assess a proximal indicator of vascular health that is one of the earliest manifestations of the atherosclerotic process (Verma, Buchanan, & Anderson, 2003). It is acknowledged that structural measures (e.g. carotid intimal medial thickness) are also important early indicators of subclinical atherosclerosis in youth (Urbina et al., 2009), but structural measures may require long-term investigation and may not be sensitive to acute perturbations in diet and exercise. The nature of our design was to establish the feasibility of high-intensity exercise to improve endothelial function in a postprandial period in the hopes of translating this evidence into future interventions for high-risk youth. However, due to the nature of the study, exclusion of female participants was necessary in this proof of concept design, as female participants require specific controls due to menstrual cycle, which can influence results (Thijssen et al., 2011). Additionally, the lack of ability to measure tanner stage is a limitation, but given the nature of the investigation, we feel that this does not limit our ability to determine the success of our aims. While alternative exercise strategies were considered, this novel and innovative exercise dosage deserves further attention for its cardioprotective properties.

CHAPTER 4: STUDY DESIGN AND METHODS

Study Design

A randomized, cross-over design by condition was used for the following study. The Arizona State University Institutional Review Board approved the study, and all participants as well as a parent/guardian provided written informed consent prior to enrollment.

Recruitment

We enrolled a total of 14 obese (BMI-percentile 98.5 ± 0.6) male adolescents between the ages of 13-16 (14.3 ± 1.0 years). Participants were recruited via a variety of efforts including flyers (Appendix H and I), newspaper ads, approved social media, re-contacting previous study participants, and word-of-mouth. Recruitment efforts were facilitated in both English and Spanish.

Participant Screening

After subjects were identified via recruitment efforts (Appendix P for recruitment consort), they were screened using a standard in-person or telephone screening process in their preferred language (either English or Spanish) by using a standardized screening form (Appendix B). If potential participants met all inclusion criteria, they were invited to come to the ASU Healthy Lifestyle Research Center (HLRC) for a screening visit. For this visit, they were asked to be fasted; however, it was not a requirement of the visit. If they were not fasted, consent and assent were received, anthropometrics were taken, and an additional fasting visit was scheduled.

Rolling Enrollment

Participants were enrolled after successful completion of initial phone screening. Enrollment began on June 2013 and was concluded on January 2014. A detailed description of enrollment based on screening are provided in Appendix P.

Eligibility

The inclusion and exclusion criteria for the present study are located in Appendix A. We recruited individuals who did not meet the current evidence-based guidelines for physical activity for youth (<60min/day) (W. B. Strong et al., 2005), as prior/current exercise training my elicit different responses due to arterial remodeling (D. J. Green, 2009). Participants must have been males aged 13-17 with a BMI percentile $\geq 97^{th}$ who had no exercise limitations. Participants were excluded if they had type 1 or type 2 diabetes. If participants were taking medication, vitamin C, or a multi-vitamin, they were excluded. During the screening visit, we excluded participants who we are unable to achieve a useable FMD image due to poor acoustic window of the ultrasound procedure. During the phone screening, we excluded participants who identified as lactose intolerant, and if they experienced meal intolerance (i.e. diarrhea), they were either excluded from the study or asked to re-do the visit in which they experienced the issue.

Randomization

A simple block-randomization was used for sequence of meal challenge visits wherein 50% of participants received the Exercise plus Meal (Ex+M) condition prior to the (meal only) MO condition and the other 50% received the MO condition prior to the Ex+M condition. This was done to control for any possible sequencing effect and was examined in data analysis.
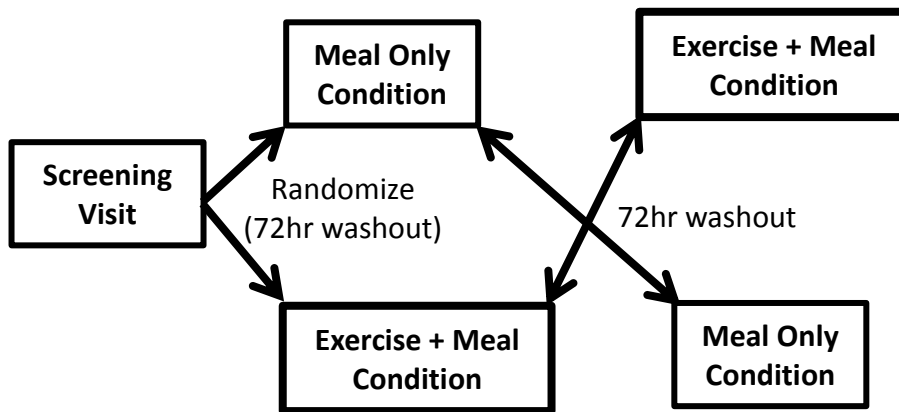
Sample Size

Sample size was based upon a previously conducted power analysis using preliminary data on lean youth (Appendix L) to test change in screening FMD versus 4hr FMD across the 2 conditions (MO and Ex+M). The effect size (*f*) used for the analysis was 0.25, which was estimated using the preliminary data for this analysis using partial η^2 for a condition x time interaction. Utilizing an $\alpha = 0.05$ at a power $(1-\beta) = 0.80$ for 2 groups with 2 measurements and a correlation between repeated measures of $r = 0.60$, we required a sample size of *n*=14 to achieve statistical significance.

Methods

Eligible participants were invited to come to the HLRC for 4 visits detailed

below, and a 100% retention rate was achieved.

Screening Visit

Participants arrived at the HLRC at ~09:00 after an overnight fast (>10 hrs). After

assent and consent were obtained, a brief physical exam was performed to assess height

and weight. Body weight was used to determine the meal composition given, in which

0.7g of fat was given per kg of body weight. Supine blood pressure was measured in

triplicate using an appropriately-sized cuff on the left arm after the subject has rested

quietly for at least 15 min. This was done in order to determine the necessary pressure

needed of the cuff during the occlusion period for the FMD procedure. Participants had

an assessment of their brachial artery FMD tested for baseline measure and as a screening

criterion using previously established methodology (Harris et al., 2010; Thijssen et al.,

2011). Cardiorespiratory fitness (VO$_2$peak) was determined using a mechanically braked

cycle ergometer and an integrated metabolic system (ParvoMedics TrueOne 2400).

Participants were oriented to the testing equipment and cycling cadence (50-70 RPMs)

prior to test initiation. The McMaster protocol was used to determine VO$_2$peak, as it

allows for height-specific workload adjustments in order to provide steady-state exercise

increases in a linear fashion. VO$_2$peak was based upon subjective (participant exhaustion

despite verbal encouragement) and objective (RER > 1.0) criteria and defined as the

highest 15-second oxygen average consumption achieved. Work rates in relation to

oxygen consumption and heart rate (HR) were recorded throughout the test and

subsequently used to determine workloads for the exercise bout. At the conclusion of the VO$_2$peak test, participants were given a snack. Upon successful completion of the screening visit, participants were randomized to either the Ex+M→M sequence or M→Ex+M sequence. A minimum washout period of 72hr after any exercise visit was required in order to minimize the effect of exercise on endothelial adaptation. This time-course was selected based on the available time-course literature on high-intensity exercise on endothelial function.(Haram et al., 2006) Regardless of sequence randomization, study procedures were identical. Table 2 gives a detailed description of the order and measures taken on each visit. A detailed study design and timeline chart is presented in Appendix C.

Exercise Visit (sequence Ex+M)

Participants returned to the HLRC at ~17:00 to complete an exercise session. A single bout of high-intensity interval exercise was performed on a cycle ergometer based upon baseline fitness levels obtained during the screening visit. The exercise bout included a 5-min warm-up period (50% of peak HR) followed by 8-intervals of 2-min at >90% peak HR and 2-min of active recovery (~50% of peak HR) between each high-intensity period. After completion of the 8-intervals, a 5-min cool down period of unloaded pedaling was performed, for a total of 42-min of exercise. This exercise stimulus was selected based on research in adults, (Tyldum et al., 2009) and a similar exercise dose has been used in obese youth to improve cardiac function (Ingul et al., 2010). Participants were instructed to refrain from further exercise and caffeinated beverages and were provided a standardized meal which was consumed prior to 22:00.

Meal Visits (regardless of sequence)

Participants returned to the HLRC at ~9:00 for assessment of fasting endothelial function measured by brachial artery FMD. Participants rested quietly in the supine position in a dim, climate controlled room for at least 20-min prior to FMD assessment. Following baseline FMD measurement, a standardized high-fat meal in milk-shake form was given. The meal was a mixture of~65% fat, ~5% protein, and ~30% carbohydrate, and was consumed at a slow pace in full within 15 min (Table 4 and Appendix M). The fat content of the meal was based upon body weight with a ratio of 0.7g/kg of weight given. This meal was selected for the high level of control, and similar fat loads and meal compositions have been shown to significantly impair endothelial function in healthy adults (Gosmanov, Smiley, Robalino, Siquiera, et al., 2010). Our pilot data suggest that a similar meal composition (a standard meal of 50g Fat, 63g CHO, and 36g Protein) is associated with endothelial dysfunction in lean and overweight adolescents. Following the meal, participants remained seated or lying down, and FMD was reassessed at 2-hr, and 4-hr post consumption. Participants were provided a standardized dinner (subway dinner, Appendix N) and instructed to refrain from any structured exercise and caffeinated beverages for 24-hr.

Brachial Artery Flow-mediated Dilatation

Images of the brachial artery were obtained using high-resolution 2D and Doppler ultrasound (Terason t3000CV ultrasound, Terason Ultrasound, Burlington, MA) with a linear-array transducer at a transmit frequency of 12 MHz. Imaging was performed following 20-min of supine rest on participants' left arm which was immobilized in an

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extended position (80° abduction). Images of the brachial artery were obtained in the longitudinal plane, proximal to the antecubital fold. The ultrasound procedure was individualized to optimize image clarity and to avoid areas of arterial branching. Probe location was marked during the first measurement of each test day and repeat measurements performed in the same region. In addition, anatomical landmarks visible on ultrasound were used to ensure proper location of the probe between trials. Following image optimization (and before cuff inflation), digital video of the procedure was captured for 1-min to determine average brachial artery diameter. A blood pressure cuff (placed 2 cm distal to the antecubital fold) was inflated to >200mmHg (or greater than 50mmHg above systolic) for 5-min. Digital video was recorded for 1-min prior to cuff releases and upon cuff release for at least 3-min in order to assess peak blood flow and determine peak dilation. Digital video was analyzed with semi-automated edge-detection software (Woodman et al., 2001) by a single investigator who was randomized and blinded to conditions. All images were analyzed in duplicate with mean values taken. Arterial diameters were calculated as the mean distance between the anterior and posterior wall at the intima-lumen interface. FMD was defined as the change in vessel diameter from rest to peak dilation as a percentage of the baseline diameter. Peak shear rate was calculated as the peak hyperemic velocity divided by the baseline vessel diameter (Pyke & Tschakovsky, 2005). We have standardized the procedures within our lab (Appendix K) and have shown good intra- and inter-user reliability (Appendix O).

Data Analysis

To test hypothesis 1.1, we used a repeated measures ANOVA to analyze the magnitude of change in FMD from screening to 4 hour FMD by condition. Additionally,

adjusted for shear stress and baseline artery diameter for each measure in order to reduce confounding. Adjusting for shear stress and baseline artery diameter was necessary, as they are known to influence FMD and may be influenced by the meal and/or exercise (Thijssen et al., 2011). Hypothesis 1.2 was evaluated in the same statistical analysis substituting baseline fasting FMD as the measure of interest. These analytical procedures are similar to those used in similarly designed postprandial endothelial function studies (Tyldum et al., 2009).

CHAPTER 5: RESULTS

Baseline Characteristics

Fourteen obese male adolescents completed all study visits and were used for subsequent

analysis. Of the 14 participants, 9 were classified as being severely obese, with their

BMI-percentile exceeding 120% of the 95%tile for age and gender.(Aaron S. Kelly et al.,

2013) Based upon screening blood pressure, which was taken while supine, we observed

that 4 participants exhibited pre-hypertension and 1 participant was classified as

hypertensive. Table 1 shows the sample demographic, anthropometric, and blood

pressure characteristics taken during the screening visit.

Table 1. Sample characteristics of adolescents who completed the study.

n=14	Mean ± SD	Range
Age	14.3 ± 1.0	13 - 16
Height (cm)	170.1 ± 8.2	156.5 - 185.0
Weight (kg)	92.6 ± 10.2	77.5 - 111.1
BMI (kg/m2)	31.9 ± 1.8	28.5 - 34.5
BMI-percentile (%)	98.5 ± 0.6	97.0 - 99.2
Obesity Status	Severely Obese (n=9)	
SBP (mmHg)	119 ± 7	106 - 133
DBP (mmHg)	65 ± 8	43 - 77
Resting HR (bpm)	68 ± 8	58 - 85
Hypertension status	Hypertensive (n=1), Pre-hypertension (n=4)	

All data are mean ± SD

Table 2 gives a details description and order of methods taken during each visit. Each

participant completed the Meal visit on 2 different occasions. One occasion was preceded

by an exercise visit while another visit was preceded by no exercise. For further

reference, a detailed study design and timeline chart appears in Appendix C.

Table 2. Detailed description of each visit.

Screening
Arrive 09:00 fasted
Consent/Assent
Anthropometrics
Lay down in dark quiet room 20min
Blood pressure (Triplicate)
Flow-Mediated Dilation
VO_2peak test
Randomization
(Meal only or Exercise + Meal)

Exercise Visit
Arrive 17:00
5 min warm-up
8 x 2min ≥90% Hrmax
2min active recovery
5 min cool-down
Subway dinner prior to 22:00

Meal Visit
Arrive 09:00 fasted
Lay down in dark quiet room 20min
Blood pressure (Triplicate)
Flow-Mediated Dilation
Milkshake consumed (65% FAT, 30% CHO, 5% PRO)
Flow-Mediated Dilation (2 hours post-meal)
Flow-Mediated Dilation (4 hours post-meal)

Table 3 displays the exercise testing results from the VO$_2$peak test. The VO$_2$peak test was

used in order to determine an exercise prescription which consisted of 8 X 2min bouts at

90%HRmax and 2 min active recovery between bouts at 50% HRmax. All participants

completed all 8 bouts of exercise which was preceded by a 5min warm-up and followed

by a 5min cool-down. On average >6 of the bouts were completed at 90%HRmax.

Table 3. Exercise testing and exercise prescription characteristics.

	Mean ± SD	Range
VO2peak (ml/kg/min)	27.7 ± 4.9	20.8 - 36.4
VO2peak (L/min)	2.6 ± 0.5	1.8 - 3.3
HRmax (bpm)	195 ± 6	186 - 207
RERpeak	1.06 ± 0.04	0.98 - 1.15
Plateau in VO$_2$	4 participants	
Peak watts	170 ± 32	121 - 221
50% HRmax (bpm)	97 ± 3	92 - 103
90% HRmax (bpm)	175 ± 6	165 - 185
50% work load (watts)	43 ± 7	30 - 50
90% work load (watts)	141 ± 28	102 - 190
Number of bouts completed	All bouts completed by each participant	
Number of bouts completed at ≥90% HRmax	6.2 ± 0.8	5 - 8

All data are mean ± SD

Table 4 shows the meal composition breakdown for the cohort. Each meal was tailored to

the body weight of each participant with a fat load of 0.7g of fat given per kg of

bodyweight.   Table 4. Weight of participants and meal characteristics.

	Mean ± SD	Range
Weight (kg)[#]	92.6 ± 10.2	77.5 - 111.1
Fat load (g)	64.9 ± 7.0	54.6 - 77.7
Total (kcal)	889 ± 95	746 - 1065
Fat (kcal) ~65%	583 ± 62	489 - 699
CHO (kcal) ~30%	264 ± 28	222 - 316
Protein (kcal) ~5%	45 ± 5	37 - 53

All data are mean ± SD
[#]Fat load used is based upon body weight of each
participant, 0.7g of fat per kg of body weight

Main Outcomes

Aim #1: To determine the effect of high-intensity interval exercise on fasting and postprandial endothelial function in obese (BMI-percentile ≥97th) adolescents.

Hypothesis #1.1: A single bout of exercise prior to consumption of a high-fat meal will protect against postprandial endothelial dysfunction as measured by sustained brachial artery flow mediated dilation.

We observed that despite consumption of a high-fat meal there was no decrement in FMD at 2hr or 4hr post-meal in either condition. No significant differences were observed between the exercise + meal condition and the meal only condition.

Table 5. Effect of exercise on postprandial endothelial function.

	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		**Meal Only**	
Screening	7.0 ± 3.0	5.2 - 8.8	7.0 ± 3.0	5.2 - 8.8
Fasting	7.5 ± 3.0	5.7 - 9.3	7.4 ± 2.8	5.6 - 9.2
2 hour	8.4 ± 3.4	6.5 - 10.2	7.6 ± 3.9	5.8 - 9.4
4 hour	8.8 ± 3.9	7.0 - 10.7	8.6 ± 4.0	6.7 - 10.4
				p-value
Linear Mixed Model		Condition		0.667
		Time		0.277
		Condition * Time		0.974

Data are mean ± SD
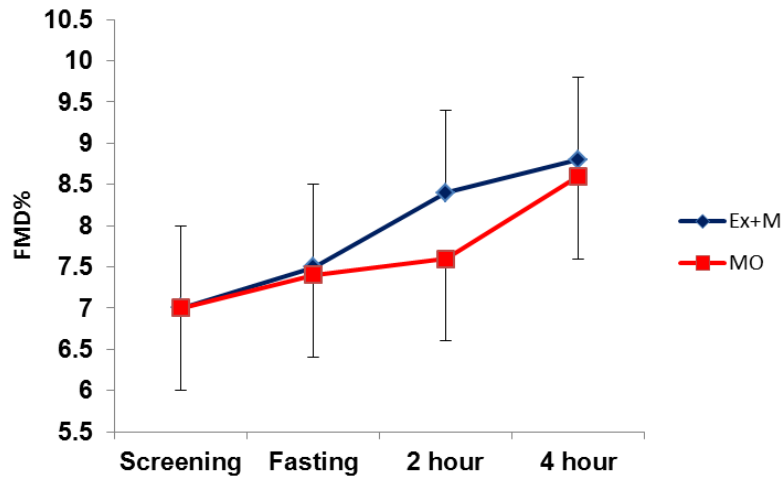
Figure 1. Effect of high-intensity interval exercise on postprandial endothelial function in obese adolescent males. Data are presented as means ± SE. Ex+M = Exercise prior to the meal condition; MO = Meal only condition without prior exercise.

Upon removal of the screening visit from the analysis we observed no difference between groups.

While FMD appears to improve over time, we observed no significant time effect of the meal.

Suggesting, the meal was unable to induce endothelial dysfunction.

Table 6. Effect of exercise on postprandial endothelial function, without screening.

	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		Meal Only	
Fasting	7.5 ± 3.0	5.7 - 9.3	7.4 ± 2.8	5.6 - 9.2
2 hour	8.4 ± 3.4	6.5 - 10.2	7.6 ± 3.9	5.8 - 9.4
4 hour	8.8 ± 3.9	7.0 - 10.7	8.6 ± 4.0	6.7 - 10.4
				p-value
Linear Mixed Model		Condition		0.632
		Time		0.418
		Condition * Time		0.928

Data are mean ± SD
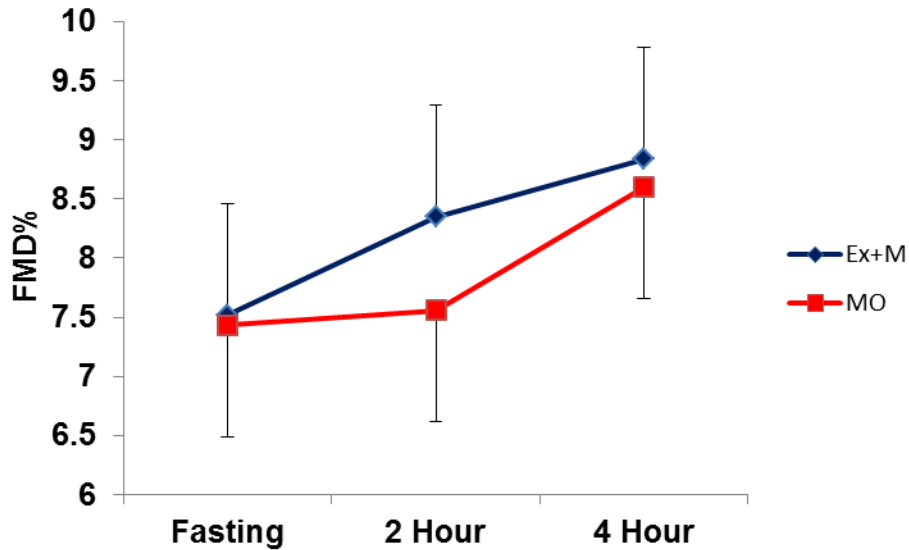
Figure 2. Effect of high-intensity interval exercise on postprandial endothelial function in obese adolescent males, without screening visit. Data are presented as means ± SE. Ex+M = Exercise prior to the meal condition; MO = Meal only condition without prior exercise.

Hypothesis #1.2: A single bout of exercise will improve fasting endothelial function.

We observed no improvement in fasting endothelial function the morning after high-intensity exercise. We observed no difference between fasting and screening endothelial function in either condition.

Table 7. Effect of exercise on fasting endothelial function.

	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		**Meal Only**	
Screening	7.0 ± 3.0	5.2 - 8.8	7.0 ± 3.0	5.2 - 8.8
Fasting	7.5 ± 3.0	5.7 - 9.3	7.4 ± 2.8	5.6 - 9.2
				p-value
RM ANOVA		Condition		0.965
		Time		0.329
		Condition * Time		0.927

Data are mean ± SD
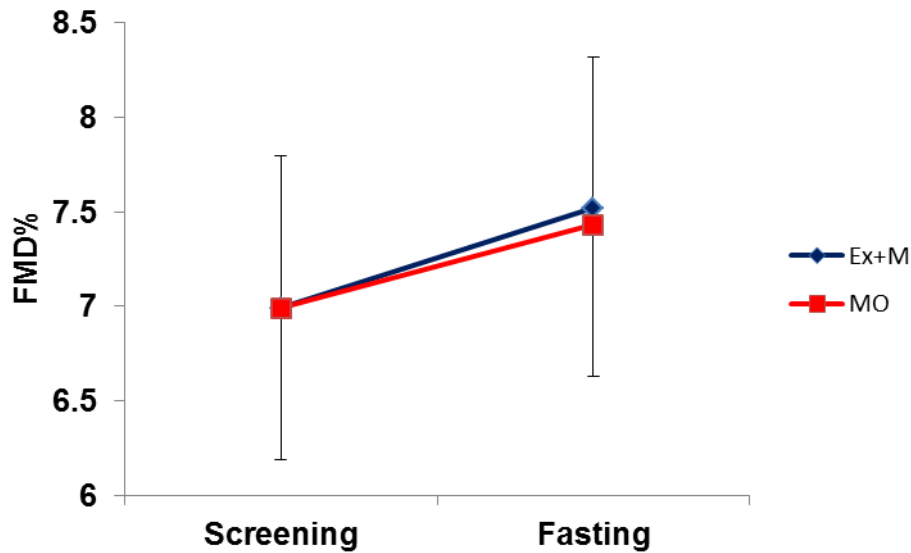
Figure 3. Effect of high-intensity interval exercise on fasting endothelial function in obese adolescent males. Data are presented as means ± SE. Ex+M = Exercise prior to the meal condition; MO = Meal only condition without prior exercise.

Other Outcomes Measures and Exploratory Hypothesis

When examining the only the exercise plus meal condition, we observed a significant

reduction in time to peak vasodilation after exercise in the fasting condition compared

with 2 hours (p=0.02) and a trend at 4 hours (p=0.08). We observed no difference in the

meal only condition and when groups were compared we found no differences.

Table 8. Effect of exercise on time to peak vasodilation.

	Time to Peak Exercise + Meal	Time to peak Meal Only
Fasting (sec)	69.8 ± 19.8	80.0 ± 19.6
2 hour (sec)	80.5 ± 18.6*	79.0 ± 21.5
4 hour (sec)	77.5 ± 19.9$^{\#}$	78.9 ± 21.5

		p-value
Linear Mixed Model	Condition	0.45
	Time	0.66
	Condition * Time	0.541

Data are mean ± SD
* p = 0.02 (paired t-test vs. fasting)
$^{\#}$ p = 0.08 (paired t-test vs. fasting)
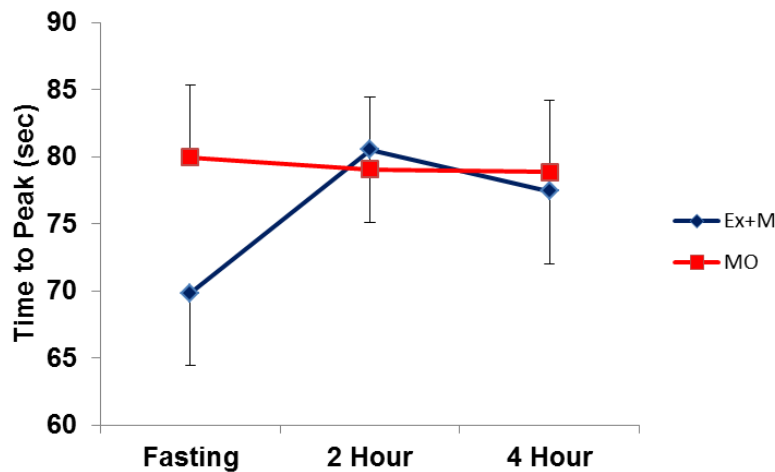
Figure 4. Effect of high-intensity interval exercise on time to peak vasodilation in obese

adolescent males. Data are presented as means ± SE. Ex+M = Exercise prior to the meal

condition; MO = Meal only condition without prior exercise.

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Despite the consumption of a high-fat meal we observed no change in shear rate (Table 9) in either condition. Despite this there remains a modest, significant relationship between shear rate and FMD and will be adjusted for in further analysis.

Table 9. Effect of exercise on average shear rate.

|	Average Shear Rate Exercise + Meal	Average Shear Rate Meal Only
Fasting (1/sec)	355 ± 100	346 ± 118
2 hour (1/sec)	326.6 ± 119	325 ± 102
4 hour (1/sec)	348 ± 98	332 ± 75
		p-value
Linear Mixed Model	Condition	0.694
	Time	0.638
	Condition * Time	0.965

Data are mean ± SD
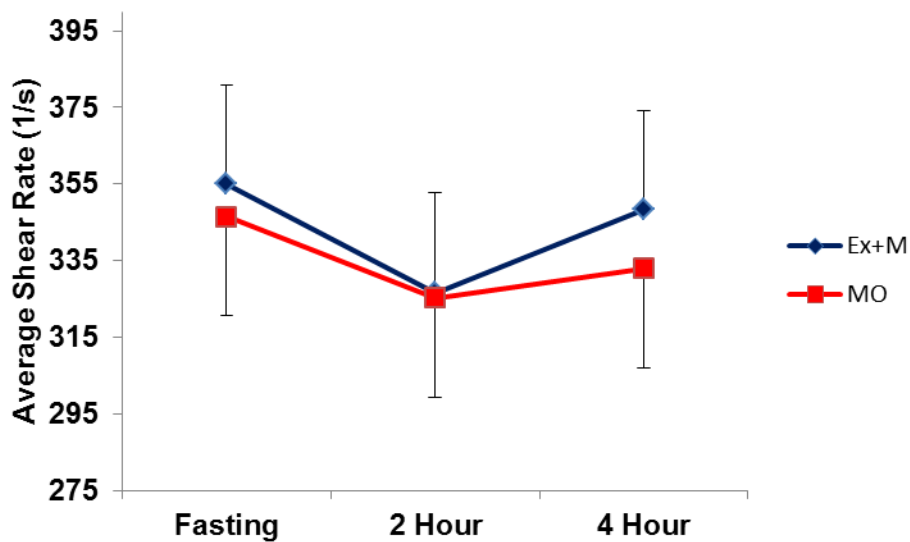
Figure 5. Effect of high-intensity interval exercise on average shear rate in obese adolescent males. Data are presented as means ± SE. Ex+M = Exercise prior to the meal condition; MO = Meal only condition without prior exercise.

Table 10. Pearson correlations of baseline (pre-occlusion and during-occulsion) and peak diameters by condtion.

Screening:	Pre-occlusion	Occulsion	Peak
Pre-occlusion	-	0.992	0.948
Occulsion	0.992	-	0.975
Peak	0.948	0.975	-
Fasting			
Pre-occlusion	-	0.932	0.941
Occulsion	0.932	-	0.975
Peak	0.941	0.975	-
2 Hour			
Pre-occlusion	-	0.980	0.967
Occulsion	0.980	-	0.974
Peak	0.967	0.974	-
4 Hour			
Pre-occlusion	-	0.965	0.947
Occulsion	0.965	-	0.962
Peak	0.947	0.962	-

All correlations are $p < 0.001$
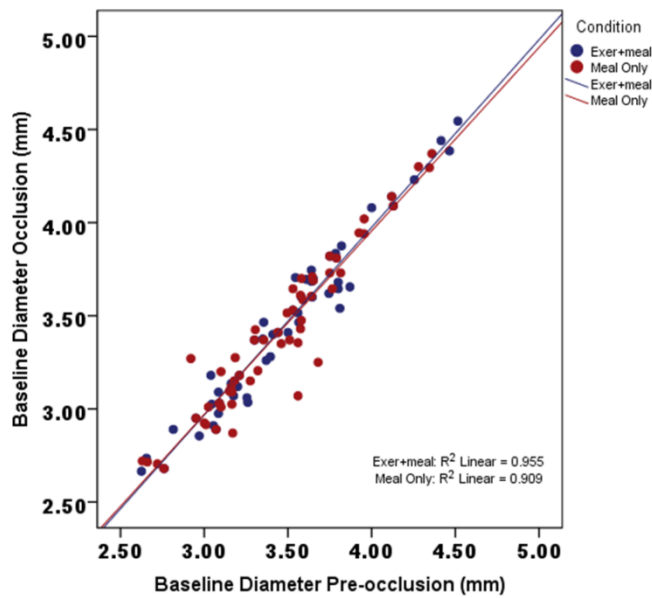
Figure 6. Relationship between pre-occlusion and occlusion baseline diameters split by condition.
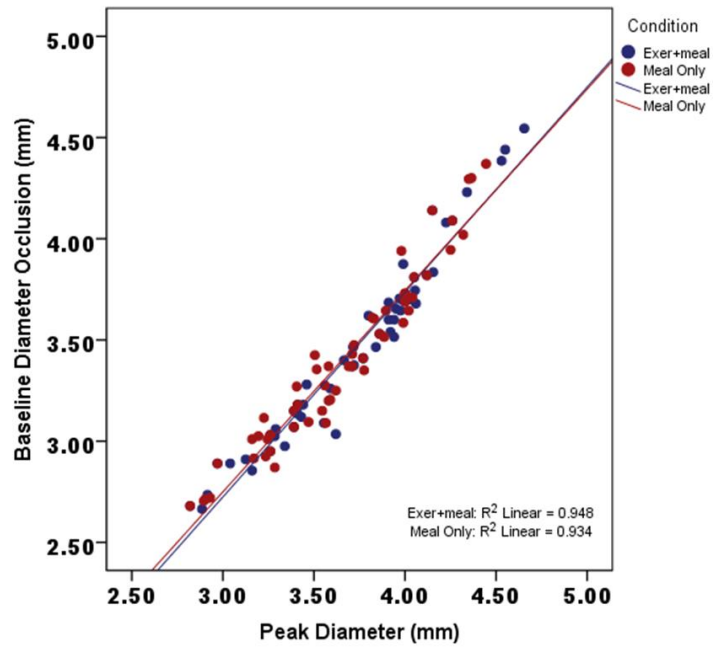
Figure 7. Relationship between peak diameter and occlusion baseline diameters split by condition.
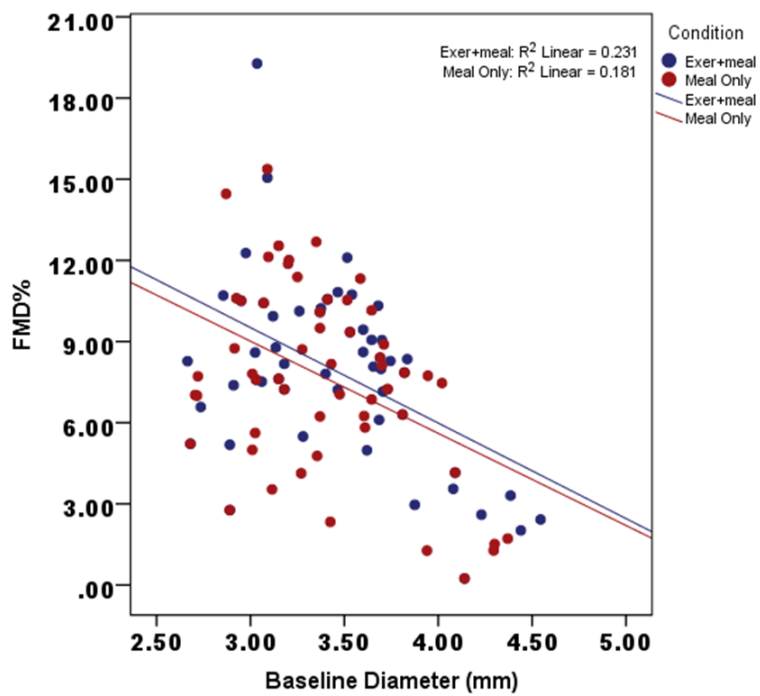
Figure 8. Relationship between FMD% and occlusion baseline diameters split by condition. Over the entire sample there was a significant correlation between FMD and baseline diameter ($r = -0.45$, $p<0.001$).
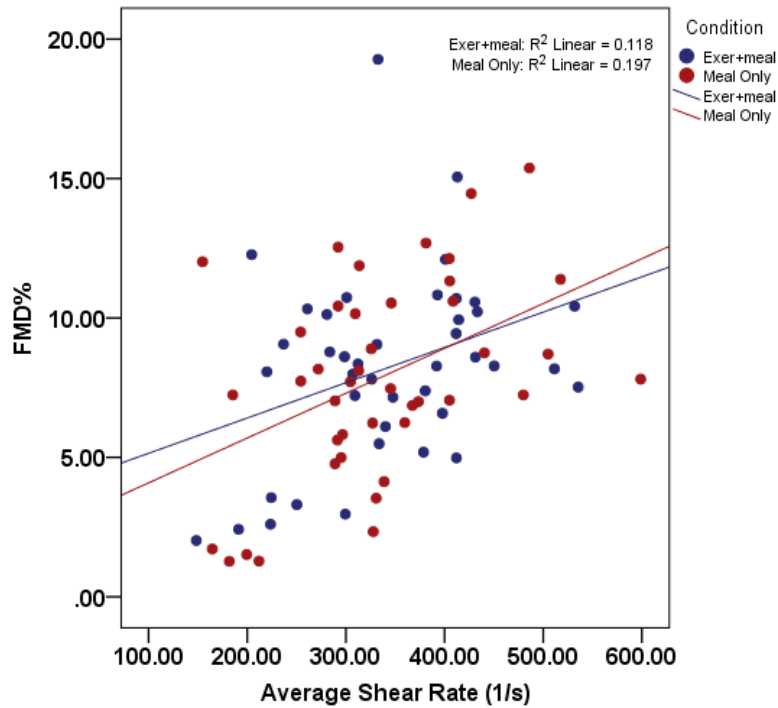
Figure 9. Relationship between FMD% and shear rate split by condition. Over the entire sample there was a significant correlation between FMD and shear rate ($r = 0.40$, $p<0.001$).

Figures 8 and 9, show a significant relationship between FMD and baseline diameter and shear rate, respectively. After adjusting for baseline diameter and shear rate, we observed no difference between the two conditions.

Table 11. Effect of exercise on postprandial endothelial function adjusted for baseline diameter and shear rate.

	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		Meal Only	
Fasting	7.7 ± 3.0	6.0 - 9.3	7.4 ± 2.8	5.8 - 9.0
2 hour	8.4 ± 3.4	6.8 - 10.1	7.5 ± 3.9	5.8 - 9.1
4 hour	8.9 ± 3.9	7.3 - 10.5	8.5 ± 4.0	7.0 - 10.1
				p-value
Linear Mixed Model		Condition		0.417
		Time		0.342
		Condition * Time		0.891
		Baseline Diameter		<0.001
		Shear Rate		0.106

Data are mean ± SD
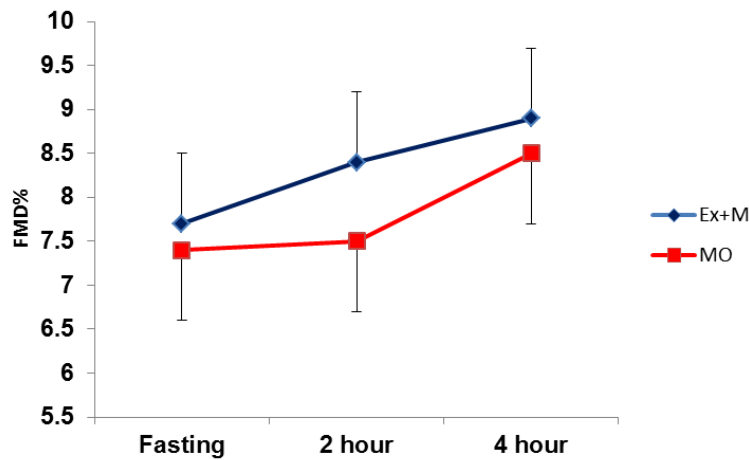
Figure 10. Effect of high-intensity interval exercise on postprandial endothelial function in obese adolescent males, adjusted for baseline diameter and shear rate. Data are presented as means ± SE. Ex+M = Exercise prior to the meal condition; MO = Meal only condition without prior exercise.

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Data were split at the median by baseline levels (screening visit) of endothelial function. Those whose FMD was >7.6% were classified as good function while those <7.6% were classified as having endothelial dysfunction. Date were analyzed as separate groups adjusting for baseline diameter and shear rate, showing no endothelial dysfunction due to the meal in either group.

Table 12. Effect of exercise on postprandial endothelial function adjusted for baseline diameter and shear rate, split by baseline endothelial dysfunction.

Good Function* ($n=7$)	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		Meal Only	
Fasting	8.6 ± 1.0	6.6 - 10.7	8.2 ± 1.0	6.2 - 10.2
2 hour	9.0 ± 1.1	6.9 - 11.2	7.9 ± 1.0	5.9 - 10.0
4 hour	8.3 ± 1.0	6.3 - 10.3	9.5 ± 1.0	7.5 - 11.5
				p-value
Linear Mixed Model		Condition		0.882
		Time		0.870
		Condition * Time		0.506
		Baseline Diameter		0.301
		Shear Rate		0.174

Dysfunctional* ($n=7$)	FMD%	95% CI	FMD%	95% CI
	Exercise + Meal		Meal Only	
Fasting	6.7 ± 1.4	4.0 - 9.4	6.5 ± 1.3	3.8 - 9.3
2 hour	7.9 ± 1.3	5.2 - 10.6	7.0 ± 1.4	4.2 - 9.8
4 hour	9.3 ± 1.3	6.6 - 12.0	7.5 ± 1.3	4.8 - 10.3
				p-value
Linear Mixed Model		Condition		0.407
		Time		0.421
		Condition * Time		0.824
		Baseline Diameter		0.006
		Shear Rate		0.477

Data are mean ± SE

*Screening visit FMD was used in order to determine endothelial dysfunction, data were split at the median with FMD < 7.6 being classified as dysfunctional

CHAPTER 6: DISCUSSION

Discussion

The present study investigated the effect of high-intensity interval exercise on postprandial endothelial function in obese male adolescents. We observed that high-intensity interval exercise, the evening prior, had no effect on fasting endothelial function and did not improve postprandial endothelial function when compared with a meal only condition. Despite consuming a high-fat meal, endothelial function in the meal only condition did not decline from fasting levels. Although with exercise endothelial function increased at 2 hours and 4 hours post-meal, the effect size was not substantial enough to show an improvement over a condition with no prior exercise.

We designed the present study based upon pilot data which suggested that exercise protected against postprandial endothelial dysfunction in lean male adolescents (Appendix L). Due to our pilot study experience, we implemented several designs as well as methodological changes which were aimed at reducing variability observed into the present investigation. Unlike our pilot study, we used a meal that was tailored to each participant instead of a fixed-dose. This allowed for an individualized approach, which we felt was more rigorous than a fixed-meal challenge. In order to reduce variability due to food consumed within the testing period, we gave participants standardized meals the night prior as an added measure of control. Although our pilot study was conducted in lean youth, we chose to examine a high-risk obese population, as the translational potential to use exercise as a prescription may be more readily applied in this population. We made rigorous attempts to standardize and refine our FMD procedures and protocol on both the assessment and the analysis end. Participants were only allowed to read, do

homework, or sleep during the 5 hour FMD test period, as we wanted to exclude any behaviors which might have influence on endothelial function. We demonstrated good inter-user and intra-observer reliability of our FMD analysis procedure. However, despite these controls and methodological refinements, we were unable to demonstrate that exercise was able to protect against postprandial endothelial dysfunction. Furthermore, large inter-individual difference still remained within our data, which makes interpretation of our findings challenging. Despite these outcomes, there are several possible explanations for our results.

Dose of Exercise and Mode

We utilized a high-intensity interval exercise bout that had been previously shown to improve postprandial endothelial function in sedentary overweight men. (Tyldum et al., 2009) The exercise protocols were very similar we used a 8 X 2min bout with 2min of active recovery, in contrast Tyldum et al. used a 4 x 4min bout with 3min of active recovery. The total amount of time spent in the high-intensity phase was the same, and the exercise was well tolerated by the youth in our study. It is possible that a single acute bout of exercise may not be sufficient to improve endothelial function at fasting or during a postprandial period in obese adolescent males. There is substantial evidence to support the efficacy of exercise training on vascular adaptation.(D. Green, 2009) Furthermore, exercise training has been shown to be effective at improving fasting endothelial function in overweight/obese adolescents.(Kelly et al., 2004) Future research should examine whether exercise training may be able to protect against postprandial endothelial dysfunction in obese adolescents.

The modality of exercise used in our study differed from other successful postprandial endothelial function studies. Our bout of exercise was completed on a cycle ergometer, whereas the Tyldum et al. used a treadmill. Various modes of exercise produce different patterns of arterial shear, and differing shear patterns result in distinct responses in vascular function (D. Green et al., 2005; Thijssen et al., 2009). While treadmill and cycling exercise exhibit similar effects on shear rate and shear pattern in lower extremities, what may be lacking is the upper limb movement and shear pattern to stimulate NO bioavailability in the brachial artery. Perhaps, more importantly, the magnitude of changes observed in shear rate during walking appears at a much lower heart rate intensity than during cycling exercise.(Thijssen et al., 2009) Therefore, in exercise that uses a prescription based upon HR, the shear rate during treadmill exercise would surpass that of cycling at the same HR value. Coupled with the effects of upper body movement and larger amounts of muscle mass used for the exercise, treadmill exercise may be a more optimal exercise mode for acute exercise studies. However, this effect could be localized to different artery sections. Therefore, future research should examine the effect that cycling exercise has on femoral flow-mediated dilation in order better determine the effect of cycling on this more localized artery. Additionally, a comparison of the same high-intensity interval exercise protocols on different modalities would be able to answer the questions posed.

This hypothesis, that treadmill walking may be superior to cycling for acute improvement in postprandial endothelial function, is supported by two studies in youth, which have been successful at inducing endothelial dysfunction and showing a protective

effect of exercise (MJ Sedgwick et al., 2013; MJ. Sedgwick et al., 2014). Each study provided youth with 2 meals (breakfast and lunch) and measured FMD at fasting, 3 hours, and 6 hours after consumption of the first meal. The exercise for one study occurred in the evening and consisted of four 15 min walking periods at 60% of VO_2peak, which included 3 min of rest between bouts. The other investigation split 60min of exercise into 6 x 10 min of bouts throughout the day with 50min of rest between each bout. Each 10min bout was at 70% of VO_2peak on a treadmill. In both studies, they observed no difference in FMD at fasting; however, at 3 hours and 6.5 hours post-meal, the exercise was able to protect against postprandial endothelial dysfunction observed in the meal only condition ($p < 0.001$). Thus, when combined with the findings from Tyldum and colleagues, perhaps, the exercise modality is a potential explanation for our findings.

Meal Dose and Composition

We observed that despite consuming a high-fat meal, the obese adolescent males did not exhibit endothelial dysfunction in either condition. While our meal was tailored to each individual with a fat load of 0.7g/kg of body weight given, this "dose" may not have been enough to induce endothelial function consistently in this population. The studies by Sedgwich et al. (MJ Sedgwick et al., 2013; MJ. Sedgwick et al., 2014) were able to induce endothelial dysfunction at 3hrs with an initial meal challenge of 22.2 kcal/ kg body weight to lean youth. While our meal dose was greater overall, we were only providing 9.6kcal/kg body weight. However, if we provided the same meal dose used by Sedgwich and colleagues our participants, on average, would have consumed ~2055kcal,

which may not be practical or "real world". The study by Tyldum et al.(Tyldum et al.,

2009) that provided a fixed meal which was 10.1 kcal/kg of body weight, much similar to

ours, suggested that this may be a function of obese youth requiring a greater dose than

healthy overweight adults in order to induce endothelial dysfunction. Additionally, our

study is not the first to show that a high-fat meal does not induce postprandial endothelial

dysfunction. Ayers et al. gave 7 healthy normal weight and 7 healthy obese young adults

(32 ± 6 yr) a standardized high-fat meal consisting of 60g of fat and 1000kcal (Ayer et al.,

2010). Despite consuming the high-fat meal, they observed no change in FMD from

baseline to 3hr post-meal in either the normal-weight (4.7 ± 4.1 to $4.3\pm3.9\%$) or obese

group (6.2 ± 1.7 to $5.8\pm4.3\%$, $p_{(group*time)} = 0.975$). Future studies may want to examine the

dose response relationship with different tailored high-fat meals in obese youth in order

to determine the optimal load of fat or kcal to provide.

Another potential explanation for why we did not observe postprandial

endothelial dysfunction in our study is due to the nature of the response to the mixed-

meal. Raitakari and colleagues examined the effect of two high-fat meals (1030kcal, 61g

fat) consisting of differing fatty acid compositions in 12 normal-weight/overweight men

and women ($33\pm7yr$, BMI = 24.3 ± 3.1 kg/m^2).(Raitakari et al., 2000) They observed no

change in FMD at 3 hours or 6 hours post-meal in either meal. However, they observed

that, with either meal, participants had increased forearm blood flow, increased

triglycerides, and increased insulin at 3 hours in response to the meal. The increase in

forearm blood flow was highly associated with the insulin response observed ($r = 0.80$,

$p<0.002$). The authors suggest that the hyperinsulinemia response observed in the

postprandial state mediated changes in forearm blood flow, which may counteract any detrimental effects of that meal on endothelial function.

While it is documented that oral and intravenous fat challenges produce postprandial endothelial dysfunction,(Gosmanov, Smiley, Robalino, Siquiera, et al., 2010) the results from mixed meal challenges are not as conclusive.(Jackson et al., 2007) This could potentially be due to the insulin response post-meal, as insulin is a potent vasodilator. In healthy persons insulin via its action on the insulin receptor on the endothelial cell surface stimulates a cascade of molecular signals through a PI3-k pathway to phosphorylate eNOS and stimulates the production of NO to increase vasodilatitory capacity.(Muniyappa et al., 2007). Thus, it is possible that compensatory hyperinsulinemia enhanced NO bioavailability in our study, which could explain the lack of postprandial endothelial dysfunction with the meal observed. This hypothesis is only supported, however, if the endothelium is insulin sensitive, as an insulin resistant endothelial cell favors a MAP-k pathway which produces higher amounts endothelin-1, a potent vasoconstrictor. Future studies should determine if the degree of insulin response during a mixed-meal is the mechanism mediating the lack of postprandial endothelial dysfunction that has been observed.

Population

The present investigation is the first to examine the effects of a mixed-meal in obese adolescents. However, a study by Metzig and colleagues examined the effects of a glucose tolerance challenge on postprandial endothelial function in obese adolescents and found no endothelial dysfunction due to the glucose load.(Metzig et al., 2011) While the

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evidence in favor of a high-fat meal inducing endothelial dysfunction is greater, this highlights the possibility that these young persons, despite being obese, may be protected against any vascular compromise, at least at this stage of the lifecycle.

The lack of arterial aging may have contributed to our findings on postprandial endothelial function. Middle aged and older adults experience losses in arterial compliance, forearm blood flow, and endothelial function over-time. Furthermore, these losses are compounded by a lack of physical activity, but they can be restored with exercise training.(DeSouza et al., 2000) However, young individuals appear to be resistant to reductions in arterial compliance and endothelial function, despite a lack physical activity or low fitness levels.(Seals, Walker, Pierce, & Lesniewski, 2009) Our fasting endothelial function values are consistent with those reported in the literature for obese youth, but there is no data for comparison during a mixed-meal post-absorptive state.(Charakida et al., 2012; Naylor et al., 2011) However, it should be noted that the arterial aging argument only applies to the fasting state, as not data are presently available on the effects of aging on postprandial endothelial function which needs to be investigated. Therefore, despite our sample being sedentary and relatively low fit, they may be protected against reduced endothelial function due to a lack of senescence.

We designed this study on the premise that physically inactive individuals may exhibit greater endothelial dysfunction and are more prone to improvements in postprandial endothelial function with exercise. Johnson and colleagues examined the effects of a high-fat meal on postprandial endothelial function in physically inactive and active normal-weight, healthy young adults. FMD was measured at fasting and 4hrs post-

meal (940kcal, 46%fat). They observed that while the active individuals were protected against endothelial dysfunction at 4hrs post-meal, the inactive persons exhibited significant endothelial dysfunction (p=0.02).(Johnson et al., 2011) While, physical inactivity may play a role in adults, the present study recruited physically inactive persons; thus, this may not be the operation in youth. Furthermore, physical fitness (VO$_2$peak) was not associated with FMD at screening or during the postprandial period at any time point in the present study. More data is needed on the relationship between fitness and endothelial function in youth. However, the present investigation suggests that while the importance of physical activity and fitness is undisputed, it may not play a role on fasting or postprandial endothelial function in obese youth.

Additionally, there were several factors that we did not or were unable to control for in our study, which may have impacted our findings. We did not account for exposure to smoking at the individual or familial/second hand level, exposure to smoke is documented to be associated with endothelial dysfunction.(D S Celermajer et al., 1993) We selected an age range of youth that are undergoing pubertal maturation which may have contributed to the variability in our results. However, recent data suggests that despite pubertal difference on baseline vessel diameter and pubertal on-set insulin resistance, puberty appears to have little effect on endothelial function. (Marlatt et al., 2013) We did not include females in this study; thus, our findings are only applicable to a single gender. The exclusion of females was necessary due to the proof of concept nature as well as the data suggesting that pre-menopausal women at all stages of the menstrual cycle may be inherently protected against postprandial endothelial dysfunction.(Harris,

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Tedjasaputra, Zhao, & Richardson, 2012) Nevertheless, future studies should include females in order to determine if gender specific differences exist in youth.

Overall Approach

There are several features of our overall approach that may have contributed to our findings which are worthy of note. We used FMD as our primary outcome measure, as this had been previously shown to be a good measure of endothelial dysfunction during a postprandial state along as well as its sensitivity to acute perturbations such as exercise. We made many attempts to standardize and reduce the variability within the measure, but despite our best efforts, the reliability and reproducibility of our data must be questioned (Appendix Q). The two fasting conditions without prior exercise, screening FMD, and fasting MO FMD should have been very similar. We observed a mean difference between conditions of 0.48±2.52%; thus, while the mean difference was small, the large standardization is troubling. We observed only a modest correlation between the fasting endothelial function values (r = 0.554, P=0.040), and they were only modestly reliable (ICC= 0.712). Upon further regression analysis, we showed that only 30.7% ($R^2 = 0.307$) of the variance in fasting FMD during the meal is explained by screening FMD, leaving 69.3% of the variance due to other factors. This lack of consistency and sensitivity within same person, despite our best efforts, may have contributed to our findings.

Perhaps, a biomarker of endothelial cell biology, which have been shown to be elevated in youth,(A. S. Kelly et al., 2010) may have been a better approach to answering the hypothesis posed. Additionally, it is possible that a more robust imaging system such

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as magnetic resonance imaging (MRI) could have been used, as its sensitivity is much higher than that of ultrasound.

Currently, to our knowledge, there are no studies detailing the effects of diurnal variation or circadian variation on endothelial function or vascular tone in youth. Data in adults suggest that endothelial function is blunted in the early morning hours (6am) and rises towards mid-day (11am) where it is sustained throughout the day.(Otto et al., 2004) This may be due to higher vascular resistance in the morning hours coupled with lower blood flow, and as the day progresses, vascular resistance is reduced, which improves the ability of vasodilation to increase.(Panza, Epstein, & Quyyumi, 1991) However, diurnal increases in endothelial function have been observed in lean young adults but not in obese young adults (Hallmark et al., 2014), whether this is applicable in youth is unknown. While we did not have a no meal condition to study the effects of diurnal variation, it is plausible that the lack of reduction in endothelial function observed with the meal could be due to a counter effect of increases in diurnal variation. Future studies should include a control condition to examine diurnal variations effect on endothelial function.

Strengths

Strengths of this study include the use of a standardized meal the night prior to meal challenge along with an individually tailored high-fat meal. The inclusion of individuals whose BMI percentile was > 97th and exclusion of physically active individuals allowed us to examine a targeted population of youth at highest-risk for premature CVD. We used a highly reproducible and translatable design by utilizing an

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exercise prescription which would be easily implementable in any setting. We used a rigorous approach to FMD analysis by examining each file in duplicate, which is not common-place in the literature and allowed us to shown good consistency, reproducibility, and reliability of results.

Limitations

While our study has many strengths, including a novel population and a readily translatable design, there are some limitations to note. The lack of inclusion of females did not allow us to access whether our findings were applicable to this gender. We were unable to measure blood samples (i.e. insulin, triglycerides, glucose, ect.), which may have given some additional insight into our findings and allowed us to examine potential mechanisms. The lack of inclusion of a control condition without a meal may have allowed us to determine if diurnal variation was the cause of some of the inter-individual variability and could have been beneficial. While we attempted to control for exercise and food the day prior, participants were free-living, which may have contributed to error. We did not examine sleep quality or duration which may have impacted our findings and should be examined in subsequent investigations. Our population was very heterogeneous and likely contributed to our biological variability.

Future Research

Our findings need to be replicated in a population of youth with existing endothelial dysfunction, as this population may exhibit greater dysfunction with the meal. Further research is needed on the impact of various meal compositions and to determine if an inclusion of a no meal control day to examine diurnal variation is needed. Research

is needed on the dose-response relationship of fat load and degree of endothelial dysfunction which will aide in researchers' ability to assume that a high-fat meal will induce endothelial dysfunction, as a lack of consistency is present within the literature. Future research should also examine the effect of exercise on other measures of endothelial function in a postprandial state.

Potential Implications

Our findings may suggest that a high-fat meal may not induce endothelial dysfunction in obese and severely obese adolescent males. While prior exercise may improve fasting endothelial function in lean youth, this may not be operational in obese male adolescents. However, further research is needed in order to determine the optimal fat and caloric dose by which obese youth exhibit endothelial dysfunction, in order to better examine whether exercise can be protective.

REFERENCES

Aggoun, Y., Farpour-Lambert, N. J., Marchand, L. M., Golay, E., Maggio, A. B., & Beghetti, M. (2008). Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure. *Eur Heart J, 29*(6), 792-799.

Ando, J., & Yamamoto, K. (2009). Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ J, 73*(11), 1983-1992.

Armon, Y., Cooper, D. M., Flores, R., Zanconato, S., & Barstow, T. J. (1991). Oxygen uptake dynamics during high-intensity exercise in children and adults. *J Appl Physiol, 70*(2), 841-848.

Ayer, J. G., Harmer, J. A., Steinbeck, K., & Celermajer, D. S. (2010). Postprandial vascular reactivity in obese and normal weight young adults. *Obesity (Silver Spring), 18*(5), 945-951. doi: 10.1038/oby.2009.331

Bacha F, Saad R, Gungor N, Arslanian SA. (2006). Are obesity-related metabolic risk factors modulated by the degree of insulin resistance in adolescents? *Diabetes Care, 29*(7), 1599-1604.

Balagopal, P. B., de Ferranti, S. D., Cook, S., Daniels, S. R., Gidding, S. S., Hayman, L. L., . . . Steinberger, J. (2011). Nontraditional Risk Factors and Biomarkers for Cardiovascular Disease: Mechanistic, Research, and Clinical Considerations for Youth: A Scientific Statement From the American Heart Association. *Circulation*.

Bansal, Sandeep, Buring, Julie E., Rifai, Nader, Mora, Samia, Sacks, Frank M., & Ridker, Paul M. (2007). Fasting Compared With Nonfasting Triglycerides and Risk of Cardiovascular Events in Women. *JAMA: The Journal of the American Medical Association, 298*(3), 309-316. doi: 10.1001/jama.298.3.309

Barker, D. J. (1995). Fetal origins of coronary heart disease. *BMJ, 311*(6998), 171-174.

Barry, Vaughn W., Baruth, Meghan, Beets, Michael W., Durstine, J. Larry, Liu, Jihong, & Blair, Steven N. (2014). Fitness vs. Fatness on All-Cause Mortality: A Meta-Analysis. *Progress in Cardiovascular Diseases, 56*(4), 382-390. doi: http://dx.doi.org/10.1016/j.pcad.2013.09.002

Bellien, J., Iacob, M., Gutierrez, L., Isabelle, M., Lahary, A., Thuillez, C., & Joannides, R. (2006). Crucial role of NO and endothelium-derived hyperpolarizing factor in human sustained conduit artery flow-mediated dilatation. *Hypertension, 48*(6), 1088-1094. doi: 10.1161/01.HYP.0000246672.72188.bd

Berenson, G. S. (2002). Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. *Am J Cardiol, 90*(10C), 3L-7L.

Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P., 3rd, Tracy, R. E., & Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med, 338*(23), 1650-1656. doi: 10.1056/NEJM199806043382302

Blair, S. N., Kohl, H. W., 3rd, Barlow, C. E., Paffenbarger, R. S., Jr., Gibbons, L. W., & Macera, C. A. (1995). Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA, 273*(14), 1093-1098.

Blair, S. N., Kohl, H. W., 3rd, Paffenbarger, R. S., Jr., Clark, D. G., Cooper, K. H., & Gibbons, L. W. (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA, 262*(17), 2395-2401.

Boo, Yong Chool, Sorescu, George, Boyd, Nolan, Shiojima, Ichiro, Walsh, Kenneth, Du, Jie, & Jo, Hanjoong. (2002). Shear Stress Stimulates Phosphorylation of Endothelial Nitric-oxide Synthase at Ser1179 by Akt-independent Mechanisms: ROLE OF PROTEIN KINASE A. *Journal of Biological Chemistry, 277*(5), 3388-3396. doi: 10.1074/jbc.M108789200

Celermajer, D S, Sorensen, K E, Georgakopoulos, D, Bull, C, Thomas, O, Robinson, J, & Deanfield, J E. (1993). Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation, 88*(5), 2149-2155. doi: 10.1161/01.cir.88.5.2149

Celermajer, D. S., Sorensen, K. E., Bull, C., Robinson, J., & Deanfield, J. E. (1994). Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol, 24*(6), 1468-1474.

Celermajer, D. S., Sorensen, K. E., Gooch, V. M., Miller, Sullivan, I. D., Lloyd, J. K., . . . Spiegelhalter, D. J. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet, 340*(8828), 1111-1115.

Charakida, M., Jones, A., Falaschetti, E., Khan, T., Finer, N., Sattar, N., . . . Deanfield, J. E. (2012). Childhood obesity and vascular phenotypes: a population study. *J Am Coll Cardiol, 60*(25), 2643-2650. doi: 10.1016/j.jacc.2012.08.1017

Cherry, P D, Furchgott, R F, Zawadzki, J V, & Jothianandan, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proceedings of the National Academy of Sciences, 79*(6), 2106-2110.

Cook, S., Weitzman, M., Auinger, P., Nguyen, M., & Dietz, W. H. (2003). Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med, 157*(8), 821-827.

Corretti, Mary C., Anderson, Todd J., Benjamin, Emelia J., Celermajer, David, Charbonneau, Francois, Creager, Mark A., . . . Vogel, Robert. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology, 39*(2), 257-265.

Corson, M. A., James, N. L., Latta, S. E., Nerem, R. M., Berk, B. C., & Harrison, D. G. (1996). Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. *Circ Res, 79*(5), 984-991.

Cortes, B., Nunez, I., Cofan, M., Gilabert, R., Perez-Heras, A., Casals, E., . . . Ros, E. (2006). Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. *J Am Coll Cardiol, 48*(8), 1666-1671. doi: 10.1016/j.jacc.2006.06.057

Davies, K. J., Quintanilha, A. T., Brooks, G. A., & Packer, L. (1982). Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun, 107*(4), 1198-1205.

Davies, P. F. (2009). Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat Clin Pract Cardiovasc Med, 6*(1), 16-26. doi: 10.1038/ncpcardio1397

Davies, P. F., Dewey, C. F., Jr., Bussolari, S. R., Gordon, E. J., & Gimbrone, M. A., Jr. (1984). Influence of hemodynamic forces on vascular endothelial function. In vitro studies of shear stress and pinocytosis in bovine aortic cells. *The Journal of Clinical Investigation, 73*(4), 1121-1129. doi: 10.1172/JCI111298

Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation, 109*(23 Suppl 1), III27-32. doi: 10.1161/01.CIR.0000131515.03336.f8

Davignon, Jean, & Ganz, Peter. (2004). Role of Endothelial Dysfunction in Atherosclerosis. *Circulation, 109*(23_suppl_1), III-27-32. doi: 10.1161/01.CIR.0000131515.03336.f8

Dawson, E. A., Whyte, G. P., Black, M. A., Jones, H., Hopkins, N., Oxborough, D., . . . Green, D. J. (2008). Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *J Appl Physiol (1985), 105*(5), 1562-1568. doi: 10.1152/japplphysiol.90837.2008

de Roos, N. M., Siebelink, E., Bots, M. L., van Tol, A., Schouten, E. G., & Katan, M. B. (2002). Trans monounsaturated fatty acids and saturated fatty acids have similar effects on postprandial flow-mediated vasodilation. *Eur J Clin Nutr, 56*(7), 674-679. doi: 10.1038/sj.ejcn.1601377

DeSouza, C. A., Shapiro, L. F., Clevenger, C. M., Dinenno, F. A., Monahan, K. D., Tanaka, H., & Seals, D. R. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation, 102*(12), 1351-1357.

Dewey, Jr C. F., Gimbrone, Jr M. A., Davies, P. F., & Bussolari, S. R. (1981). The Dynamic Response of Vascular Endothelial Cells to Fluid Shear Stress. *Journal of Biomechanical Engineering, 103*(3), 177-185. doi: 10.1115/1.3138276

Dietz, William H. (2005). Physical Activity Recommendations: Where do we go from here? *The Journal of pediatrics, 146*(6), 719-720.

Dimmeler, Stefanie, Fleming, Ingrid, Fisslthaler, Beate, Hermann, Corinna, Busse, Rudi, & Zeiher, Andreas M. (1999). Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature, 399*(6736), 601-605.

Ebenbichler, C. F., Kirchmair, R., Egger, C., & Patsch, J. R. (1995). Postprandial state and atherosclerosis. *Curr Opin Lipidol, 6*(5), 286-290.

Eriksson, B. O. (1980). Muscle metabolism in children--a review. *Acta Paediatr Scand Suppl, 283*, 20-28.

Farpour-Lambert, Nathalie J., Aggoun, Yacine, Marchand, Laetitia M., Martin, Xavier E., Herrmann, François R., & Beghetti, Maurice. (2009). Physical Activity Reduces Systemic Blood Pressure and Improves Early Markers of Atherosclerosis in Pre-Pubertal Obese Children. *Journal of the American College of Cardiology, 54*(25), 2396-2406.

Fernhall, B., & Agiovlasitis, S. (2008). Arterial function in youth: window into cardiovascular risk. *J Appl Physiol, 105*(1), 325-333.

Fisslthaler, Dimmeler, Hermann, Busse, & Fleming. (2000). Phosphorylation and activation of the endothelial nitric oxide synthase by fluid shear stress. *Acta Physiologica Scandinavica, 168*(1), 81-88. doi: 10.1046/j.1365-201x.2000.00627.x

Flegal, K. M., Carroll, M. D., Kit, B. K., & Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA, 307*(5), 491-497. doi: 10.1001/jama.2012.39

Flegal, K. M., Kit, B. K., & Graubard, B. I. (2013). OVerweight, obesity, and all-cause mortality—reply. *JAMA, 309*(16), 1681-1682. doi: 10.1001/jama.2013.3101

Fleming, Ingrid, Fisslthaler, Beate, Dixit, Madhulika, & Busse, Rudi. (2005). Role of PECAM-1 in the shear-stress-induced activation of Akt and the endothelial nitric oxide synthase (eNOS) in endothelial cells. *Journal of Cell Science, 118*(18), 4103-4111. doi: 10.1242/jcs.02541

Force, U. S. Preventive Services Task. *Screening for Obesity in Children and Adolescents: US Preventive Services Task Force Recommendation Statement*. Retrieved from http://pediatrics.aappublications.org/content/early/2010/01/18/peds.2009-2037.abstract

Freedman, D. S., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics, 103*(6 Pt 1), 1175-1182.

Freedman, D. S., Khan, L. K., Serdula, M. K., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (2005). The relation of childhood BMI to adult adiposity: the Bogalusa Heart Study. *Pediatrics, 115*(1), 22-27. doi: 10.1542/peds.2004-0220

Freedman, D. S., Mei, Z. G., Srinivasan, S. R., Berenson, G. S., & Dietz, W. H. (2007). Cardiovascular risk factors and excess adiposity among overweight children and adolescents: The Bogalusa Heart Study. *Journal of Pediatrics, 150*(1), 12-17. doi: DOI 10.1016/j.jpeds.2006.08.042

Furchgott, RF, & Vanhoutte, PM. (1989). Endothelium-derived relaxing and contracting factors. *The FASEB Journal, 3*(9), 2007-2018.

Furchgott, RF, & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, 288*(5789), 373-376.

Gaesser, Glenn A., & Angadi, Siddhartha S. (2011). High-intensity interval training for health and fitness: can less be more? *Journal of Applied Physiology, 111*(6), 1540-1541. doi: 10.1152/japplphysiol.01237.2011

Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol, 590*(Pt 5), 1077-1084. doi: 10.1113/jphysiol.2011.224725

Gill, J. M., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Packard, C. J., Sattar, N., . . . Caslake, M. J. (2004). Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol, 44*(12), 2375-2382. doi: 10.1016/j.jacc.2004.09.035

Gill, Jason M. R., Al-Mamari, Ali, Ferrell, William R., Cleland, Stephen J., Packard, Chris J., Sattar, Naveed, . . . Caslake, Muriel J. (2004). Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol, 44*(12), 2375-2382. doi: 10.1016/j.jacc.2004.09.035

Gosmanov, A. R., Smiley, D. D., Robalino, G., Siquiera, J., Khan, B., Le, N. A., . . . Umpierrez, G. E. (2010). Effects of oral and intravenous fat load on blood pressure, endothelial function, sympathetic activity, and oxidative stress in obese healthy subjects. *Am J Physiol Endocrinol Metab, 299*(6), E953-958. doi: ajpendo.00469.2010 [pii]

10.1152/ajpendo.00469.2010

Gosmanov, A. R., Smiley, D., Robalino, G., Siqueira, J. M., Peng, L., Kitabchi, A. E., & Umpierrez, G. E. (2010). Effects of intravenous glucose load on insulin secretion in patients with ketosis-prone diabetes during near-normoglycemia remission. *Diabetes Care, 33*(4), 854-860. doi: dc09-1687 [pii]

10.2337/dc09-1687

Graham, T. E. (2004). Exercise, Postprandial Triacylglyceridemia, and Cardiovascular Disease Risk. *Canadian Journal of Applied Physiology, 29*(6), 781-799. doi: doi:10.1139/h04-051

Green, D. J. (2009). Exercise training as vascular medicine: direct impacts on the vasculature in humans. *Exerc Sport Sci Rev, 37*(4), 196-202. doi: 10.1097/JES.0b013e3181b7b6e3

00003677-200910000-00008 [pii]

Green, D. J., Dawson, E. A., Groenewoud, H. M., Jones, H., & Thijssen, D. H. (2014). Is flow-mediated dilation nitric oxide mediated?: a meta-analysis. *Hypertension, 63*(2), 376-382. doi: 10.1161/HYPERTENSIONAHA.113.02044

Green, D. J., Maiorana, A., O'Driscoll, G., & Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol, 561*(Pt 1), 1-25. doi: 10.1113/jphysiol.2004.068197

Green, D. J., Rowley, N., Spence, A., Carter, H., Whyte, G., George, K., . . . DH, J. Thijssen. (2013). Why isn't flow-mediated dilation enhanced in athletes? *Med Sci Sports Exerc, 45*(1), 75-82. doi: 10.1249/MSS.0b013e318269affe

Green, DJ. (2009). Exercise Training as Vascular Medicine: Direct Impacts on the Vasculature in Humans. *Exercise and Sport Sciences Reviews, 37*(4), 196-202 110.1097/JES.1090b1013e3181b1097b1096e1093.

Green, DJ, Bilsborough, William, Naylor, Louise H., Reed, Chris, Wright, Jeremy, O'Driscoll, Gerry, & Walsh, Jennifer H. (2005). Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *The Journal of Physiology, 562*(2), 617-628. doi: 10.1113/jphysiol.2004.075929

Griffith, T. M., Edwards, D. H., Lewis, M. J., Newby, A. C., & Henderson, A. H. (1984). The nature of endothelium-derived vascular relaxant factor. *Nature, 308*(5960), 645-647.

Hallmark, R., Patrie, J. T., Liu, Z., Gaesser, G. A., Barrett, E. J., & Weltman, A. (2014). The effect of exercise intensity on endothelial function in physically inactive lean and obese adults. *PLoS One, 9*(1), e85450. doi: 10.1371/journal.pone.0085450

Hambrecht, R., Adams, V., Erbs, S., Linke, A., Kränkel, N., Shu, Y., . . . Schuler, G. (2003). Regular Physical Activity Improves Endothelial Function in Patients With Coronary Artery Disease by Increasing Phosphorylation of Endothelial Nitric Oxide Synthase. *Circulation, 107*(25), 3152-3158. doi: 10.1161/01.cir.0000074229.93804.5c

Haram, P. M., Adams, V., Kemi, O. J., Brubakk, A. O., Hambrecht, R., Ellingsen, O., & Wisloff, U. (2006). Time-course of endothelial adaptation following acute and regular exercise. *Eur J Cardiovasc Prev Rehabil, 13*(4), 585-591. doi: 10.1097/01.hjr.0000198920.57685.76

Harris, Ryan A., Nishiyama, Steven K., Wray, D. Walter, & Richardson, Russell S. (2010). Ultrasound Assessment of Flow-Mediated Dilation. *Hypertension, 55*(5), 1075-1085. doi: 10.1161/hypertensionaha.110.150821

Harris, Ryan A., Tedjasaputra, Vince, Zhao, Jia, & Richardson, Russell S. (2012). Premenopausal Women Exhibit an Inherent Protection of Endothelial Function Following a High-Fat Meal. *Reproductive Sciences, 19*(2), 221-228. doi: 10.1177/1933719111418125

Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., . . . Bauman, A. (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc, 39*(8), 1423-1434.

Hopkins, N., Stratton, G., Maia, J., Tinken, T. M., Graves, L. E., Cable, T. N., & Green, D. J. (2010). Heritability of arterial function, fitness, and physical activity in youth: a study of monozygotic and dizygotic twins. *J Pediatr, 157*(6), 943-948. doi: 10.1016/j.jpeds.2010.06.005

Hubert, H B, Feinleib, M, McNamara, P M, & Castelli, W P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of

participants in the Framingham Heart Study. *Circulation, 67*(5), 968-977. doi: 10.1161/01.cir.67.5.968

Ignarro, LJ, Buga, G M, Wood, K S, Byrns, R E, & Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences, 84*(24), 9265-9269.

Ignarro, Louis J, & Kadowitz, Philip J. (1985). The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annual review of pharmacology and toxicology, 25*(1), 171-191.

Ingul, C. B., Tjonna, A. E., Stolen, T. O., Stoylen, A., & Wisloff, U. (2010). Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch Pediatr Adolesc Med, 164*(9), 852-859. doi: 164/9/852 [pii]

10.1001/archpediatrics.2010.158

Jackson, K. G., Armah, C. K., & Minihane, A. M. (2007). Meal fatty acids and postprandial vascular reactivity. *Biochem Soc Trans, 35*(Pt 3), 451-453. doi: 10.1042/bst0350451

Johnson, Blair D., Padilla, Jaume, Harris, Ryan A., & Wallace, Janet P. (2011). Vascular consequences of a high-fat meal in physically active and inactive adults. *Applied Physiology, Nutrition, and Metabolism, 36*(3), 368-375. doi: 10.1139/h11-028

Juonala, M, Kähönen, Mika, Laitinen, Tomi, Hutri-Kähönen, Nina, Jokinen, Eero, Taittonen, Leena, . . . Raitakari, Olli T. (2008). Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: The Cardiovascular Risk in Young Finns Study. *European Heart Journal, 29*(9), 1198-1206. doi: 10.1093/eurheartj/ehm556

Juonala, M., Viikari, J. S., Laitinen, T., Marniemi, J., Helenius, H., Ronnemaa, T., & Raitakari, O. T. (2004). Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation, 110*(18), 2918-2923. doi: 10.1161/01.cir.0000147540.88559.00

Juonala, M., Viikari, J. S., Ronnemaa, T., Helenius, H., Taittonen, L., & Raitakari, O. T. (2006). Elevated blood pressure in adolescent boys predicts endothelial dysfunction: the cardiovascular risk in young Finns study. *Hypertension, 48*(3), 424-430. doi: 10.1161/01.hyp.0000237666.78217.47

Kaiser, L., & Sparks, H. V., Jr. (1986). Mediation of flow-dependent arterial dilation by endothelial cells. *Circ Shock, 18*(2), 109-114.

Katsanos, C. S. (2006). Prescribing aerobic exercise for the regulation of postprandial lipid metabolism : current research and recommendations. *Sports Med, 36*(7), 547-560.

Kaufman, C. L., Kaiser, D. R., Steinberger, J., & Dengel, D. R. (2007). Relationships between heart rate variability, vascular function, and adiposity in children. *Clin Auton Res, 17*(3), 165-171. doi: 10.1007/s10286-007-0411-6

Kaufman, FR. (2005). Screening for abnormalities of carbohydrate metabolism in teens. *J Pediatr, 146*(6), 721-723.

Kavey, Rae-Ellen W., Allada, Vivek, Daniels, Stephen R., Hayman, Laura L., McCrindle, Brian W., Newburger, Jane W., . . . Steinberger, Julia. (2006). Cardiovascular Risk Reduction in High-Risk Pediatric Patients: A Scientific Statement From the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: Endorsed by the American Academy of Pediatrics. *Circulation, 114*(24), 2710-2738. doi: 10.1161/circulationaha.106.179568

Kelly, Wetzsteon, R. J., Kaiser, D. R., Steinberger, J., Bank, A. J., & Dengel, D. R. (2004). Inflammation, insulin, and endothelial function in overweight children and adolescents: the role of exercise. *J Pediatr, 145*(6), 731-736.

Kelly, A. S., Hebbel, R. P., Solovey, A. N., Schwarzenberg, S. J., Metzig, A. M., Moran, A., . . . Steinberger, J. (2010). Circulating activated endothelial cells in pediatric obesity. *J Pediatr, 157*(4), 547-551. doi: 10.1016/j.jpeds.2010.04.069

Kelly, Aaron S., Barlow, Sarah E., Rao, Goutham, Inge, Thomas H., Hayman, Laura L., Steinberger, Julia, . . . Daniels, Stephen R. (2013). Severe Obesity in Children and Adolescents: Identification, Associated Health Risks, and Treatment Approaches: A Scientific Statement From the American Heart Association. *Circulation, 128*(15), 1689-1712. doi: 10.1161/CIR.0b013e3182a5cfb3

Lee, D C, Sui, X, & Blair, S N. (2009). Does physical activity ameliorate the health hazards of obesity? *British Journal of Sports Medicine, 43*(1), 49-51. doi: 10.1136/bjsm.2008.054536

Mahmud, F. H., Hill, D. J., Cuerden, M. S., & Clarson, C. L. (2009). Impaired vascular function in obese adolescents with insulin resistance. *J Pediatr, 155*(5), 678-682.

Marlatt, K. L., Steinberger, J., Dengel, D. R., Sinaiko, A., Moran, A., Chow, L. S., . . . Kelly, A. S. (2013). Impact of pubertal development on endothelial function and arterial elasticity. *J Pediatr, 163*(5), 1432-1436. doi: 10.1016/j.jpeds.2013.07.002

Mattsson, E. J., Kohler, T. R., Vergel, S. M., & Clowes, A. W. (1997). Increased blood flow induces regression of intimal hyperplasia. *Arterioscler Thromb Vasc Biol, 17*(10), 2245-2249.

Metzig, A. M., Schwarzenberg, S. J., Fox, C. K., Deering, M. M., Nathan, B. M., & Kelly, A. S. (2011). Postprandial endothelial function, inflammation, and oxidative stress in obese children and adolescents. *Obesity (Silver Spring), 19*(6), 1279-1283. doi: 10.1038/oby.2010.318

Meyer, Andreas A., Kundt, Günther, Lenschow, Ute, Schuff-Werner, Peter, & Kienast, Wolfgang. (2006). Improvement of Early Vascular Changes and Cardiovascular Risk Factors in Obese Children After a Six-Month Exercise Program. *Journal of the American College of Cardiology, 48*(9), 1865-1870. doi: 10.1016/j.jacc.2006.07.035

Mikus, Catherine R., Fairfax, Seth T., Libla, Jessica L., Boyle, Leryn J., Vianna, Lauro C., Oberlin, Douglas J., . . . Thyfault, John P. (2011). Seven days of aerobic exercise training improves conduit artery blood flow following glucose ingestion in patients with type 2 diabetes. *Journal of Applied Physiology, 111*(3), 657-664. doi: 10.1152/japplphysiol.00489.2011

Morrison, J. A., Friedman, L. A., & Gray-McGuire, C. (2007). Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. *Pediatrics, 120*(2), 340-345. doi: 10.1542/peds.2006-1699

Muniyappa, Ranganath, Montagnani, Monica, Koh, Kwang Kon, & Quon, Michael J. (2007). Cardiovascular Actions of Insulin. *Endocrine Reviews, 28*(5), 463-491. doi: 10.1210/er.2007-0006

Naghavi, Morteza, Libby, Peter, Falk, Erling, Casscells, S. Ward, Litovsky, Silvio, Rumberger, John, . . . Willerson, James T. (2003). From Vulnerable Plaque to Vulnerable Patient: A Call for New Definitions and Risk Assessment Strategies: Part II. *Circulation, 108*(15), 1772-1778. doi: 10.1161/01.cir.0000087481.55887.c9

Napoli, C., D'Armiento, F. P., Mancini, F. P., Postiglione, A., Witztum, J. L., Palumbo, G., & Palinski, W. (1997). Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest, 100*(11), 2680-2690. doi: 10.1172/jci119813

Naylor, Louise H., Green, Daniel J., Jones, Timothy W., Kalic, Rachelle J., Suriano, Katie L., Shah, Mark, . . . Davis, Elizabeth A. (2011). Endothelial Function and Carotid Intima-Medial Thickness in Adolescents with Type 2 Diabetes Mellitus. *The Journal of pediatrics, 159*(6), 971-974.

Newman, William P., Wattigney, Wendy, & Berenson, Gerald S. (1991). Autopsy Studies in United States Children and Adolescentsa. *Annals of the New York Academy of Sciences, 623*(1), 16-25.

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2012). Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA, 307*(5), 483-490. doi: 10.1001/jama.2012.40

Osanai, T., Fujita, N., Fujiwara, N., Nakano, T., Takahashi, K., Guan, W., & Okumura, K. (2000). Cross talk of shear-induced production of prostacyclin and nitric oxide in endothelial cells. *Am J Physiol Heart Circ Physiol, 278*(1), H233-238.

Otto, M. E., Svatikova, A., Barretto, R. B., Santos, S., Hoffmann, M., Khandheria, B., & Somers, V. (2004). Early morning attenuation of endothelial function in healthy humans. *Circulation, 109*(21), 2507-2510. doi: 10.1161/01.CIR.0000128207.26863.C4

01.CIR.0000128207.26863.C4 [pii]

Paffenbarger, Ralph S., Hyde, Robert, Wing, Alvin L., & Hsieh, Chung-cheng. (1986). Physical Activity, All-Cause Mortality, and Longevity of College Alumni. *New England Journal of Medicine, 314*(10), 605-613. doi: doi:10.1056/NEJM198603063141003

Palmer, R. M., Ashton, D. S., & Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature, 333*(6174), 664-666. doi: 10.1038/333664a0

Panza, Julio A., Epstein, Stephen E., & Quyyumi, Arshed A. (1991). Circadian Variation in Vascular Tone and Its Relation to α-Sympathetic Vasoconstrictor Activity. *New England Journal of Medicine, 325*(14), 986-990. doi: doi:10.1056/NEJM199110033251402

Pyke, Kyra E., & Tschakovsky, Michael E. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *Journal of Physiology, 568*(2), 357-369. doi: 10.1113/jphysiol.2005.089755

Raitakari, O. T., Lai, N., Griffiths, K., McCredie, R., Sullivan, D., & Celermajer, D. S. (2000). Enhanced peripheral vasodilation in humans after a fatty meal. *J Am Coll Cardiol, 36*(2), 417-422.

Rapoport, R. M., & Murad, F. (1983). Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ Res, 52*(3), 352-357.

Ratel, S., Lazaar, N., Williams, C. A., Bedu, M., & Duché, P. (2003). Age differences in human skeletal muscle fatigue during high-intensity intermittent exercise. *Acta Pædiatrica, 92*(11), 1248-1254.

Reilly, J. J., & Kelly, J. (2010). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes (Lond)*.

Reilly, J. J., & Kelly, J. (2011). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes (Lond), 35*(7), 891-898. doi: 10.1038/ijo.2010.222

Rodriguez, B. L., Fujimoto, W. Y., Mayer-Davis, E. J., Imperatore, G., Williams, D. E., Bell, R. A., . . . Linder, B. (2006). Prevalence of cardiovascular disease risk factors in U.S. children and adolescents with diabetes: the SEARCH for diabetes in youth study. *Diabetes Care, 29*(8), 1891-1896.

Romero-Corral, A., Montori, V. M., Somers, V. K., Korinek, J., Thomas, R. J., Allison, T. G., . . . Lopez-Jimenez, F. (2006). Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. *Lancet, 368*(9536), 666-678. doi: 10.1016/s0140-6736(06)69251-9

Rubanyi, G. M., Romero, J. C., & Vanhoutte, P. M. (1986). Flow-induced release of endothelium-derived relaxing factor. *American Journal of Physiology - Heart and Circulatory Physiology, 250*(6), H1145-H1149.

Sarkola, T., Manlhiot, C., Slorach, C., Bradley, T. J., Hui, W., Mertens, L., . . . Jaeggi, E. (2012). Evolution of the arterial structure and function from infancy to adolescence is related to anthropometric and blood pressure changes. *Arterioscler Thromb Vasc Biol, 32*(10), 2516-2524. doi: 10.1161/atvbaha.112.252114

Seals, D. R., Walker, A. E., Pierce, G. L., & Lesniewski, L. A. (2009). Habitual exercise and vascular ageing. *J Physiol, 587*(Pt 23), 5541-5549. doi: 10.1113/jphysiol.2009.178822

Sedgwick, MJ, Morris, JG, Nevill, ME, Tolfrey, K, Nevill, A, & Barrett, LA. (2013). Effect of exercise on postprandial endothelial function in adolescent boys. *British Journal of Nutrition, 110*(02), 301-309. doi: doi:10.1017/S0007114512004977

Sedgwick, MJ., Morris, J. G., Nevill, M. E., & Barrett, L. A. (2014). The accumulation of exercise and postprandial endothelial function in boys. *Scand J Med Sci Sports, 24*(1), e11-19. doi: 10.1111/sms.12101

87

Srinivasan SR, Myers L, Berenson GS. (2006). Changes in metabolic syndrome variables since childhood in prehypertensive and hypertensive subjects: the Bogalusa Heart Study. *Hypertension, 48*(1), 33-39.

Strong, J. P., & McGill Jr, H. C. (1969). The pediatric aspects of atherosclerosis. *Journal of Atherosclerosis Research, 9*(3), 251-265. doi: http://dx.doi.org/10.1016/S0368-1319(69)80020-7

Strong, JP, & McGill. (1962). The natural history of coronary atherosclerosis. *Am J Pathol, 40*, 37-49.

Strong, JP, & McGill, H. C., Jr. (1963). The Natural History of Aortic Atherosclerosis: Relationship to Race, Sex, and Coronary Lesions in New Orleans. *Exp Mol Pathol, 52*, SUPPL1:15-27.

Strong, W. B., Malina, R. M., Blimkie, C. J., Daniels, S. R., Dishman, R. K., Gutin, B., . . . Trudeau, F. (2005). Evidence based physical activity for school-age youth.[see comment]. *Journal of Pediatrics, 146*(6), 732-737.

Tanasescu, Mihaela, Leitzmann, Michael F., Rimm, Eric B., Willett, Walter C., Stampfer, Meir J., & Hu, Frank B. (2002). Exercise Type and Intensity in Relation to Coronary Heart Disease in Men. *JAMA: The Journal of the American Medical Association, 288*(16), 1994-2000. doi: 10.1001/jama.288.16.1994

Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., . . . Green, D. J. (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol, 300*(1), H2-12.

Thijssen, D. H., Dawson, E. A., Black, M. A., Hopman, M. T., Cable, N. T., & Green, D. J. (2009). Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci Sports Exerc, 41*(5), 1072-1079. doi: 10.1249/MSS.0b013e3181923957

Thijssen, D. H., Rongen, G. A., Smits, P., & Hopman, M. T. (2008). Physical (in)activity and endothelium-derived constricting factors: overlooked adaptations. *J Physiol, 586*(2), 319-324. doi: 10.1113/jphysiol.2007.145698

Trigona, B., Aggoun, Y., Maggio, A., Martin, X. E., Marchand, L. M., Beghetti, M., & Farpour-Lambert, N. J. (2010). Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. *J Pediatr, 157*(4), 533-539. doi: 10.1016/j.jpeds.2010.04.023

Trost, S. G., Pate, R. R., Sallis, J. F., Freedson, P. S., Taylor, W. C., Dowda, M., & Sirard, J. (2002). Age and gender differences in objectively measured physical activity in youth. *Med Sci Sports Exerc, 34*(2), 350-355.

Tushuizen, M. E., Diamant, M., & Heine, R. J. (2005). Postprandial dysmetabolism and cardiovascular disease in type 2 diabetes. *Postgrad Med J, 81*(951), 1-6.

Tyldum, Gjertrud Aunet, Schjerve, Inga Ekeberg, Tjonna, Arnt Erik, Kirkeby-Garstad, Idar, Stolen, Tomas O., Richardson, Russell S., & Wisloff, Ulrik. (2009). Endothelial Dysfunction Induced by Post-Prandial Lipemia: Complete Protection Afforded by High-Intensity Aerobic Interval Exercise. *J Am Coll Cardiol, 53*(2), 200-206. doi: 10.1016/j.jacc.2008.09.033

Urbina, E. M., Williams, R. V., Alpert, B. S., Collins, R. T., Daniels, S. R., Hayman, L., . . . McCrindle, B. (2009). Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension, 54*(5), 919-950.

Vanhoutte, P. M. (2009). Endothelial dysfunction: the first step toward coronary arteriosclerosis. *Circ J, 73*(4), 595-601.

Verma, Subodh, Buchanan, Michael R., & Anderson, Todd J. (2003). Endothelial Function Testing as a Biomarker of Vascular Disease. *Circulation, 108*(17), 2054-2059. doi: 10.1161/01.cir.0000089191.72957.ed

Vita, Joseph A., & Keaney, John F., Jr. (2002). Endothelial Function: A Barometer for Cardiovascular Risk? *Circulation, 106*(6), 640-642. doi: 10.1161/01.cir.0000028581.07992.56

Wang, Youfa, Beydoun, May A., Liang, Lan, Caballero, Benjamin, & Kumanyika, Shiriki K. (2008). Will All Americans Become Overweight or Obese? Estimating the Progression and Cost of the US Obesity Epidemic. *Obesity, 16*(10), 2323-2330.

Watts, K., Beye, P., Siafarikas, A., Davis, E. A., Jones, T. W., O'Driscoll, G., & Green, D. J. (2004). Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. *J Am Coll Cardiol, 43*(10), 1823-1827. doi: 10.1016/j.jacc.2004.01.032

S0735109704004632 [pii]

Watts, K., Beye, P., Siafarikas, A., O'Driscoll, G., Jones, T. W., Davis, E. A., & Green, D. J. (2004). Effects of exercise training on vascular function in obese children. *J Pediatr, 144*(5), 620-625.

Whitlock, Evelyn P., O'Connor, Elizabeth A., Williams, Selvi B., Beil, Tracy L., & Lutz, Kevin W. (2010). Effectiveness of Weight Management Interventions in Children: A Targeted Systematic Review for the USPSTF. *Pediatrics, 125*(2), e396-418. doi: 10.1542/peds.2009-1955

Woo, KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, . . . DS., Celermajer. (2004). Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. *Int J Obes Relat Metab Disord. , 28*, 852-857.

Woodman, R. J., Playford, D. A., Watts, G. F., Cheetham, C., Reed, C., Taylor, R. R., . . . Green, D. (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol, 91*(2), 929-937.

Zarins, C K, Giddens, D P, Bharadvaj, B K, Sottiurai, V S, Mabon, R F, & Glagov, S. (1983). Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circulation Research, 53*(4), 502-514. doi: 10.1161/01.res.53.4.502

APPENDIX A

INCLUSION AND EXCLUSION CRITERION

Inclusion
Age: 13-17
Weight-status: BMI percentile >97th
Male
Less than 60 min/day of physical activity
Exclusion
Female
Type 1 or 2 diabetes
Taking medication (s)
Acute illness
Regular multivitamin / Vitamin C use
Recent smoking history
Lactose intolerance
Diagnosed with a condition that could influence exercise capacity, vascular function, or lipid metabolism

APPENDIX B

ELIGIBILITY SCREENING FORM

Eligibility Data Form
(Exercise and Postprandial Endothelial Function in Obese Adolescent Males)

ED1: Today's Date: ___/___/___
                mm dd   yy

ED2: Staff Initials: _____

Complete on own:

ED3: Child's Name: _____

ED4: Child's Age: _____

ED5: Child's Date of Birth: _____

ED6: Approximate Weight _____ Approximate Height_____
 Calculate BMI _____
Calculate BMI percentile_____*
* An answer below a BMI percentile of 97th makes the person ineligible for the study
Eligibility Criteria:

SUBJECTS ARE INELIGIBLE IF ANY ITEM IN A SHADED BOX IS CHECKED

1. Child is 13-17 years old (ED7)	|__| YES	|__| NO
2. Documented diagnosis of Type 2 Diabetes (ED8)	|__| YES	|__| NO

Complete with Parent/Caregiver (ask in person or via phone)
"If you have a few minutes now I would like to ask for some information to help me determine whether you might be eligible to participate in the study."

3. Is your child able to perform physical activity without any difficulty? (ED9)	|__| YES	|__| NO
4. Does your child participate in exercise/ physical activity for more than 60min/day everyday of the week? (ED10)	|__| YES	|__| NO
5. Is your child lactose intolerant or have any food allergies to dairy products? (ED11)	|__| YES	|__| NO
6. Does your child have any medical conditions for which he/she receives treatment from a health care professional? ** (ED12) Specifically: Asthma, Type 1 diabetes	|__| YES*** List:_____ _____ _____ _____ ___	|__| NO
7. Is your child taking any medication currently? (ED13)***	|__| YES*** List:_____ _____	|__| NO

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***Medications will have to be examined to determine if they will interfere with any of the measured outcomes	_____ _____ _____ _____	
8. Is your child taking any dietary supplements? (ED14) Specifically: Multivitamin, Vit. C supplement, or L-arginine containing proteins (e.g. Soy, ect) **Are they willing to discontinue use for at least 7 days prior to any visit? If not, they will need to be excluded.	|__| YES** List:_____ _____ _____ _____ _____ _____ _____	

If the answer is "Yes", compare the list of medical conditions to the exclusion criteria below: (ED15)

Orthopedic limitation	|__| YES
Physical Disability	|__| YES
Chronic Obstructive Lung Disease	|__| YES
Emphysema	|__| YES
Angina	|__| YES
Prior Myocardial Infarction	|__| YES
Heart Surgery of any kind	|__| YES
Other Pulmonary Disease: Specify _____	|__| YES
Cardiovascular Disease: Specify _____	|__| YES
Neurological Disease: Specify _____	|__| YES
Organic brain syndrome	|__| YES
Mental retardation	|__| YES

SUBJECTS ARE INELIGIBLE IF ANY ITEM IN A SHADED BOX IS CHECKED

Complete with Parent/Caregiver (ask in person or via phone)

Parent/Caregiver Contact Information

ED16: Name: _____

ED17: Home Address:_____

City, State, Zip:_____

ED18: Phone Number:_____

ED19: Alternative Phone Number: _____

ED20: Best time to reach you: _____

ED21: Email address: _____

ED22: Can you please give me the name, address, and telephone number of a relative or friend who would always know how to contact you if we can't reach you?

 Name:

 _____

 Telephone #: (_ _ _)-(_ _ _)-(_ _ _ _)

 Address:

Are you currently participating in any other ASU-sponsored studies?   YES   NO

If YES, how much money can you potentially earn by participating in these studies per year?

_____

Appendix C

STUDY DESIGN AND TIMELINE CHART

Study Design Timeline Chart

Baseline Visit

Time of Arrival: 9:00 AM

Procedures:
Consent and Assent
Height and Weight

If fasted:
(if not on a return visit at the same time)
Seated Blood Pressure
Flow-mediated Dilation (FMD)
Fitness testing
Snack

≥ 72 Hour washout

Visit 2

Time of Arrival: 5:00 PM

Procedures:
High Intensity Exercise
Snack

Subject requirments:
Eat pre-selected Meal
Fasted: No food after 10pm

Option 1: Ex+M --> M

Randomization

Option 2: M --> Ex+M

---

16 Hours between visits

Visit 3

Time of Arrival: 9:00 AM

Procedures:
Resting BP taken
Fasted FMD
Milkshake Given
FMD 2hr post-meal
FMD 4hr post-meal

Subject requirments:
Eat pre-selected meal day prior
Fasted: No food after 10pm

Visit 2 (Option 2)

≥ 72 Hour washout

Time of Arrival: 9:00 AM

Procedures:
Resting BP taken
Fasted FMD
Milkshake Given
FMD 2hr post-meal
FMD 4hr post-meal

Subject requirments:
Eat pre-selected meal day prior
Fasted: No food after 10pm

Visit 3 (Option 2)

≥ 72 Hour washout

Time of Arrival: 5:00 PM

Procedures:
High Intensity Exercise
Snack

Subject requirments:
Eat pre-selected Meal
Fasted: No food after 10pm

---

16 Hours between visits

Visit 4

Time of Arrival: 9:00 AM

Procedures:
Resting BP taken
Fasted FMD
Milkshake Given
FMD 2hr post-meal
FMD 4hr post-meal

APPENDIX D

CHILD ASSENT FORM - ENGLISH

CHILD ASSENT for RESEARCH

Study Title: Exercise and Post-Prandial Endothelial Function in Obese Adolescent Males

Principal Investigator: Gabriel Q. Shaibi, PhD
Arizona State University
Day Time Phone: 602-496-0909

RESEARCHERS

Gabriel Shaibi, PhD and Glenn Gaesser, PhD, from Arizona State University (ASU) have invited you to be part of a research study.

STUDY PURPOSE

The purpose of this study is to see how blood vessels work with exercise and diet.

What we will do

You will come to the Healthy Lifestyle Research Center (HLRC) at ASU for 4 visits described below.

Baseline Visit (1 hour).

You will come to ASU in the morning at 9:00 AM before you eat breakfast.

We will perform the following:
- A medical and family history.
- Measure your height, weight, blood pressure, and pulse.
- We will take a picture of the blood vessels in your arm using an ultrasound.
- We will put a blood pressure cuff on your arm and pump it up for 5 minutes and then release it and take another picture of your blood vessels for 3 minutes.
- You will perform an exercise test on a stationary bicycle. This test will tell us how fit you are.
- After the exercise test, we will give you a snack and set up your next appointment.

Evening Exercise Visit (~1 hour)

You will come back to ASU at 5:00 PM for an exercise session on a stationary bicycle.

- The exercise session will be based upon the exercise test we did at the first visit. We will start with a 5-minute warm-up followed by 8 exercise periods that will last for 2 minutes each and will feel like you are pedaling a bike up a steep hill. Each hard exercise period will be followed by 2 minutes of easy pedaling. The total exercise session will last 42 minutes and will include a 5-minute cool down.

- We will give you a subway gift card and have you pick out a meal to eat for that night.

Morning Blood Vessel Measurement and Meal Test after Exercise (~5 hours)

You will come back to ASU at 9:00 AM the next morning after the exercise test. You will not be able to eat after 10pm the night before until you come to ASU.
- We will take a picture of the blood vessels in your arm using an ultrasound.
- We will put a blood pressure cuff on your arm and pump it up for 5 minutes and then release it and take another picture of your blood vessels.
- We give you a vanilla milkshake for breakfast and ask you to drink it in 10-minutes.
- Then we will take 2 more pictures of your arm over the next 4 hours.
- During this time you will be able to read but not watch TV or use a computer

Blood Vessel Measurement and Meal test without Exercise (~5 hours)

You will come back to ASU at 9:00 AM during the course of the study. You will not be able to eat after 10pm the night before and will have the same subway dinner the night before.
- We will take a picture of the blood vessels in your arm using an ultrasound.
- We will put a blood pressure cuff on your arm and pump it up for 5 minutes and then release it and take another picture of your blood vessels.
- We give you a milkshake for breakfast and ask you to drink it in 10-minutes.
- Then we will take 2 more pictures of your arm over the next 4 hours.
- During this time you will be able to read but not watch TV or use a computer
- After the last picture, you will walk for about 10min on a treadmill wearing a heart rate monitor.

ARE THERE ANY RISKS.
- You may find parts of this study uncomfortable.
- The exercise test may make your muscles hurt or feel sore. The exercise test may make you sweat, breathe hard, and tired. Some people may get dizzy or nauseous (feel like they are going to throw up) when they exercise hard.
- You will perform hard exercise, will be asked to fast overnight, and will be asked to consume a large breakfast.
- You will have to remain seated or lying down for about 4 hours and will not be allowed to walk around during this time except to go to the bathroom.
- We will give you a milkshake that may make your stomach hurt or feel like throwing up.

DO I HAVE TO DO THIS

You do not have to be in this project. The choice is yours. No one will be mad at you if you decide not to do this. Even if you start the project, you can stop later if you want. You may ask questions about the study at any time.

WHAT DO I GET FOR DOING THIS
- You will receive up to $80 for being in this study.
- You will get information about your fitness level.
- You will get to feel what it is like to participate in a research project.

If you have any questions about the research study, before or after your consent, please call Dr. Shaibi, 500 N. 3rd Street, Phoenix, AZ 85004, 602-496-0909.

Your signature below means that you have read this form (or it has been read to you) and you want to participate in this study.

Signature of participant_____

Participant's printed name _____

Date_____

_____   _____   _____
Parent/legal guardian's Signature   Printed Name.   Date

CONSENT TO BE CONTACTED FOR FUTURE RESEARCH STUDIES.

Dr. Shaibi and his colleagues at ASU have other research projects. You may be eligible to participate in. If you would like to be notified of other opportunities for research participation please sign below. You can still be in this study even if you do not wish to be contacted in the future.

I ☐ DO consent to be re-contacted for future studies.

I ☐ DO NOT consent to be re-contacted for future studies.
_____

Participant's initials

INVESTIGATOR'S STATEMENT.

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have offered the participant a copy of this signed consent document."

_____ _____ _____
Investigator's Signature /   Printed Name   Date
Person Obtaining Consent

APPENDIX E

PARENTAL CONSENT FORM – ENGLISH

PARENT/LEGAL GUARDIAN INFORMED CONSENT for RESEARCH

Study Title: Exercise and Post-Prandial Endothelial Function in Obese Adolescent Males

Principal Investigator: Gabriel Q. Shaibi, PhD
Arizona State University
Day Time Phone: 602-496-0909

INTRODUCTION

The purpose of this form is: 1) Give you information to help you decide whether to let your child participate in this project and 2) Record your agreement for your child to be part of this study.

RESEARCHERS

Gabriel Shaibi, PhD and Glenn Gaesser, PhD, from Arizona State University (ASU) have invited your child to be part of a research study.

STUDY PURPOSE

The purpose of this study is to compare the blood vessels of obese youth, and see how their blood vessels respond to exercise and diet. We are asking your child to take part in this study because he is obese. Up to 30 children will be in this study.

DESCRIPTION OF RESEARCH STUDY

If you and your child agree to participate, you both will come to the Healthy Lifestyle Research Center (HLRC) at ASU for 4 visits described below.

Baseline Visit (~1 hour).

You and your child will be scheduled to come to ASU in the morning around 9:00 AM. Your child will be asked not to eat anything for 10 hours before the visit with only having water in the morning.
We will perform the following:
- A medical and family history.
- Measure your child's height, weight, blood pressure, and pulse.
- We will take a picture/video of the blood vessels in your child's arm using an ultrasound. This is a similar procedure doctors use on pregnant women. For this test your child will have to lie quietly in a bed for about 25 minutes. We will take a video of your child's blood vessel for 1 min then we will inflate a blood pressure cuff on their arm for 5 minutes. After we release the blood pressure cuff, we will take another picture of your child's upper arm for about 3 more minutes.
- Your child will perform an exercise test on a stationary bicycle. For this test, your child will breathe through a mouthpiece while they pedal on the

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bike. The exercise test will last about 15 minutes and we will increase the resistance during the test until your child will no longer be able to continue. We will measure your child's breathing and heart rate before, during, and after the exercise test. This test will tell us how fit your child is.

- After a 10 minute rest period your child will perform an "all-out" bout of exercise at the same resistance they ended at on the previous test. Your child will push themselves as hard as they feel comfortable with until they can no longer continue. This test usually lasts about 2-3 minutes.

- We will give your child a snack and set up a time for their next visit.

Evening Exercise Visit (~1 hour)

- Your child will be asked to come back to ASU at 5pm for an exercise session. The exercise given will be based upon the exercise test we did during the baseline visit.

- The exercise session will be on a stationary bike, after a 5 minute warm-up your child will pedal against resistance in 8 stages. Each stage will last 4 minutes long and they will pedal hard for 2 minutes and easy for 2 minutes. They will cool down for 5 minutes afterwards. The total time of the exercise session will be about 42 minutes.

- After the exercise session, your child will be given a subway gift card for dinner that night. Your child will get to pick out a specific meal to eat, and the meal must be eaten prior to 10pm.

- Your child will be instructed to not eat any food after 10pm but will be allowed to drink water.

Morning Blood Vessel Measurement and Meal Test following Exercise (~5 hours)

Your child will be asked to return to ASU at 9:00 AM the morning after the exercise visit. We will ask your child not to eat or drink anything after 10 PM the night before and not to eat breakfast on this day.

- We will repeat the ultrasound test described under the baseline visit.

- We will give your child a vanilla milkshake for breakfast that contains about 900 calories (60% Fat, 10% Protein 30% Carbohydrate). We will ask them to drink the milkshake slowly in under 10 minutes.

- We will repeat the ultrasound test at 2 hours and 4 hours, after the meal. Between measures, we will ask that your child remain seated or lying down except to use the restroom. They should bring a book to read or something to keep them occupied during this time. We will not allow the use of TV, computers, iPad, ect. during this time as they may effect the measures we are taking.

- After this visit, your child will be given a subway gift card for dinner the night prior to the next visit. Your child will eat the same meal as the night prior to the other meal visit.

Morning Blood Vessel Measurement and Meal Test without Exercise

Your child will be asked to return to ASU at 9:00 AM. We will ask your child to eat the standard selected subway meal the night prior. We will ask your child not to eat or drink anything after 10 PM the night before, not to exercise the day before, and not to eat breakfast on this day.

- We will repeat the ultrasound test described previously.

- We will give your child a vanilla milkshake for breakfast that contains about 900 calories (60% Fat, 10% Protein 30% Carbohydrate). We will ask them to drink the milkshake slowly in under 10 minutes.

- We will repeat the ultrasound test at 2 hours, and 4 hours, after the meal. Between measures, we will ask that your child remain seated or lying down except to use the restroom. They should bring a book to read or something to keep them occupied during this time. We will not allow the use of TV, computers, iPad, ect. during this time as they may effect the measures we are taking.

- At the end of the visit we will ask your child to walk on a treadmill for ~10 minutes while wearing a heart rate monitor.

RISKS.

There may be parts of this study your child finds uncomfortable. Your child will perform vigorous exercise, will be asked to fast overnight, and will be asked to consume a high-fat milkshake. Also, your child will have to remain seated or lying down for about 4 hours and will not be allowed to walk around during this time except to go to the bathroom.

General Discomforts.

The exercise is likely to make your child sweat, breathe hard, feel tired and make their muscles sore. With any hard exercise, they may feel light-headed, out of breath, or nauseas. In rare cases people do vomit and / or pass-out after exercise testing. Your child will be asked to eat a high fat milkshake and could experience diarrhea, vomiting, nausea, stomachache, allergic reaction or food poisoning.

COMPENSATION FOR ILLNESS AND INJURY

If you give permission for your child to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside

to compensate you in the event of injury. However, if any injury occurs due to the experimental procedures, first aid will be provided. If there is a situation that the researchers believes needs attention by a primary care practitioner, you will be referred to the Nurse Practitioner Clinic on the downtown ASU campus. If any injury occurs after hours when to the Nurse Practitioner Clinic is closed, you should seek attention at an urgent care facility. If a medical emergency were to occur during the study, we will call "911" to bring emergency medical personnel to the HLRC. You will be responsible for any costs incurred.

ALTERNATIVE TREATMENTS.

There are no alternative procedures available for this study.

BENEFITS.

There may be no direct benefit to you or your child for participating. The information from this study will help researchers better understand how exercise and diet impact cardiovascular health of youth. Your child will have the opportunity to learn about their fitness levels and how scientists use exercise to understand how the body works.

NEW INFORMATION.

If we find new information during the study that would change your or your child's choice about participating, then we will give you this information.

CONFIDENTIALITY.

All information obtained in this study is confidential unless the law makes us disclose it. The results of the study may be published or presented but your child's name or identity will be kept private. To keep information private, we will use codes to identify research volunteers and only the study team will have access to personal information. The study records will be kept locked in Dr. Shaibi's office.

WITHDRAWAL PRIVILEGE.

It is ok for you and your child to say no. Even if you say yes now, you both are free to say no later, and leave the study at any time. Your choice will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

COST AND COMPENSATION.

The researchers want your family's decision about participating in the study to be your choice. They understand that participation may cost your family in terms of time and travel. Your child will receive compensation for their time and effort and free parking will be provided.

Your child will receive up to $80 in compensation ($20 Screening Visit and $30 for each meal visit). If your child does not finish the tests, they will receive $10 per hour cash for the time spent, but will not go over the amount stated above for each visit. The researchers may ask your child to leave the study at anytime without consent. Your child will still be compensated for their time.

VOLUNTARY CONSENT.

Any questions you have concerning the research study or your child's participation, before or after your consent, will be answered by Dr. Shaibi, 500 N. 3rd Street, Phoenix, AZ 85004, 602-496-0909.

If you have questions about your child's rights as a participant in this research, or if you feel your family has been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit except as stated. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you. **Your signature below means that you consent for your child to participate in this study.**

Your child's name (please print): _____

_____   _____   _____
Parent/legal guardian's Signature.   Printed Name.   Date

CONSENT TO BE CONTACTED FOR FUTURE RESEARCH STUDIES.

Dr. Shaibi and his colleagues at ASU have other research projects. Your child may be eligible to participate in. If you would like to be notified of other opportunities for research participation please sign below. Your child can still be in this study even if you do not wish to be contacted in the future.

I ☐ DO consent to have my child re-contacted for future studies.
I ☐ DO NOT consent to have my child re-contacted for future studies.
_____

Parent's initials
INVESTIGATOR'S STATEMENT.

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have offered the participant a copy of this signed consent document."

_____   _____   _____
Investigator's Signature /   Printed Name   Date
Person Obtaining Consent

APPENDIX F

CHILD ASSENT FORM – SPANISH

CONSENTIMIENTO DEL ADOLESCENTE PARA LA INVESTIGACIÓN CIENTÍFICA

Título del estudio: El ejercicio y la función del endotelio después de comer en varones adolescentes obesos

Investigador Principal: Gabriel Shaibi, PhD
Universidad Estatal de Arizona
Teléfono durante el día: 602-496-0909

INVESTIGADORES

Gabriel Shaibi, PhD y Glenn Gaesser, PhD, de la Universidad Estatal de Arizona (ASU) te invitan para que seas parte de un estudio de investigación científica.

PROPÓSITO DEL ESTUDIO

El propósito de este estudio científico es para ver cómo funcionan los vasos sanguíneos con el ejercicio y la dieta.

Lo que nosotros haremos

Tu vendrás al Centro de Investigaciones Científicas Estilo de Vida Saludable (HLRC) en ASU por 4 visitas relatadas a continuación.

Visita de fundamento (~1 hora).

Tu vendrás a ASU en la mañana a las 9:00 am antes de desayunar.

Juntos vamos a hacer lo siguiente:
- Un historial medico y familiar.
- Medir tu estatura, peso, presión arterial y pulso.
- Vamos a tomar una imagen de los vasos sanguíneos del brazo utilizando un ultrasonido.
- Vamos a poner un manguito de presión arterial en tu brazo y inflarlo para mantener la presión en el brazo por 5 minutos, luego liberarla y tomar otra imagen de los vasos sanguíneos durante 3 minutos consecutivos.
- Tu llevarás a cabo una prueba de ejercicio en una bicicleta estacionaria. Esta prueba nos dirá tu estado de condición física.
- Después de la prueba de ejercicio, te daremos una botana y estableceremos tu próxima visita.

Visita de Ejercicio por la Tarde (aproximadamente 1 hora)

Tu volverás a ASU a las 5:00 pm de la tarde para una sesión de ejercicio en una bicicleta estacionaria.

- La sesión y la intensidad del ejercicio se basara en los resultados obtenidos en la prueba de ejercicio que hicimos en la primera visita. Vamos a empezar con 5 minutos de calentamiento seguido por un total de 8 períodos de ejercicio que tendrán una duración de 2 minutos cada uno y se sentirá como si estuvieras pedaleando una bicicleta en una montaña. Cada periodo de ejercicio severo será seguido por 2 minutos más fáciles de pedaleo. El total de la sesión de ejercicio tendrá una duración de 42 minutos y incluirá un enfriamiento de 5 minutos.
- Te daremos una tarjeta de regalo para el restaurante Subway y tendrás que escoger una comida para comer de cena esa noche.

Medidas de los Vasos Sanguíneos por la Mañana y la Prueba de Comida Después del Ejercicio (aproximadamente 5 horas)

Tu volverás a ASU a las 9:00 am de la mañana siguiente de la prueba de ejercicio. Tu no podrás comer después de las 10:00 pm de la noche anterior, tendrás que esperar hasta llegar a ASU.
- Tomaremos una imagen de los vasos sanguíneos del brazo utilizando un ultrasonido.
- Vamos a poner un manguito de presión arterial en el brazo y mantendremos la presión por 5 minutos, luego de liberarla tomaremos otra imagen de los vasos sanguíneos.
- Te daremos un licuado de vainilla para el desayuno, te pediremos que la termines de beber en 10 minutos.
- Después, vamos a tomar 2 más imágenes de tu brazo durante un periodo de 4 horas.
- Durante este tiempo, tu puedes leer o dormir pero no ver la televisión o usar la computadora.

Medidas de los Vasos Sanguíneos y la Prueba de Comida Sin Ejercicio (~ 5 horas)

En el transcurso del estudio tu volverás a ASU a las 9:00 am de la mañana. La noche anterior tu no podrás comer después de las 10:00 pm de la noche.
- Vamos a tomar una imagen de los vasos sanguíneos en el brazo utilizando un ultrasonido.
- Vamos a poner un manguito de presión arterial en tu brazo e inflarla para mantener la presión en el brazo por 5 minutos, luego liberarla y tomar otra imagen de los vasos sanguíneos.

- Te daremos un licuado de vainilla para el desayuno y te pediremos que te la termines de tomar en 10 minutos.
- Después, vamos a tomar 2 más imágenes de tu brazo durante las siguientes 4 horas.
- Durante este tiempo, tu podrás leer o dormir pero no ver la televisión o usar la computadora.
- Después de la última imagen, tu tendrás que caminar durante unos 10 minutos en la caminadora usando un monitor de frecuencia cardíaca.

EXISTE ALGÚN RIESGO.

- Tu puedes encontrar algunas partes un poco incomodas en este estudio científico.
- La prueba del ejercicio puede hacer que los músculos se sienten adoloridos o que duelan. La prueba de ejercicio tal vez te va a hacer sudar, respirar con mas fuerza, y a cansarte. Algunas personas cuando hacen ejercicio mas duro pueden experimentar mareos o náuseas (sentir que tienen que vomitar).
- Tu vas a hacer ejercicio intenso, también te pediremos que estés en ayunas toda una noche, y que consumas un desayuno grande.
- Tu tendrás que permanecer sentado o acostado por alrededor de 4 horas y no podrás caminar durante este tiempo, excepto para ir al baño.
- Te daremos un licuado que tal vez cause dolor en el estomago o ganas de vomitar.

TENGO QUE HACER ESTO

Tu no tienes que ser parte de este proyecto. Tu decides. Si decides no hacerlo nadie va a estar enojado con tigo. Incluso si tu decides iniciar el proyecto, si tu lo deseas puedes terminar a cualquier momento durante tu participación. Tu puedes hacer preguntas sobre el estudio científico en cualquier momento.

QUÉ PUEDO GANAR POR PARCIPAR EN ESTO

- Tu recibirás hasta $80 dólares por tu participación en este estudio científico.
- Tu recibirás información acerca de tu nivel de condición física.
- Tu vas a experimentar lo que se siente participar en un proyecto de investigaciones científicas.

Si tu tienes alguna pregunta sobre el estudio de investigaciones científicas, antes o después de tu consentimiento, por favor llama a Dr. Shaibi, 500 N. 3rd Street, Phoenix, AZ 85004, 602-496-0909.

Tu firma se significa que tu has leído este formulario (o que se te ha leído a ti) y deseas participar en este estudio científico.

Firma del Participante _____

Nombre del Participante _____

Fecha _____

_____   _____   _____
Firma del tutor legal.   Nombre (letra de molde).   Fecha

CONSENTIMIENTO DE CONTACTO PARA FUTUROS ESTUDIOS DE INVESTIGACION CIENTIFICA.

Dr. Shaibi y sus colegas en ASU tienen otros proyectos de investigaciones científicas. Tu puedes estar elegible para participar. Si tu quisieras enterarte de otras oportunidades para participar en investigaciones científicas, por favor firma abajo. Incluso si tu no deseas ser contactado en el futuro, no te afectaría tu participación en este estudio.

YO ☐ SI doy mi consentimiento para ser contactado nuevamente para estudios científicos en el futuro.

YO ☐ NO doy mi consentimiento para ser contactado nuevamente para estudios científicos en el futuro.

_____
Iniciales de el participante

DECLARACIÓN DEL INVESTIGADOR.

"Certifico que he explicado al individuo de arriba la esencia y el propósito, los posibles beneficios y los posibles riesgos asociados con la participación en este estudio de investigación científica, se han contestado todas las preguntas que se han echo, y he sido testigo de la firma de arriba. Estos elementos del Informado Consentimiento se ajustan a las garantías dadas por la Universidad Estatal de Arizona a la Oficina de Investigación de Protecciones Humanas para proteger los derechos de los seres humanos. He ofrecido al participante una copia firmada de este documento de consentimiento."

_____   _____   _____
Firma del Investigador/   Nombre (letra de molde)   Fecha
Persona obteniento el concentimiento

APPENDIX G

PARENTAL CONSENT FORM – SPANISH

EL CONSENTIMIENTO INFORMADO DE EL TUTOR LEGAL PARA LA INVESTIGACIÓN CIENTIFICA

Título del estudio: El ejercicio y la función del endotelio después de comer en varones adolescentes obesos

Investigador Principal: Gabriel Shaibi, PhD
Universidad Estatal de Arizona
Teléfono durante el día: 602-496-0909

INTRODUCCIÓN
El propósito de este formulario es: 1) Le dará información que le ayudara a decidir si desea permitir que su hijo que participe en este proyecto y 2) Registrar que usted esta de acuerdo para que su hijo participe en este estudio científico.

INVESTIGADORES
Gabriel Shaibi, PhD y Glenn Gaesser, PhD, de la Universidad Estatal de Arizona (ASU)
 han invitado a su hijo para que sea parte de un estudio de investigaciones científicas.

PROPÓSITO DEL ESTUDIO
El propósito de este estudio científico es para comparar los vasos sanguíneos de los jóvenes obesos, y ver cómo sus vasos sanguíneos responden al ejercicio y la dieta. Porque su hijo es clasificado obeso, estamos pidiendo que el participe en este estudio científico. Un máximo de 30 niños serán parte de este estudio científico.

DESCRIPCIÓN DEL ESTUDIO DE INVESTIGACIÓN CIENTÍFICA
Si usted y su hijo están de acuerdo en participar, usted y su hijo vendrán al Centro de Investigaciones Científicas Estilo de Vida Saludable (HLRC) en ASU por 4 visitas relatadas a continuación.

Visita de fundamento (aproximadamente 1 hora).

Una cita será programada para que usted y su hijo visiten a ASU en la mañana alrededor de las 9:00 am. Se le pedirá a su hijo que no coma nada durante 10 horas antes de la visita, sólo podrá tomar agua por la mañana.
Vamos a hacer lo siguiente:
* Un historial médico y familiar.
* Medir la altura de su hijo, el peso, la presión arterial y el pulso.
* Vamos a tomar una imagen/video de los vasos sanguíneos en el brazo de su hijo por medio de un ultrasonido. Es un procedimiento similar al que los médicos utilizan en las mujeres embarazadas. Para esta prueba su hijo

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tendrá que quedarse acostado en una cama durante unos 25 minutos. Vamos a tomar un video de los vasos sanguíneos de su niño durante 1 min y luego vamos a inflar un manguito de presión arterial en el brazo durante 5 minutos. Después de que liberemos el manguito de presión arterial, vamos a tomar mas imágenes del brazo de su hijo durante 3 minutos consecutivos.

- Su hijo llevara a cabo una prueba de ejercicio en una bicicleta estacionaria. Para esta prueba, su niño respira a través de una boquilla mientras pedalea en la bicicleta. La prueba de ejercicio durara unos 15 minutos, y durante la prueba vamos a aumentar la resistencia hasta que su hijo ya no pueda continuar. Vamos a medir la respiración de su hijo y la frecuencia cardíaca antes, durante y después de la prueba de ejercicio. Esta prueba nos dirá el estado de forma física de su hijo.
- Después de un período de 10 minutos de descanso su hijo hará ejercicio "con toda fuerza" en la misma resistencia que terminó en la prueba anterior. Su hijo se motivara a si mismo para darle duro en la bicicleta hasta que ya no pueda continuar. Esta prueba en general dura alrededor de 2-3 minutos.
- Vamos a darle a su hijo una botana y estableceremos el tiempo y fecha para la próxima visita.

Visita De Ejercicio Por La Tarde (aproximadamente 1 hora)
- Su hijo regresara a ASU a las 5 pm de la tarde para una sesión de ejercicio. La sesión e intensidad del ejercicio se basara en los resultados obtenidos en la prueba de ejercicio que hicimos en la primera visita.
- La sesión de ejercicio será en una bicicleta estacionaria, después de 5 minutos de calentamiento su hijo va a pedalear contra la resistencia por un total de 8 etapas. Cada etapa tendrá una duración de 4 minutos que será pedalear fuerte durante 2 minutos y mas fácil por los otros 2 minutos. Después se le dará 5 minutos para enfriamiento. En general el tiempo de la sesión de ejercicio será unos 42 minutos.
- Después de la sesión del ejercicio, su hijo recibirá una tarjeta de regalo para el restaurante Subway para que compre su cenar esa noche. Su hijo tendrá que elegir una comida específica para que cene, la cena tendrá que comerse antes de las 10:00 pm de la noche.
- Se le indicará a su hijo que no coma ningún alimento después de las 10:00 de la noche, pero se le permitirá beber agua.

Medidas de los Vasos Sanguíneos por la Mañana y la Prueba de Comida Después del Ejercicio (aproximadamente 5 horas)

Se le pedirá a su hijo que regrese a ASU a las 9:00 AM de la mañana después de la visita del ejercicio. Vamos a pedirle a su hijo que no coma ni beba nada después de las 10:00 pm de la noche anterior y esto también incluye no comer desayuno el día de esta visita.

- Vamos a repetir la prueba del ultrasonido que describimos en la parte de la primera visita.
- Vamos a darle a su hijo un licuado de vainilla para el desayuno que contiene alrededor de 900 calorías (60% de grasa, 10% de proteínas 30% de carbohidratos). Le pediremos que se lo tome lentamente y en menos de 10 minutos.
- Vamos a repetir el ultrasonido después de la comida a las 2 horas y a las 4 horas. Entre las dos medidas, vamos a pedirle a su hijo que permanezca sentado o acostado, con la excepción de ir al baño. El puede traer un libro para leer o algo para mantenerse ocupado durante este tiempo. Durante este tiempo no vamos a permitir el uso de la televisión, computadoras, iPad, etc. ya que puede afectar las medidas que estamos tomando.
- Después de esta visita, su hijo recibirá una tarjeta de regalo para el restaurante Subway para   que compre su cena la noche antes de su próxima visita. Su hijo va a comer la misma cena que comió la noche anterior de la otra visita.

<u>Medidas de los Vasos Sanguíneos y la Prueba de Comida Sin Ejercicio</u>

Se le pedirá a su hijo que vuelva a ASU a las 9:00 AM de la mañana. Vamos a pedirle a su hijo que la noche anterior coma la cena que el selecciono del restaurante Subway. Vamos a pedirle a su hijo que no coma ni beba nada después de la 10:00 de la noche anterior, que no haga ejercicio el día anterior, y que no coma desayuno el día de esta visita en ASU.

- Vamos a repetir el ultrasonido que anteriormente describimos.
- Vamos a darle a su hijo una malteada de leche de vainilla para el desayuno que contiene alrededor de 900 calorías (60% de grasa, 10% de proteínas 30% de carbohidratos). Le pediremos que se lo tome lentamente y se lo termine en menos de 10 minutos.
- Vamos a repetir el ultrasonido después de la comida a las 2 horas y a las 4 horas. Entre las dos medidas, vamos a pedirle a su hijo que permanezca sentado o acostado, con la excepción de ir al baño. El puede traer un libro para leer o algo para mantenerse ocupado durante este tiempo. Durante este tiempo no vamos a permitir el uso de la televisión, computadoras, iPad, etc. ya que puede afectar las medidas que estamos tomando.
- Al final de la visita, se le pedirá a su hijo que camine en una caminadora durante unos 10 minutos usando un monitor de frecuencia cardíaca.

RIESGOS.

Es posible que haya partes de este estudio que su hijo encuentre incómodas. Su hijo realizará un ejercicio vigoroso, se le pedirá que ayune durante la noche y se le pedirá que consuma un licuado alta en grasas. Además, su hijo tendrá que permanecer sentado o acostado por alrededor de 4 horas y no será permitido caminar durante este tiempo, excepto para ir al baño.

MOLESTIAS GENERALES.

Es probable que con el ejercicio su hijo empiece a sudar, a respirar con mas fuerza, a sentirse cansado y con los músculos adoloridos. Como con cualquier tipo de ejercicio mas duro, su hijo puede experimentar mareos, fatiga, o nauseas. En raras ocasiones algunas personas experimentan vómito y/o desmayos después de la prueba de ejercicio. Se le pedirá a su hijo que se coma un licuado alta en grasa y tal vez podría experimentar diarrea, vómitos, náuseas, dolor de estómago, reacciones alérgicas o intoxicaciones alimentarias.

COMPENSACIÓN POR ENFERMEDAD Y LESIONES

Si usted le da permiso a su hijo para que participe en este estudio científico, su consentimiento no cede ninguno de sus derechos legales. Sin embargo, no se han reservado fondos financieros para compensar en caso de lesión. Sin embargo, si alguna lesión ocurre debido a los procedimientos experimentales, se proporcionará primeros auxilios. Si hay una situación en la que los investigadores creen que se necesita mas atención de un médico, se le remitirá a la Clínica de Enfermeras en el campo de ASU. Si alguna lesión ocurre después de que la Clínica de Enfermeras en ASU ya este cerrada, usted debe buscar atención en un centro de emergencia medica. Si una emergencia fuera a pasar durante el estudio, le llamaremos a "911" para que el personal médico de emergencia vengan a el edificio HLRC. Usted será responsable de los gastos ocurridos.

TRATAMIENTOS ALTERNATIVOS.

No existen otros procedimientos alternativos disponibles para este estudio.

BENEFICIOS.

Puede que no haya ningún beneficio directo para usted o para su hijo por su participación. La información de este estudio científico ayudará a los investigadores a entender mejor cómo el ejercicio y la dieta tiene impacto en la salud cardiovascular de los jóvenes obesos. Su hijo tendrá la oportunidad de aprender acerca de sus niveles de condición física y cómo los científicos usan el ejercicio para entender cómo el cuerpo funciona.

NUEVA INFORMACIÓN.

Si nosotros encontramos nueva información durante el estudio que pueda cambiar su decisión o la de su hijo acerca de seguir participando, nosotros le daremos saber esta información.

CONFIDENCIALIDAD.

Toda la información obtenida en este estudio es confidencial al menos que la ley nos haga que la revelemos. Los resultados del estudio podrán ser publicados o presentados pero el nombre o la identidad de su hijo se mantendrá privada. Para mantener la información privada, vamos a utilizar códigos para identificar los voluntarios de la investigación y sólo el equipo de estudio tendrá acceso a la información personal. Los registros del estudio se mantendrán cerrados con llave en la oficina del Dr. Shaibi.

PRIVILEGIO DE RETIRO.

Esta bien si usted y su hijo dicen que no. Incluso si usted dice que sí ahora, los dos tienen la libertad de decir no durante el estudio y dejar de participar en cualquier momento. Su decisión no afectará su relación con la Universidad Estatal de Arizona o tampoco causara una pérdida de beneficios a los que de otro modo pueda tener derecho.

COSTO Y COMPENSACIÓN.

Los investigadores quieren que la opción de participar en el estudio científico sea la decisión de su familia. Ellos entienden que su participación puede costarle a su familia en términos de tiempo y viajes. Su hijo recibirá una compensación por su tiempo y esfuerzo y se le ofrecerá estacionamiento gratuito.

Su hijo recibirá una compensación de hasta $80 Dólares ($20 en la primera visita de selección y $30 por cada visita de comida). Si su hijo no termina los exámenes, el recibirá $10 dólares en efectivo por cada hora de su tiempo, pero no recibirá mas de la cantidad indicada anteriormente por cada visita. En cualquier momento los investigadores pueden pedirle a su hijo que abandone el estudio sin previo consentimiento. Su hijo todavía será compensado por su tiempo.

CONSENTIMIENTO VOLUNTARIO.

Cualquier pregunta que tenga sobre el estudio de investigación Científica o de la participación de su hijo, antes o después de su consentimiento, serán contestadas por Dr. Shaibi, 500 N. 3rd Street, Phoenix, AZ 85004, 602-496-0909.
Si usted tiene preguntas sobre los derechos que su hijo tiene como participante en esta investigación, o si usted siente que su familia ha sido puesta en riesgo, usted puede ponerse en contacto con el Presidente de la Junta Institucional de Revisión de Sujetos Humanos, a través de la Oficina de Integridad de la Investigación y Aseguramiento de ASU, al 480-965 6788.
Esta forma explica la esencia, las necesidades, los beneficios y los riesgos del proyecto. Al firmar este formulario, usted acepta con la capacitación que asumirá cualquier riesgo involucrado. Recuerde que su participación es voluntaria. Usted puede optar por no participar o de retirar su consentimiento y dejar de participar en cualquier momento sin

penalización o pérdida de beneficios, excepto lo que estén indicados aquí. Al firmar este formulario de consentimiento, usted no esta cediendo alguna reclamación legal, derechos o recursos. Una copia de este formulario se le entregará a usted.

Su firma abajo significa que usted autoriza a que su hijo participe en este estudio científico.

El nombre de su hijo (letra de molde): _____

_____   _____   _____
Firma del tutor legal.   Nombre (letra de molde)   Fecha

CONSENTIMIENTO PARA SER CONTACTADO/A PARA FUTUROS ESTUDIOS DE INVESTIGACIÓN CIENTÍFICA.

Dr. Shaibi y sus colegas en ASU tienen otros proyectos de investigaciones científicas. Su hijo puede ser elegible para participar en ellos. Si usted quisiera ser notificado/a de otras oportunidades de participación en investigaciones científicas, por favor firme abajo. Su hijo todavía puede participar en este estudio, aun si usted no quiere ser contactado/a en el futuro.

YO ☐ SI, doy mi consentimiento para que mi hijo sea contactado para futuros estudios.

YO ☐ NO, doy mi consiento para que mi hijo vuelva a ser contactado para futuros estudios.

_____
Iniciales del tutor legal

DECLARACIÓN DEL INVESTIGADOR.
"Certifico que he explicado al individuo de arriba la esencia y el propósito, los posibles beneficios y los posibles riesgos asociados con la participación en este estudio de investigación científica, se han contestado todas las preguntas que se han echo, y he sido testigo de la firma de arriba. Estos elementos del Informado Consentimiento se ajustan a las garantías dadas por la Universidad Estatal de Arizona a la Oficina de Investigación de Protecciones Humanas para proteger los derechos de los seres humanos. He ofrecido al participante una copia firmada de este documento de consentimiento."

_____   _____   _____
Firma del Investigador/   Nombre (letra de molde)   Fecha
Persona obteniento el concentimiento

APPENDIX H

FLYER-ENGLISH

ASU | School of Nutrition & Health Promotion

ARIZONA STATE UNIVERSITY

We are looking for adolescent males for a research project about diet, exercise and health.

Adolescents Qualify If:

- They are between 13-17 years old
- Are overweight but otherwise healthy

What's involved?

- Coming to ASU on 4 different occasions
- Measuring height and weight
- Exercising on a stationary bike
- Drinking a milkshake for breakfast
- Ultrasound measures of their arm
                Compensation!!!

- If you complete the 4 visits you will get paid **$80 in cash**
- We will also provide 2 breakfasts and 2 dinners

For more information please contact:

Jrryder@asu.edu

(602) 910-4997

APPENDIX I

FLYER-SPANISH

ASU School of Nutrition & Health Promotion
ARIZONA STATE UNIVERSITY

Estamos buscando a varones adolescentes para que participen en un proyecto de investigaciones científicas acerca de la dieta, el ejercicio y la salud

Los adolescentes califican si:

- Tienen entre 13 a 17 años de edad
- Están sobrepeso pero a la misa vez sanos

¿Qué se implica?

- Venir a ASU en 4 diferentes ocasiones
- Tomar medidas de la altura y el peso
- Hacer ejercicio en una bicicleta estacionaria
- Beber un licuado para el desayuno
- Tomar medidas de su brazo con un ultrasonido

Compensación

- Los participantes que terminen las 4 visitas recibirán $80 dólares en efectivo
- También les proveeremos 2 desayunos y 2 cenas

Para más información, por favor comunicarse con
Estela Barraza
por correo electrónico a estela.barraza@asu.edu
o por teléfono al (602) 888-3046

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APPENDIX J

DATA COLLECTION SHEETS

<u>Visit 1: Screening</u>   **<u>Date:</u>**

Consent |_____|_____|_____| **Notes:**
                      Complete   Initial   Time

Assent |_____|_____|_____| **Notes:**
                      Complete   Initial   Time

Anthropometrics |_____|_____|_____| **Notes:**
                                Complete   Initial   Time

Blood Pressure |_____|_____|_____| **Notes:**
                              Complete   Initial   Time

FMD |_____|_____|_____|_____|
Notes:
                  Complete   Initial   Time   Eligible?

VO2peak |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

Verification Phase |_____|_____|_____| **Notes:**
                                    Complete   Initial   Time

<u>Exercise Visit</u>   **<u>Date:</u>**

Completion of visit |_____|_____|_____|
Notes:
                                  Complete   Initial   Time

Number of bouts completed = _____ (tally)_____#

Number of bouts completed within HR range
=_____(tally)_____#

<u>Meal Visit Post Exercise</u>   **<u>Date:</u>**

Blood Pressure |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

Baseline FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

Meal Ingestion
|_____|_____|_____|_____| **Notes:**
                          Complete   Initial   Time
Tolerated?

2 Hour FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

4 Hour FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

<u>Meal Visit NO Exercise</u>   **<u>Date:</u>**

Blood Pressure |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

Baseline FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

Meal Ingestion
|_____|_____|_____|_____| **Notes:**
                          Complete   Initial   Time
Tolerated?

2 Hour FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

4 Hour FMD |_____|_____|_____| **Notes:**
                          Complete   Initial   Time

SUBEJCTS #:_____ **DOB:**_____/_____/_____

AGE:_____**(yr)**

WEIGHT:_____ **(lb)**_____**(kg)**

HEIGHT:_____**(in)**_____**(cm)**

BMI (kg/m^2):_____

      BMI(percentile):_____

	TRIAL 1	**TRIAL 2**	**TRIAL 3**

MEAN   HR

Morning BP (Screening): _____/_____   _____/___   _____/_____
_____/_____ _____

Morning BP (M+Ex): _____/_____   _____/___   _____/_____
_____/_____ _____

Morning BP (M only): _____/_____   _____/___   _____/_____
_____/_____ _____

Meal Dose

G fat needed:_____ **Meal break down:** _____(g
HWC)_____(g Ice Cream)

Exercise day (V1): Time of arrival:_____ **Confirm fasted: Yes /
No**

McMaster Protocol

RATE: 50-60rpm Initial load (Watts):_____

VO2peak begin time:_____ **VO2peak end
time:**_____

Rest time: Start_____**End**_____**Total amount of rest:**
_____

Verification phase: Work load:_____

Start time:_____**End:**_____ **Total time:**_____

VO2peak (ml/kg/min):_____   **HRmax(bpm):**_____

Work$_{max}$_____

50% HRmax:_____   **Power (Watts):**_____

90%HRmax:_____   **Power (Watts):**_____

Notes:

Exercise + Meal Day (V2): Time of arrival:_____   **Confirm fasting: Y / N**

Approximate time of last meal consumption:_____

Baseline FMD (Pre-meal): Time:_____

Meal consumption----Time: Start_____**End**_____**Total eat time:**_____

2 hour post-meal consumption FMD: Time:_____

4 hour post-meal consumption FMD: Time:_____

Notes:

Meal Only Day (V3): Time of arrival:_____   **Confirm fasting: Y / N**

Approximate time of last meal consumption:_____

Baseline FMD (Pre-meal): Time:_____

Meal consumption----Time: Start_____**End**_____**Total eat time:**_____

2 hour post-meal consumption FMD: Time:_____

4 hour post-meal consumption FMD: Time:_____

Notes:

<u>Submaximal VO$_2$ test:</u>

Weight: _____ (kg)   Height: _____ (cm) Resting HR: _____(bpm)

Start Time:_____   End Time:_____

<u>Measured during test:</u>

4 min HR: _____ (bpm)

HR Difference: _____ (bpm)

Speed: _____(mph)

Estimated VO2max:_____

VO2= -1772.81 + 318.64 * sex (F=0, M=1) + 18.34 * weight (kg) + 24.45 * Height (cm) – 8.74 * 4minHR – 0.15 * Weight (kg) * HR Difference + 4.41 * Speed (mph) * HR Difference.

APPENDIX K

LABORATORY FLOW-MEDIATED DILATION ASSESSMENT PROTOCOL

I. Subject Preparation
    a. Controls:
        i. Time of day: diurnal variation can effect FMD so Pre-Post measurements should be taken at the same time of day
        ii. Caffeine: no caffeine intake for 24 hours
        iii. Alcohol: no alcohol intake for 48 hours
        iv. Smoking: Refrain from smoking for > 12 hours if smokers are allowed in study
        v. Vitamins or supplements: no vitamins or supplements for 72 hours
        vi. Medications: all medications should be avoided for > 4 half-lives of the drug. Especially beta blockers, nitrates, and calcium channel blockers. Also NSAIDS should be avoided for 1 day and aspirin for 3 days.
        vii. Menstrual cycle: FMD should occur during days 1-7 of the menstrual cycle (ie: early follicular phase). Day 1 is when menses begins.
        viii. Prior exercise: subjects should avoid vigorous exercise for 24 hours also subjects should be instructed to reduce physical activity to as little as possible 24 hours prior.
        ix. Fasting measurements: should be taken at least 6 hours fasted
        x. Non-dominant arm: For cross-sectional studies the non-dominant arm should be used for FMD measurement
    b. Procedures:
        i. Lay down in a quiet dimly lit room, with no distractions, and temperature controlled for at least 20 minutes
            1. No cell phone use or music during this time
            2. Lay on back with legs fully extended
            3. Subjects should refrain from watching TV or engaging in any activity which might cause emotion before the FMD
        ii. Make sure subject is comfortable with arm and head fully supported
            1. Towel or foam rolls should be placed proximal to the elbow and at the mid forearm just distal to the cuff placement
                a. The towels/foam should hold the arm off of the table so that the cuff is not resting on the table while inflated
            2. The shoulder should be slightly externally rotated and at 80 degrees of abduction
            3. If the subject cannot keep his/her arm externally rotated the opposite side of the table can be lifted to support the body to allow the subject to lay on their side

II. Device Preparation

a. Ultrasound gel should be used and preferable warmed with a gel warmer to increase subject comfort
b. The blood pressure cuff (of appropriate size) should be placed just distal to the elbow (~1cm) and tightened snugly around the forearm
    i. If the subject has a very small arm (ie child or very small adult) then a child size cuff should be used
c. Table height should be adjusted so the sonographer can maintain a neutral angle at the wrist while imaging
d. The sonography chair should be used so that the arms are utilized as arm rests
e. The ultrasound probe should be held so that the notch on the top portion faces medially
    i. Reversal of this will make arteries blue and veins red in color mode

III. Image Preparation and Terason Settings
  a. Color Mode:
    i. Find artery
      1. Red colored pulsating blood flow
      2. May need to move yellow box to encompass artery
      3. Slow, subtle movements are needed in order to find artery the best
    ii. Adjust depth to find optimal image quality
      1. Larger subjects will require deeper imaging (ie: 4-6 cm), very lean subjects will require shallow imaging (ie: 2-3 cm)
    iii. Adjust focal zone to find optimal view of the artery
    iv. Find a section of the artery that is clear across the whole screen if possible
      1. Slide proximal and distal until desired artery section is found
      2. Once optimal location is found measure the distance from the medial epicondyle to the distal end of the probe and record this distance
      3. Also draw a small line at the distal end of the probe, so you have a reference point to come back to as well as seeing if you are moving the probe during recording
  b. 2-D Mode:
    i. Adjust frequency to find optimal setting (typical optimal setting is "High", but sometimes "Medium" results in a better image)
    ii. Increase rejection to decrease the amount of noise in the arterial lumen
    iii. Decrease compression to make the artery walls brighten
    iv. Adjust time gain compensation (TGC) sliders to brighten artery walls and decrease noise in lumen

          1. These are positional adjustments that align with the portion of the image that is just left of the slider on the screen

     v. Turn the "zoom" on then select a region about 0.5 - 1 cm above and below the artery encompassing the entire length of the artery if possible (if the entire screen length is not clear then only select the section that is clear)

     vi. Readjust compression, rejection, and TGC sliders now that the image is zoomed (it is normal to have to increase rejection more and decrease the TGC sliders that are aligned with the lumen after zooming)

     vii. Once the image is clear with crisp upper and lower artery walls and a noiseless lumen you can move on to Pulse Wave Mode

  c. Pulse Wave Mode:

     i. Place SV cursor in the artery lumen all the way on one side or the other (do not place the cursor in the center of the artery, the software will mistake the cursor for artery walls)

     ii. Decrease the "sound volume", the default is very loud

     iii. Increase SV size to encompass the entire artery lumen without touching the walls

     iv. Decrease the Baseline so that there is only a small portion of the negative blood flow velocity region visible (you will need as much visible in the positive direction for immediately after cuff release)

     v. Adjust the Gain knob in a clockwise direction to increase the sensitivity of the SV cursor or in a counterclockwise direction to decrease.

          1. Decrease gain when extra noise is being picked up outside of the typical pulse waves; Increase gain when the waves are dim and quiet

          2. This can be adjusted continuously while recording

IV. Flow-Mediated Dilation and Image Recording

  a. Baseline image

     i. Once both the image and the blood velocity signal are clear you are ready to record the baseline image

     ii. With the cuff not inflated record a 1 minute video at rest

     iii. Click "stop" then save the video file

     iv. Alternatively you can continue the recording and record all the way through cuff inflation up until 3 to 5 minutes post cuff release.

  b. Occlusion

     i. After recording the baseline image inflate the cuff to ~250 mmHg (supra-systolic is all that is necessary, but at least 200 is suggested)

     ii. Start a stop watch as soon as the target pressure is reached

     iii. Start imaging again soon after inflation

1. Some arteries can move substantially during occlusion so it may take a while to obtain a good image again
2. If substantial artery movement occurs then reset the TGC sliders (since these are positional)
3. Go through all 3 modes again and make sure you have a clear image and velocity signal

    iv. Start recording again at 4 minutes of occlusion, this will allow you to obtain 1 minute of post-occlusion baseline image before cuff deflation

c. FMD
    i. After you start recording the post-occlusion baseline video click the SV cursor to obtain control so that you can move the cursor in case the artery moves with cuff deflation
    ii. Have an assistant release the cuff at exactly 5 minutes of occlusion (longer or shorter occlusion times will result in different outcomes)
    iii. Record the video for at least 3 minutes after cuff deflation
        1. For patients with cardiovascular disease and elderly patients 5 minutes of post-deflation video is necessary
    iv. After the recording time is complete click "stop" and save the video file

V. Analysis using the lab view based Dicom encoder software by Ryan Harris
    a. Blinding, randomizing, and assessing reliability
        i. Before analysis begins a non-biased investigator should change all of the file names in order to blind the investigator who is conducting the analysis
        ii. The subject ID, condition, and/or time of the file should not be known to the investigator conducting the analysis
        iii. For each study it is recommended that a random sample of 10 images should be analyzed twice in order to assess intra-rater reliability
            1. Reliability should be assessed with coefficients of variation and Intra-class correlation coefficients
    b. Settings tab
        i. Select the folder you want to access the AVI files from in the AVI tab
        ii. Select the folder you want to save the analysis file to or access an analysis file from using the analysis tab
        iii. Select correct analysis options: ultrasound machine type, analysis type, etc
        iv. Input changes on patient files
    c. Acquire tab
        i. Choose avi file → input start and stop times → input patient name, DOB, and ID number

  ii. Choose csv file and name the file (already should be selected) → click return

  iii. Click on "show graph" (this is best on a dual monitor system, then you can have one monitor with the graph and the other with the acquire tab)

  iv. Review entire video to identify good ROI

  v. Click on the X for Diameter ROI → select ROI → click save ROI

   1. Rules on ROI selection:

    a. Select the largest ROI possible while still maintaining stable walls

    b. The upper and lower lines can be adjusted while analysis is going, but the length of the ROI box (right and left lines) should not be changed

   2. Scroll through video to see if you need to change ROI for different sections of the video

   3. Click "calibrate" next to ROI

    a. Each large tick mark is 1.0 cm

    b. Use automatic if the lines snap to the large tick marks correctly, if not then manually place the lines in the correct location

  vi. Click the X next to velocity ROI

   1. Select the entire velocity graph (inside of upper and lower tick marks and outside of right and left tick marks)

   2. Click Save ROI and the right and left lines should snap to the graph borders and you should see yellow dots tracing the waves

   3. Next click calibrate next to velocity

    a. Select an appropriate range on the y-axis, choose the value range that you are selecting (or type in a user defined range) then click calibrate

    b. Make sure the lines snap to the range you chose

    c. This can be done manually too if auto is not working

  vii. Click "analyze" → watch the entire video and graphing carefully to make sure the diameter ROI is optimal. You can change the upper and lower walls as necessary throughout the analysis process

  viii. Once it finishes it should ask you to save file; if it doesn't then click analyze again then immediately click exit, it will then ask you if you want to save

 d. Analysis tab

  i. Select file

  ii. Select the appropriate cursor control:

   1. FMD only: when you have baseline and FMD in the same video

          2. Beginning/end only: when you have only a baseline image at rest
- iii. For beginning/end only put one blue line on one end of the image and leave the other at the beginning
- iv. For FMD only:
    1. The first red line is baseline begin, put this just after the start of baseline
    2. The second red line is baseline end, put this just before cuff release
    3. The first green line is FMD begin, put this just right at cuff release
    4. The second green line is FMD end, put this after the peak dilation
    5. The next line is the dashed blue line which should go at the end of the video
- v. Manual outlier removal:
    1. By placing the two dashed blue lines around a section and pressing the space bar you can remove sections of the data
    2. This should be done for spikes in the data that are obviously errant data
        a. If the spike is greater than 0.01 cm above the rest of the data around that point then it should be manually removed
        b. This should be done before the automatic outlier removal
- vi. Next, click data control and select remove outliers (do this once only)
- vii. Above calculate choose the analysis type
    1. Polyfit is the most conservative and our preferred method
- viii. Click calculate
- ix. Make sure the software chose the appropriate peak diameter location if there is an obvious peak
- x. Scroll through the output on the bottom to obtain baseline diameter, peak diameter, FMD %, blood flow values, and shear rate values
- xi. Click "copy" then past the results into an excel sheet
- xii. Make sure you use the pre-cuff inflation baseline to calculate FMD.
    1. You can do this manually if your baseline and FMD files are separate
    2. Or if you continued recording all the way through you can have the software calculate this for you.

APPENDIX L

PRELIMINARY DATA
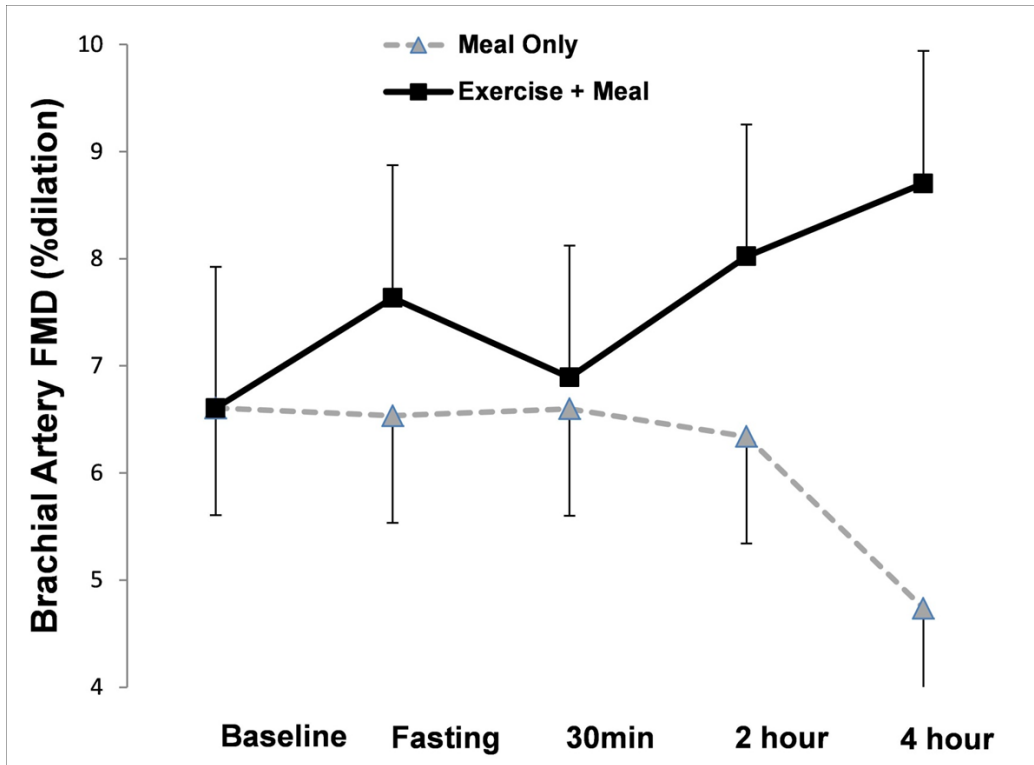
Preliminary data from pilot study on lean youth aged 11-16 years old (n=8). Data are presented as mean ± SE. Consumption of a high-fat breakfast was associated with a 28% decrease in FMD at 4hr when high-intensity exercise was "prescribed" 16hrs prior to consuming the high-fat breakfast, we observed an improvement of a 16% increase in FMD at 4hr. The exercise appeared to blunt or mitigate any postprandial endothelial dysfunction which was observed.

APPENDIX M

MILKSHAKE BREAKDOWN BY BODY WEIGHT

Body Weight (kg)	Fat Dose Needed (g)	Mix (g)	HWC (g)	VIC (g)	Total Kcal	Kcal Fat	kcal CHO	kcal PRO	%Fat	%CHO	%Pro	kcal HWC	Fat (g) HWC	CHO (g) HWC	PRO (g) HWC	kcal VIC	Fat (g) VIC	CHO (g) VIC	PRO (g) VIC
78	54.6	284.79	40.68	244.11	746	489.06	222.12	37.44	65.6	29.8	5.0	138	14.8	1.12	0.82	608	39.54	54.41	8.54
80	56	292.10	41.73	250.37	767	503.82	227.8	38.44	65.7	29.7	5.0	144	15.43	1.16	0.85	623	40.55	55.79	8.76
82	57.4	299.40	42.77	256.63	787	516.69	233.56	39.44	65.7	29.7	5.0	148	15.84	1.19	0.88	639	41.57	57.2	8.98
84	58.8	306.70	43.81	262.89	805	529.02	239.2	40.4	65.7	29.7	5.0	151	16.21	1.22	0.9	654	42.57	58.58	9.2
95	66.5	346.86	49.55	297.31	911	598.59	270.6	45.68	65.7	29.7	5.0	171	18.35	1.38	1.02	740	48.16	66.27	10.4
96	67.2	350.52	50.07	300.44	921	604.8	273.44	46.16	65.7	29.7	5.0	173	18.54	1.4	1.03	748	48.66	66.96	10.51
97	67.9	354.17	50.60	303.57	931	611.01	276.24	46.64	65.6	29.7	5.0	175	18.72	1.41	1.04	756	49.17	67.65	10.62
98	68.6	357.82	51.12	306.70	940	617.4	279.16	47.12	65.7	29.7	5.0	176	18.91	1.43	1.05	764	49.69	68.36	10.73
107	74.9	390.68	55.81	334.87	1027	674.01	304.76	51.44	65.6	29.7	5.0	193	20.65	1.56	1.14	834	54.24	74.63	11.72
111	77.7	405.28	57.90	347.39	1065	699.3	316.24	53.4	65.7	29.7	5.0	200	21.42	1.62	1.19	865	56.28	77.44	12.16

* Body Weight is for each participant

The amounts of each item were given to each participant based upon body weight

HWC = Heavy Whipping Cream

VIC = Vanilla Ice Cream

CHO= Carbohydrate

PRO = Protein

Appendix N

STANDARD MEAL DESCRIPTION

Participants were allowed to choose from any of the following meals to consume on 2 evenings prior to morning meal visits. These meals were consumed prior to 22:00 and were asked to be consumed with water. Instruction on to maintain the same bread type, cheese, and toppings were given. Participants were asked to refrain from chips or soda. Participants were asked to consume the entire foot-long subway sandwich. The nutrition data for each sandwich allowed is provided below. Note: these are for 6 inch sandwich's with no toppings.

	SERVING SIZE (g)	CALORIES	CALORIES FROM FAT	TOTAL FAT (g)	SATURATED FAT (g)	TRANS FATS (g)	CHOLESTEROL (mg)	SODIUM (mg)	CARBOHYDRATES (g)	DIETARY FIBER (g)	SUGARS (g)	PROTEIN (g)	VITAMIN A (%DV)	VITAMIN C (%DV)	CALCIUM (%DV)	IRON (%DV)
Black Forest Ham	216	290	40	4.5	1.0	0.0	20	800	46	5	8	18	8	20	30	15
Roasted Chicken	230	320	40	5.0	1.5	0.0	40	610	47	5	8	23	8	30	30	15
Roast Beef	230	320	40	5.0	1.5	0.0	40	660	45	5	7	24	8	20	30	25
Subway Club®	237	310	40	4.5	1.5	0.0	40	800	46	5	7	23	8	20	30	20
Sweet Onion Chicken Teriyaki	265	370	40	4.5	1.0	0.0	50	770	57	5	16	25	10	25	35	20
Turkey Breast	216	280	30	3.5	1.0	0.0	20	670	46	5	7	18	8	20	30	15
Turkey Breast & Ham	216	280	35	4.0	1.0	0.0	20	730	46	5	8	18	8	20	30	1

Appendix O

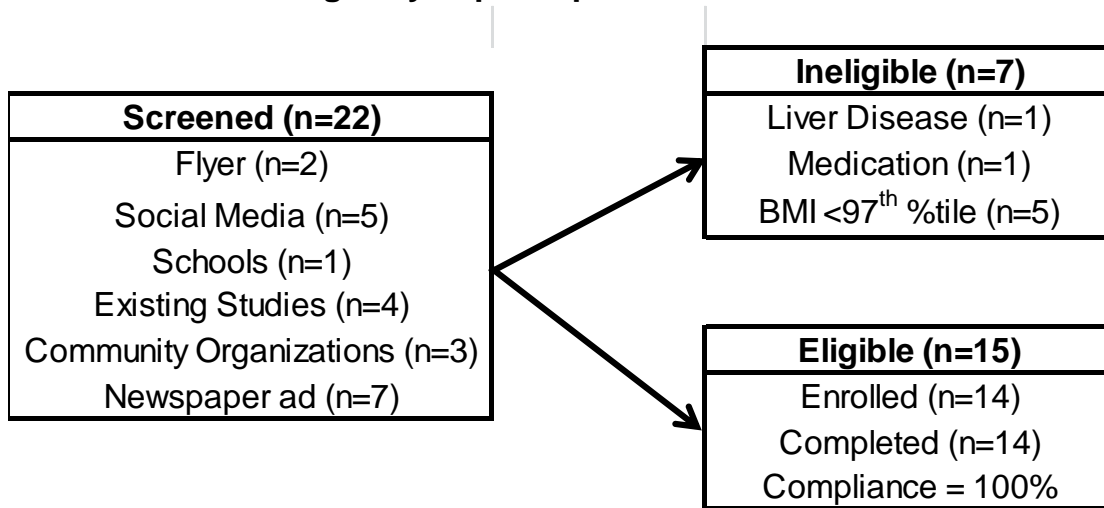FMD ANALYSIS PROCEDURES AND RELIABILITY

FMD analysis procedures and reliabilty
- All FMD files were blinded and randomized from the sonographer prior to analysis
- All files were analyzed in duplicate, blinded with CV% and ICC calculated
- If files CV% was >50%, the files was analyzed a third time
    - This was conducted on 5 files (of 168) with 4/5 showing close agreement with one file
    - The one file which could not show agreement was excluded from analysis (2hr Ex+M)
- The mean value for each files baseline diameter and peak diameter was used
- 10 peak and 10 baseline images were randomized and blinded
    - Images were analyzed by 3 different observes
    - Images were un-blinded and CV% and ICC with original sonographer were calculated
- **Intra-user reliability (n=168)**
    - Baseline diameter: CV% = 0.51% ; ICC = 0.998
    - Peak diameter: CV% = 0.64%; ICC = 0.997
    - FMD%: CV% = 11.31%; ICC = 0.965
- **Inter-rater reliability (n=20, range with 3 observers)**
    - Baseline diameter: CV% = 0.65-0.73%; ICC = 0.993-0.995
    - Peak diameter: CV% = 0.59-0.82; ICC = 0.993-0.996
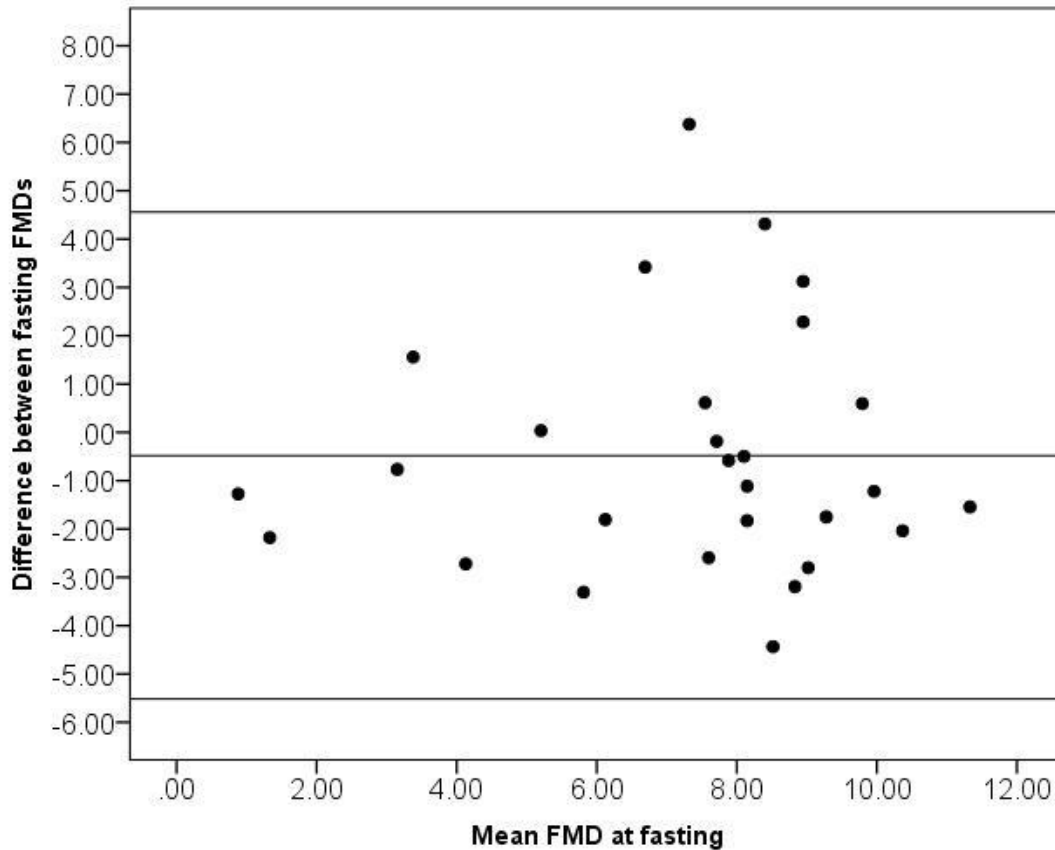   - FMD%: CV% = 5.8-7.7%; ICC = 0.931-0.989

Appendix P

RECRUITMENT AND PARTICIPANT ENROLLMENT

Recruitment and eligibility of participants

Screened (n=22)
Flyer (n=2)
Social Media (n=5)
Schools (n=1)
Existing Studies (n=4)
Community Organizations (n=3)
Newspaper ad (n=7)

Ineligible (n=7)
Liver Disease (n=1)
Medication (n=1)
BMI <97th %tile (n=5)

Eligible (n=15)
Enrolled (n=14)
Completed (n=14)
Compliance = 100%

Appendix Q

BLAND-ALTMAN PLOT OF FASTING FMD VALUES

Appendix Q is a Bland-Altman plot of fasting FMD values, with the difference between values being screening FMD – fasting FMD during the meal only visit. There is a difference between fasting values of 0.48±2.5%. The values were modestly associated with each other, r = 0.554, P=0.040. These values had an inter-class correlation coefficient of 0.712, suggesting modest reliability. The R^2 of this relationship is 0.307, or 30.7% of the variance in fasting FMD during the meal is explained by screening FMD, leaving 69.3% of the variance due to other factors.

BIOGRAPHICAL SKETCH

Justin Ross Ryder was born in Houston, Texas on November 23rd, 1984. At age 4 he moved to St. Louis, Missouri where he spent the remainder of his youth. He graduated from Parkway Central High School in Chesterfield, Missouri in May of 2003. He completed a Bachelor of Arts Degree in Exercise Science with a concentration in Exercise Physiology at Drury University in Springfield, Missouri in December of 2007. Upon completion of the degree he enrolled at the University of Missouri-Columbia in a Master of Science program in Exercise Physiology, which he completed in August of 2010. In August of 2010 he enrolled in an interdisciplinary program of Physical Activity, Nutrition and Wellness at Arizona State University. His research focuses on cardiovascular disease risk in youth. This dissertation is the culminating work of his graduate experience and the launching point of a career as a pediatric researcher. Upon completion of his Ph.D. he will become a postdoctoral fellow at the University of Minnesota on the Minnesota Obesity Prevention Training program to further his research training.