Children Active To Stay Healthy (CASH): Exercise as a Tool for Reducing Inflammation and Cardiovascular Risk in Sedentary, Pubescent Adolescents With Obesity

Dissertation

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By

Brooke Erin Starkoff, M. Ed.
Graduate Program in Physical Activity & Educational Services

The Ohio State University
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Dissertation Committee:
Steven T. Devor, Advisor
Ihuoma U. Eneli
Andrea E. Bonny
Lei Cao
Abstract

Obesity, even in children, is generally accompanied by a state of chronic inflammation. To combat childhood obesity, clinicians and scientists recommend lifestyle interventions that include increased physical activity and exercise in an attempt to promote weight loss and, consequently, decrease comorbidities associated with excess adiposity. More importantly, it appears that the influence of regular exercise may offer children with obesity a multitude of health benefits, independent of weight loss. However, the intensity of exercise required to elicit significant health benefits is still unclear. Therefore, the aim of the present project was to study the influence of high intensity interval exercise (HIIE) on the existing inflammatory state and endothelial dysfunction found in obesity. Adolescents with obesity were randomized into either moderate exercise (MOD) or HIIE groups, and attended sessions 3 times per week for 6 weeks. The moderate group cycled continuously for 30 minutes at 65%-70% of age predicted maximal heart rate (APMHR) and the HIIE group performed ten, 2-minute bouts at 90%-95% of APMHR. Outcome measures of body composition, aerobic capacity, blood lipids, glucose metabolism, endothelial function, and inflammation were measured pre- and post-intervention. 27 (17 females and 10 males; mean age 14.7 ± 1.5 years) completed the exercise intervention. A significant decrease in waist-to-hip ratio was
detected in both groups (p = 0.020), with no difference between groups. No other significant changes in body composition were noted within or between groups from pre- to post-intervention. For all subjects, BMIz score at baseline was strongly and positively associated with hsCRP (p = 0.006; r= 0.511). The HIIE group demonstrated a significant and negative relationship between endothelin-1 (ET-1) and percent of APMHR achieved during the intervention (p = 0.035; r = -0.567). Venous occlusion plethysmography (VOP) did not indicate significant changes to endothelial function from pre- to post-intervention for either group or between groups. However, the vasoconstrictor, ET-1 was found to decrease with increasing exercise intensity. Therefore, HIIE may elicit greater improvements to endothelial function by decreasing the enzyme responsible for vasoconstriction. These results may help establish exercise protocols not only for children with obesity, but also other inflammatory diseases such as diabetes, cancer, and arthritis.
Dedication

To grandpa Harvey Feinberg. Thank you for teaching me to continue to learn and always open new doors.
Acknowledgments

This process of personal growth and development would have never occurred without the support and encouragement I have received from many individuals. First, I want to thank my family for the continued love and positive thoughts as I traveled this winding road. I would also like to thank Dr. Devor for calming my nerves and keeping me on track. Likewise, I truly appreciate the mentorship of Drs. Bonny and Eneli who have put in plenty of ‘extra hours’ to help me build this project and see it through to the end. I also need to acknowledge Dr. Hoffman and the staff and nurses in the Clinical Research Services at NCH for the time and energy they also put into the CASH study.

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Vita

1997 ........................................ Laureal School

2001 .......................................... B.S. Communication Studies, The College of Wooster

2008 ........................................... M.Ed. Exercise Science, Cleveland State University

2007-2010 ................................... Exercise Physiologist, University Hospitals Case Medical Center, Rainbow Babies and Children’s Hospital

2010-present ............................... Graduate Associate, Department of Health and Exercise Science, The Ohio State University

2012 ........................................... Selected as Columbus-Athens Albert Schweitzer Fellow

Fields of Study

Major Field:  Physical Activity & Educational Services
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Chapter 1: Introduction

Childhood obesity rates among children and adolescents remain elevated in most industrialized countries. While recent studies have shown a plateau in the rise of obesity rates in children, the prevalence is still indicative of an epidemic. Research has shown that increased BMI at even 2 weeks of age is associated with a significant increase in risk of overweight or obesity in childhood (213). Subsequently, data also shows that childhood obesity is a major predictor for obesity in adulthood. Adult diseases such as atherosclerosis, type II diabetes mellitus (T2DM), and hypertension are becoming more prevalent in children and adolescents, with evidence of these diseases identified as early as the age of 2 (93).

The dangers of obesity in childhood are glaringly obvious. Recently, a plethora of research has recognized an increased risk of morbidity and mortality among children with obesity compared to their normal weight peers (48, 71, 75, 157, 184). Specifically, a recent study by Franks et al. (71) concluded that the current obese children are the first generation that will not live longer than their parents and, in fact, are 2 times more likely to die before the age of 55. While many contributing factors are responsible for this epidemic, positive energy balance is ultimately the culprit. Currently, The American College of Sports Medicine (ACSM) recommends children 6-18 years old accumulate at least 60 minutes, and up to
several hours, of moderate to vigorous daily physical activity. Yet, some research shows that 58% of children (ages 6-11) fail to attain the recommended amount of physical activity, a pattern that continues to decline rapidly through adolescence (195).

While inactivity is present in healthy weight and obese children alike, the dangers of future health complications are greater in the obese population. In fact, physical inactivity not only contributes to obesity and associated comorbidities in childhood, but it also sets the stage for a host of diseases in adulthood, including hypertension, T2DM, cardiovascular disease (CVD), and even premature death (71, 177, 185). Not only are many of these risk factors responsible for initiating an inflammatory response, but childhood obesity is independently associated with systemic low-grade inflammation (75). However, whether this inflammatory state is initiated solely by obesity or if physical inactivity also plays a role is not well understood (85, 123).

Inflammation is a normal physiological response to injury or infection in the body. Yet, in the case of obesity, a state of chronic, low-grade inflammation can be deleterious, leading to dysregulated adipocyte production of factors involved in the inflammatory cascade (75). Increasing adiposity is accompanied by increased inflammation via multiple mechanisms, involving adipose tissue generation of oxidative stress, endoplasmic reticulum stress (ER stress), and adipocyte secretions. Furthermore, obesity and subsequent inflammation are associated with dysregulated glucose metabolism resulting in hyperinsulinemia, insulin resistance,
and T2DM, eventually contributing to endothelial dysfunction (ED) and CVD, even in children (102).

Regular participation in exercise has been shown to increase anti-inflammatory mediators and decrease pro-inflammatory markers. A significant number of studies also show that regular exercise improves endothelial function (100, 123, 192, 205) and that continuous training is necessary to maintain vascular and anthropometric benefits (205). Exercise causes an increase in blood flow across the endothelium, which leads to shear stress mediated upregulation of endothelial nitric oxide synthase (eNOS) expression. The increased nitric oxide (NO) bioavailability thus allows for more efficient vasodilation and improved blood flow (100). In fact, Tjonna et al. (192) demonstrated that aerobic exercise improved HDL, blood glucose, and insulin, all of which directly influence NO bioavailability (192). It, therefore, seems crucial to use exercise as a tool to improve endothelial function in children with obesity.

Due to the overwhelming dangers of obesity many children now face, determining effective frequency, intensity and duration of exercise has become increasingly important. While many interventions include an exercise component, there is a lack of clarity concerning the proper intensity of exercise required to produce the greatest health benefits. In adults, both moderate and high intensity interval exercise (HIIE) have been shown to produce significant cardiovascular benefits, however, the improvements appear to be greater with higher intensity exercise. HIIE has been found to reduce inflammation and improve endothelial
function in adult populations, often times more significantly than moderate or lower intensity exercise (192).
Chapter 2: Review of the Literature

Adipose Tissue

White adipose tissue (WAT) is responsible for storing and releasing energy in the form of lipids. This system permits mammals to fast between meals by allowing for a backup of energy supply at all times. The white adipocyte is spherical in shape to accommodate maximal storage in a minimum amount of space. In fact, 90% of the white adipocyte is filled with a lipid droplet, comprised of triglycerides. These cells are specifically capable of secreting multiple hormones and cell signaling proteins known as cytokines, or adipokines.

Brown adipose tissue (BAT) is also capable of storing and releasing energy, but is mainly responsible for thermogenesis and the release of chemical energy as heat. By storing triglycerides in multiple, small cytoplasmic lipid droplets, brown adipocytes are able to rapidly burn large amounts of fatty acids. Brown adipocytes are activated by the action of sympathetic nerves on beta3-adrenoceptors (β3-AR). Once activated, brown adipocytes burn fatty acids within the mitochondria due to the uncoupling of fatty acid oxidation from ATP production via uncoupling proteins (UCP1). Subsequently, energy is dissipated as heat and allows the sympathetic nervous system to maintain thermoregulation, specifically when the individual is exposed to sub-thermoneutral temperatures. Uncoupling proteins have been
identified in WAT depots, and, therefore, demonstrate the ability of WAT to convert
to BAT (31, 45, 78).

During exposure to cold temperatures, transdifferentiation of white to brown
adipocytes has been documented (16). Following cold acclimatization, wild type
mice demonstrated a 20-fold increase in brown adipocytes in subcutaneous WAT.
This increase was accompanied by induction of thermogenic genes, such as UCP1,
peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), and
CCAAT/enhancing binding protein-β (C/EBPβ), in visceral and subcutaneous WAT.
The cold weather, subsequently, was found to increase the amount of brown
adipocytes present in WAT. Likewise, β3-AR knockout mice demonstrated a blunted
increase in number of brown adipocytes found in WAT after cold-acclimatization
(16). This highlights the significance of β3-AR in the phenotypic switch of white to
brown adipocytes.

Physical activity has also been found to aid in the transdifferentiation of
white to brown adipocytes. Mice exposed to larger living spaces, running wheels,
and mazes also demonstrated upregulation of multiple brown adipocyte proteins in
WAT. 4 weeks of exposure to this enriched environment resulted in some changes in
BAT gene expression, but a large change in the number of genes expressed in WAT.
The majority of these genes are responsible for switching adipocytes from energy
storage to energy expenditure, including PGC-1α. Multiple forms of β-adrenergic
receptors were also upregulated in WAT with a concomitant downregulation in
BAT, further implicating the role of physical activity in converting white adipocytes
to brown adipocytes. Even under the condition of diet-induced obesity, the mice in the enriched environment gained less weight, exhibited smaller fat pads, demonstrated greater body temperatures than the control group, further highlighting increased energy expenditure and, subsequent resistance to obesity (32).

It is hypothesized that the social, cognitive, motor, and social stimulation of the environmentally enriched mice contributed to activation of the sympathoneuronal system, resulting in the induction of brain derived neurotropic factor (BDNF). In turn, BDNF activates the sympathetic nervous system, contributing to the activation of proteins involved in brown adipocyte differentiation and subsequent adaptive thermoregulation (32). The role of exercise in the conversion of WAT to BAT has not yet been elucidated, but may be a viable option for helping to reduce obesity.

**Adipocyte Hypertrophy**

Made up of lipid-filled adipocytes, endothelial cells, blood cells, and macrophages, WAT serves functions apart from simply fat storage. WAT is now widely known as an endocrine organ, responsible for not only triglyceride storage, but also for regulation of glucose homeostasis, energy balance, and inflammation. By producing and secreting adipokines, WAT specifically regulates the inflammatory state of the body. When disturbed, as in obesity, adipocytes and nearby macrophages are able to initiate the inflammatory cascade, a mechanism implicated in the altered endocrine function associated with obesity (65).
Energy imbalance due to greater consumption of calories versus caloric expenditure, leads to storage of excess energy in adipocytes resulting in adipocyte hypertrophy and/or hyperplasia. Both hypertrophy and hyperplasia result in intracellular abnormalities of the adipocyte leading to subsequent functional abnormalities of the endoplasmic reticulum (ER) and mitochondria (41). Ultimately, adipocyte dysfunction results in increased secretion of pro-inflammatory adipokines and concurrent decreased secretion of anti-inflammatory mediators. The subsequent increased release of free fatty acids (FFA) and inflammatory proteins often result in dyslipidemia, hypertension, insulin resistance, T2DM, and CVD.

Positive energy balance sustained over a significant period of time often results in increased cell size or cell number. Hypertrophy is the enlargement of existing adipocytes, while hyperplasia is an increase in adipocyte number via differentiation of preadipocytes (57). Accumulation of fat from either of these mechanisms can occur in subcutaneous or visceral adipose tissue, yet differ in fat metabolism based on depot. Women, for example, with higher amounts of body fat mass tend to accumulate more fat cells predominantly in the subcutaneous region versus the visceral depot. Women with obesity also had larger adipocytes in both depots, yet they were significantly larger in abdominal subcutaneous regions, demonstrating greater adipocyte hypertrophy in subcutaneous adipose tissue. These observations show that obesity is associated with adipocyte hypertrophy in both depots, but adipocyte hyperplasia mainly in subcutaneous regions (57).
Hypertrophic cells may not receive adequate blood supply required to function properly. When the oxygen availability does not keep up with oxygen demand, the adipocytes become hypoxic and dysfunctional. Oxygen diffusion is limited to 100 μm, however, hypertrophic cells swell to nearly 180 μm (86). Therefore, the cell becomes hypoxic due to reduced blood flow and responds through several pathways. One such pathway results via the release of hypoxia inducible factor -1 (HIF-1), a transcription factor that accumulates during hypoxia and increases mRNA expression for genes involved in erythropoiesis and angiogenesis. In one study, low oxygen concentrations were identified in the WAT of mice with obesity, which also had less oxygen perfusion than lean controls. The expression of hypoxia inducible genes and ER stress molecules was also significantly elevated in these mice. Also notable was the significant suppression of adiponectin transcription factors identified in obesity, a characteristic that ultimately leaves the subject susceptible to CVD (86).

The hypertrophied adipocyte in obesity is heavily stressed and triggers inflammatory cascades to aid in repair of itself. The stress of obesity contributes to increased generation of reactive oxygen species (ROS) due to mitochondrial dysfunction, ER stress and unfolded protein response (UPR), adipose tissue hypoxia, and increased production and secretion of adipokines. Excess nutrient intake induces increased Kreb’s cycle activity. In normal conditions, the electron transport chain produces ROS, but in amounts easily managed by the antioxidant system. In
obesity, increased oxidative stress at the mitochondria due to processing of excess fatty acids results in mitochondrial uncoupling and increased release of ROS (41).

A similar process occurs in the ER during protein synthesis and subsequent folding, which requires a substantial amount of energy. After proteins are synthesized, they are then folded in the ER to ensure proper functioning. However, excessive nutrient intake places a greater demand on the adipose tissue ER to synthesize and subsequently fold more proteins. The process is slowed and often leads to misfolded or unfolded proteins, triggering the UPR. This cellular stress response is designed to activate a signaling pathway to increase production of molecular chaperones to assist in proper protein folding. If not performed in a timely fashion or if the system is overwhelmed, the UPR will initiate cell death, or apoptosis. During this process, C-Jun N-terminal kinase (JNK) is activated, phosphorylating serine residues of insulin receptor substrate-1 (IRS-1) and ultimately inhibiting insulin signaling (41, 49). JNK can also initiate inflammation via activation of nuclear factor-kappa b (NF-κB) and result in enhanced expression of pro-inflammatory mediators.

**Adipokines**

Adipose tissue produces and secretes a myriad of proteins that act as major regulators of metabolism, including satiety signaling, energy expenditure, and nutrient regulation. Adipokines are proteins secreted by the adipose tissue that act as cell signals both receiving information and consequently initiating a system of reaction. There are more than 50 identified adipokines responsible for adipocyte
regulation, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and adiponectin. These adipokines specifically play a role in inflammation, insulin sensitivity, and endothelial function.

The increased adipocyte volume in obesity can result in deleterious effects due to increased production and secretion of these inflammatory markers (14, 83, 178, 211). In a study comparing healthy controls to subjects who were obese non-diabetic, obese type 2 diabetic, and non-obese type 1 diabetic, obesity was associated with increased adipocyte volume and inflammation. The inflammatory markers TNF-α, IL-6, and high sensitivity C-reactive protein (hsCRP) were elevated, and adiponectin was lower in subjects with obesity, regardless of diabetic status (14). This significant shift in the pro-inflammatory/anti-inflammatory adipokine secretion occurs in hypertrophied adipocytes to favor inflammation (116). As cell size increases so does the secretion of the pro-inflammatory adipokines, TNF-α, IL-6 and IL-8, and monocyte chemotactic protein-1 (MCP-1) (178, 211). The impaired secretion of hypertrophied adipocytes is the first step in the pathogenesis of many inflammatory diseases associated with obesity, including insulin resistance, T2DM, and atherosclerosis (14, 83, 178, 211).

Excess adipose tissue that accompanies obesity contributes to the amount of circulating concentrations of adipokines, which are capable of a myriad of biological processes. These proteins are generally synthesized and/or secreted by adipocytes and are, therefore, dysregulated when the adipocyte is hypertrophied. Specifically, TNF-α, adiponectin, and IL-6 have been implicated as major contributors to the
inflammatory state found in obesity (75, 102, 122). These inflammatory markers are capable of triggering other pro-thrombotic mediators and acute phase reactants, such as C-reactive protein (CRP), which may ultimately induce CVD (146). The links between childhood obesity, CVD, and inflammation have been documented in recent research (19, 75, 100, 102, 146, 157). In a study comparing obese to healthy weight children, the obese group was found to have significantly higher values of the pro-inflammatory markers hsCRP and TNF-α, and lower values of the anti-inflammatory marker, adiponectin (75). Other studies have also shown this link between increased pro-inflammatory and decreased anti-inflammatory markers in childhood obesity (67, 100, 102, 133, 158, 176).

*Tumor Necrosis Factor- alpha (TNF-α)*

TNF-α is a 17 kD polypeptide hormone involved in the regulation of homeostasis and inflammation. Secreted primarily from macrophages within adipose tissue, TNF-α is a pro-inflammatory adipokine, rapidly synthesized and released into circulation where it is quickly degraded (64, 186). In injury or stress, TNF-α promotes white blood cell activity to aid in the repair of damaged tissue. However, in obesity, hypertrophied adipocytes contribute to increased levels of TNF-α, continuously being produced and secreted into circulation (178, 211). Ultimately, altered levels of TNF-α may lead to further inflammation, vascular injury, and atherosclerosis.

Insulin resistance and associated atherosclerosis are particularly deleterious effects of increased levels of serum TNF-α (24). Once secreted, TNF-α can cross talk
with adipocytes and contribute to the breakdown and release of FFA (182). FFA and TNF-α released from adipocytes play a significant role in insulin resistance at the adipocyte, in the liver, and at other peripheral tissues. When secreted in excess, this protein can block the site of insulin binding at the tyrosine kinase insulin receptor, resulting in hyperinsulinemia and dysfunction of glucose and lipid metabolism. More specifically, TNF-α is capable of modifying insulin signal transduction and altering glucose uptake, ultimately impacting rates of gluconeogenesis and glycogenolysis.

When values are elevated, TNF-α increases serine phosphorylation of IRS-1, which then inhibits insulin receptor tyrosine kinase activity. The result is a decrease in skeletal muscle glucose transporter type 4 (GLUT4) translocation to the plasma membrane, and subsequent reduction in cellular glucose uptake. Following 4 hours of TNF-α infusion, whole body glucose uptake was inhibited in healthy men. However, there were no significant changes in hepatic glucose production, demonstrating that TNF-α may initially exert its effects on solely skeletal muscle. The administration of TNF-α prior to infusion of insulin also resulted in increased phosphorylation of JNK at the skeletal muscle along with an increase in serine phosphorylation of IRS-1 (152). Again, these results demonstrate that TNF-α interferes with and prevents insulin-stimulated tyrosine phosphorylation of IRS-1. Therefore, TNF-α is a significant contributor to insulin resistance and subsequent inflammation.
Secreted in response to injury and stress, TNF-α signals monocytes and other cytokines to aid in repairing the damage. This protein is responsible for triggering major inflammatory cascades such as the JNK and NF-kB pathways (64, 152). NF-kB and JNK pathways link inflammation, obesity, and insulin resistance. Obesity-induced activation of NF-kB is via transcriptional regulation of target genes, while JNK activation is through serine phosphorylation of IRS-1, inhibiting normal tyrosine phosphorylation of IRS-1 and blocking insulin signaling. These pathways thus, are capable of transcriptional regulation of target genes, which then act on smooth muscle to initiate atherosclerosis and contribute to cardiovascular disease (CVD).

TNF-α levels in children with obesity are higher compared to their normal weight peers, confirming the existence of low-grade systemic inflammation in children with obesity (24, 50). However, the relationship between TNF-α levels and inflammation is not necessarily based on the magnitude of obesity. Rather, the degree of physical fitness plays a significant role in TNF-α levels in children. While obesity contributes to increased synthesis and secretion of inflammatory markers, physical activity is inversely correlated with these biomarkers. In a study comparing obese and lean children with varying levels of fitness, the obese-fit group had lower levels of TNF-α compared to the obese-unfit group. In fact, the obese-fit group had levels similar to the lean-fit group (81). It is apparent, therefore, that while obesity is accompanied by inflammation, this pathology can be remedied through increasing physical activity.
Interleukin-6 (IL-6)

IL-6 is a circulating adipokine involved in inflammation and injury. While it is produced by many cell types, in a normal state, 15-30% of circulating IL-6 is derived from adipose tissue (128). As the amount of adipose tissue increases, such as in the state of obesity, levels of circulating IL-6 also increase (94, 133). Recently, IL-6 has been identified as a key regulator in glucose and fat metabolism. Most likely due to the low-level inflammation associated with obesity, this rise in circulating IL-6 can potentially alter metabolism of fat and glucose.

Current studies have shown paradoxical effects of IL-6 on glucose and lipid metabolism. While some studies have found a relationship between increased IL-6 and impaired insulin function, others show IL-6 levels linked to enhanced glucose uptake and fat oxidation in the liver. Research on children with obesity found that IL-6 levels rise linearly with increasing adiposity (94, 133). This is of particular concern in obesity, as excess visceral fat is mobilized through the hepatic portal vein and distributed to the liver. The accumulation of fat in the liver not only leads to the release of acute phase proteins, such as CRP, but can also interfere with hepatic glucose uptake and fat oxidation (94).

During exercise, however, IL-6 has been identified as an anti-inflammatory agent. Induced by contraction of the skeletal muscle, exercise leads to a rapid release of IL-6. Once in circulation, IL-6 acts as an anti-inflammatory agent, inhibiting pro-inflammatory mediators like TNF-α and interleukin-1 (IL-1) (145, 161). Greater muscle oxidative capacity is another significant benefit to habitual
endurance exercise. The improved inflammatory condition coupled with mitochondrial biogenesis as a result of endurance exercise is beneficial for glucose and fat metabolism.

Ji et al. (91) examined the effects of IL-6 on lipolysis, mitochondrial function and biogenesis, and mitochondrial contents in differentiated 3T3-L1 adipocytes. The study sought to determine changes in glucose uptake within the adipocyte, following IL-6 treatment. The results showed that IL-6 treatment led to increased lipolysis, ROS, basal glucose uptake, and mitochondrial biogenesis and decreased ATP production and mitochondrial membrane potential in 3T3-L1 adipocytes compared to control cells. In fact, treated cells were found to have significantly fewer lipids and abnormal morphology. Oxidative phosphorylation was also decreased in adipocytes treated with IL-6 as seen by a decrease in ATP production by the mitochondria. This study showed that IL-6 had a lipolytic effect on mitochondria, increasing the amount of free fatty acids, and inhibiting normal metabolic function. These findings not only elucidate the regulation of glucose and fat metabolism by IL-6, but also highlight the dangers of obesity.

IL-6 is produced in higher levels by abdominal adipocytes compared to subcutaneous, and has a dichotomous role in inflammation. When produced by adipocytes, IL-6 contributes to inflammation by acting on the liver to produce acute-phase reactants such as CRP (133). CRP acts on the endothelium and decreases eNOS expression, reducing NO production. The higher production in abdominal adipocytes, implicate IL-6 as a major contributor to insulin resistance. Adipocytes in
the visceral depot are not only more active and lipolytic, but also have direct access to the liver via the hepatic portal vein. Increased flux of fatty acids and adipokines to the liver inhibit normal insulin signaling and result in insulin resistance.

**C-reactive Protein (CRP)**

C-reactive protein (CRP) is an acute phase reactant produced solely by the liver. This protein was originally identified as a protein that triggered the C-polysaccharide of the pneumococcus cell wall in streptococcus pneumonia infections. CRP also was found at high levels during the initial phase of an infection, but returned to undetectable levels once the infection disappeared (95, 188). Primarily created in the liver, CRP is produced in response to IL-6, which is enhanced by IL-1β via the NF-κB mediators p50 and p65. IL-6, when bound to its receptor, phosphorylates JAK kinases resulting in subsequent phosphorylation of STAT3. STAT3 then binds to response elements on the promoter region of specific cytokines, such as IL-6, and leads to enhanced CRP transcription. IL-1β has no effect by itself, but does synergistically enhance CRP transcription in the presence of IL-6 (218).

Baseline values of this marker of inflammation are used as an independent predictor of CVD and T2DM in adults and children. CRP values are positively associated with measures of fatness including BMI, with extreme obese children (defined as 1.2 times the 95th percentile) showing significantly greater levels than obese and normal weight children (133). This linear relationship between obesity and CRP is due to the actions of TNF-α and IL-6 on the liver (75). Increased levels of
these circulating adipokines found in obesity act on the liver and increase production and secretion of CRP.

Once secreted, CRP is capable of inducing complement activation, enhancing monocyte infiltration and stimulating tissue factor production resulting in thrombosis and atherosclerotic lesions. After incubation with CRP, human endothelial cells express a 10-fold increase in vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) expression. This response appears to be dose-dependent until 100 μg/mL with similar results after incubation with IL-1β (144). Increased expression of adhesion molecules in the vasculature is indicative of the role of CRP in modulation of the vessel walls and its role in the development of inflammation and atherosclerosis.

Increased concentrations of CRP have been linked to CVD, T2DM, and the development of atherosclerosis. Specifically, levels between 3 and 10 mg/L indicate high risk for CVD in adult subjects. Circulating levels of CRP may also be closely associated with an inflammatory profile and early atherosclerotic development. In a group of children with obesity, CRP levels were inversely related to measures of endothelial function, demonstrating increased endothelial dysfunction with greater levels of CRP (99). Another study examining the effects of obesity on Native Canadian children, found significantly higher CRP levels compared to previously reported NHANES III data. In fact, 15.8% of the children in the study had levels indicative of high risk of CVD in adults while 21.9% demonstrated average adult risk of CVD (159). These studies ultimately demonstrate that the deleterious effects of
obesity expose children, as young as 10 years of age, to the same risk as adults for inflammatory conditions, contributing to atherosclerosis and associated CVD.

High-sensitivity CRP (hsCRP) is an assay with greater sensitivity of determining levels of inflammation within a normal range. Plasma levels of hsCRP have been identified as a strong, independent predictor of myocardial infarction, stroke, and vascular death among those without known CVD. In children with obesity, hsCRP was significantly related to intima media thickness (IMT) of the common carotid arteries, demonstrating the role of chronic inflammation in the progression of atherosclerotic disease (157).

Habitual participation in physical activity may result in reduced levels of CRP in children. While some studies show that participation in vigorous physical activity was associated with lower CRP levels in adolescent boys, but not girls (164), others demonstrate reductions in CRP regardless of gender or pubertal status (158). The sex differences may be attributed to the greater amount of visceral fat in males versus females. Subsequently, visceral adiposity contributes to the production of pro-inflammatory cytokines, such as IL-6, resulting in the activation of CRP production in the liver. Therefore, reductions in adiposity may be responsible for the associated changes in CRP due to the subsequent decreased production of pro-inflammatory cytokines.

Physical activity often contributes to weight loss and subsequent fat loss, which may be the mechanism responsible for decreased CRP production. A one-year intervention designed to induce weight loss in children with obesity through diet,
exercise, and behavioral therapy, resulted in significant reductions in hsCRP. Compared to those children who maintained their weight, those who achieved significant weight loss (defined as a drop in BMI-z score of .66) demonstrated reductions in hsCRP, blood pressure, triglycerides, and measures of insulin resistance with a concomitant increase in HDL (158). Body weight is therefore believed to pay a significant role in the circulating values of CRP. Many studies have identified a significant correlation between hsCRP and measures of adiposity (75, 94, 100, 158, 164, 175, 183), highlighting the mediation of cytokines on the metabolic effects of obesity. The reduction in CRP levels was even identified in as little as 6 weeks of aerobic exercise, independent of significant weight loss (99). It is possible that exercise alone may contribute to decreasing levels of pro-inflammatory adipokines, potentially via mechanisms that promote anti-inflammatory proteins.

**Adiponectin**

Adiponectin is a protein secreted into the plasma exclusively and abundantly from adipose tissue. Consisting of an N-terminal signal sequence, variable domain, collagenous domain, and a highly conserved C-terminal globular domain (gAd), adiponectin plays a role in metabolic processes and protects against structural disorders of the vasculature. It is abundantly present in human plasma circulating at a range of 2-30 µg/ml, and making up 0.05% of the total plasma protein (10). Recent studies have shown that the full-length protein is proteolytically cleaved, yielding a
globular domain believed to have more extensive biological activities than the full form (52).

One of the first groups to discover the protein also identified a paradoxical decrease in obesity. Fat samples were taken from obese mice and humans and adiponectin levels were examined. Significant reductions in adipoQ mRNA expression in fat tissue from the mice sample and a 50-80% reduction in the human sample were found (87). This exemplifies how obesity leads to a dysregulation in the secretion of adiponectin. More specifically, adiponectin has been found at significantly reduced levels in obese subjects compared to non-obese subjects (10, 37). Following gastric bypass surgery, morbidly obese individuals demonstrated increased adiponectin levels, greater than those still awaiting surgery, and similar to non-obese individuals (37). In individuals with obesity, hypertrophied adipocytes and subsequent adipocyte dysfunction are responsible for decreased levels of adiponectin production and secretion.

With adipose tissue now being identified as an endocrine organ, adipokines, specifically adiponectin, have been identified as important regulators of energy homeostasis. In particular, adiponectin has been shown to increase glucose uptake and stimulate fatty acid oxidation in human skeletal muscle. The chronic inflammatory state associated with obesity, however, has been found to blunt many of the beneficial effects of adiponectin. Research has found a significant negative correlation between BMI and plasma adiponectin concentration, thereby decreasing the overall positive benefits of the adipokine (10, 37). As the adipocyte
hypertrophies in obesity, TNF-α secretion increases which ultimately antagonizes and reduces adiponectin production. The subsequent reduction in circulating adiponectin equates to increased inflammation and decreased production of NO, contributing to impaired glucose and fat metabolism and endothelial dysfunction.

Bruce et al. (26) identified a blunting of fat oxidation in subjects with obesity. The attenuated response was due to decreased AMP protein kinase (AMPK) activation in those with obesity. Subjects in this study were also found to have decreased levels of adiponectin, potentially implicated in the attenuated rates of fat oxidation. Adiponectin directly phosphorylates AMPK to initiate the cascade that leads to improved fat oxidation. Once activated by phosphorylation, AMPK phosphorylates and thus inhibits Acetyl-CoA carboxylase (ACC) activity, an essential enzyme for fatty acid synthesis. Subsequently, ACC activity on malonyl-CoA is inhibited, allowing carnitine palmitoyltransferase 1 (CPT1) to proceed in fatty acid oxidation (26). Increased fat oxidation thus allows for greater lipid mobilization and less accumulation of ectopic lipid.

Adiponectin also acts as an anti-atherogenic and anti-inflammatory protein, specifically by inhibiting the actions of TNF-α. While the protein is still produced and secreted, TNF-α action is inhibited at the post-receptor level. Therefore, TNF-α may bind to its receptor, but its activity is halted by the presence of adiponectin (139). The inhibition of TNF-α also attenuates the formation of atherosclerotic plaque within the vessel walls. The process of atherosclerosis, one of the first signs of CVD, begins with injury to the endothelial cells lining the walls of the vasculature.
The injured site induces adhesion molecules like vascular cell adhesion molecule (VCAM-1) and intracellular adhesion molecule (ICAM-1), which ultimately attract circulating monocytes to the injured cells. The monocytes infiltrate into the subendothelial space and differentiate into macrophages. Subsequently, large amounts of lipids accumulate at this space and become foam cells, eventually impairing synthesis of NO by eNOS and hindering blood flow through the vessels. Adiponectin, however, antagonizes the inflammatory adipokine TNF-α, thereby reducing the inflammatory cascade (118).

Along with its antagonistic effects on TNF-α, adiponectin, in a fashion similar to insulin, activates eNOS in vascular endothelium to increase the production of NO. Insulin phosphorylates and activates Akt at Ser473, which then phosphorylates and activates eNOS at Ser1179. Adiponectin activates AMPK, which in turn phosphorylates eNOS at Ser1179 via a phosphoinositide-3-kinase (PI3K) pathway, activating the enzyme and leading to increased production of NO. Both insulin and adiponectin phosphorylate eNOS via PI3K dependent pathways, but adiponectin does not require the phosphorylation of Akt for the production of NO (36). Therefore, adiponectin and insulin act synergistically to increase vasorelaxation and improve endothelial function.

Consequently, decreases in both insulin and adiponectin contribute to the significant reduction in endothelial function and can lead to CVD. In one study of Japanese men, those with hypoadiponectinemia were found to have significantly reduced rates of forearm blood flow (FBF) and flow debt repayment. In these men,
the reduced rate of blood flow following occlusion was less than that of those with normal adiponectin levels (174). Reduced adiponectin, therefore, has a significant role in decreasing NO production, resulting in endothelial dysfunction.

Adiponectin levels have been found to be an independent predictor of changes to the vasculature and thus, endothelial function. In obese male rats, maximal endothelial-dependent relaxation was impaired compared to normal weight rats. Furthermore, exposure to the globular domain of adiponectin, gAd increased vasodilation through increased production of NO in the obese rat. More specifically, gAd phosphorylates AMPK at Thr^{172}, allowing for activation of eNOS. Before treatment with gAd, the obese rats demonstrated impaired vasodilation and NO production as a result of hypoadiponectinemia, an initial indication of endothelial dysfunction (52).

In obesity, when inflammatory markers are elevated and adiponectin is decreased, a substantial amount of oxidative stress is placed on the vascular system. The increased production of TNF-α, MCP-1, and plasminogen activator inhibitor 1 (PAI-1) by hypertrophied adipocytes overtake adiponectin production and reduce its functionality, promoting the generation of ROS. The increase in the generation of ROS is responsible for the decreased production and availability of NO. ROS destroys NO and therefore reduces vasomotor tone within the vessels (33).

Adiponectin is a potent anti-thrombotic and anti-atherogenic protein secreted in abundance from the adipose tissue. Its effects on fat oxidation, insulin sensitivity, and endothelial function are demonstrated in a myriad of studies using
humans and animals. The reduction of this protein can have significantly deleterious effects to vascular function and may result in the development of CVD. It is therefore, essential to maintain a healthy weight through proper diet and exercise to avoid positive energy balance. However, individuals with hypoadiponectinemia may consider treatment with gAd to improve insulin sensitivity and endothelial function.

**Macrophage Infiltration**

The mechanism contributing to atherosclerosis involves TNF-α induced activation of MCP-1(24). Dysregulated production and secretion of MCP-1 results in macrophage infiltration of adipose tissue (73, 83, 148). Macrophages are white blood cells, derived from monocytes that function as immune cells by initiating the inflammatory cascade. Yet, when levels are increased due to obesity, the inflammation can be more deleterious than beneficial.

As levels of MCP-1 rise in adipose tissue, macrophages are recruited from circulating monocytes. In obesity, increased levels of macrophages are capable of producing and secreting inflammatory mediators. Transcription factors associated with macrophages are upregulated in direct proportion to adiposity (206).

However, macrophages exist in two different phenotypes, M1 and M2, and are always present in white adipose tissue. M1 is the pro-inflammatory phenotype, characterized by greater ROS generation and active secretion of inflammatory markers such as TNF-α and IL-6. On the contrary, the M2 phenotype generates anti-inflammatory adipokines, offering a protective feature to the adipocyte.
In obesity, there tends to be a switch of phenotypes from M2 to M1 that is differentially activated depending on the degree of adiposity (116, 181). As the ratio of M1 to M2 cells substantially increases, a coinciding recruitment of M1 macrophages to the area occurs, inducing platelet aggregation and contributing to the creation of foam cells (116). Foam cells are the beginning stages of atherosclerotic development that occur at the endothelium. Injury to the endothelial cells, including that seen in response to obesity, contributes to MCP-1 macrophage recruitment and subsequent initiation of CVD.

Research has found that macrophage infiltration of the adipose tissue contributes to the low-grade chronic inflammation, linking obesity to its many comorbidities (148, 182). Macrophages, in particular, stimulate production of chemokines, adhesion molecules, and adipokines (73, 148, 182). These macrophages have been found to be the major producer of TNF-α and a significant producer of IL-6, therefore, contributing to inflammation in obesity (182, 206). The inflammatory process is further developed when accumulated fat in adipose tissue and subsequent production of ROS trigger the NF-κB pathway. NFκB further increases production of MCP-1 and contributes to inflammation, acting as a key mediator in the role of oxidative stress on adipocyte gene expression. Adipocytes treated with agents that induce oxidative stress show increased levels of phosphorylated IκBα initially. Soon thereafter, the phosphorylated subunit decreases, demonstrating the proteosomal degradation specific to the NF-κB pathway. Likewise, 3T3-L1 adipocytes pretreated with macrophages have also been
found to induce p65, a subunit of the NF-κB pathway (148). This implicates the NF-κB pathway in the inflammation process that occurs with increased ROS in obesity (64). The release of inflammatory markers indicates crosstalk between adipocytes and macrophages that further increases the inflammatory profile (148, 182).

Excess TNF-α and the resultant increase in FFA also contribute to significant secretion of inflammatory proteins and macrophage recruitment. Subsequently, greater numbers of macrophages aggregate around dead adipocytes, resulting in crown-like structures (CLS), the first sign of atherosclerosis. In a cohort of adults with obesity, a positive significant association was identified between presence of CLS and TNF-α. These individuals also demonstrated a significantly greater amount of genes involved in the NF-κB pathway, further enhancing the synthesis of the pro-inflammatory markers, IL-6 and IL-8 (110).

The increased secretion of MCP-1 tends to occur in the presence of adipose tissue cell death. There is a significant positive association between adipocyte size and rate of cell death so that as the cell hypertrophies in obesity, there is an increased chance of cell death. Adipose tissue macrophages are responsible for clearance of the dead cell in order to remodel and repair the adipose tissue, ultimately activating the macrophages and leading to an inflammatory condition (181). In a normal, healthy state, the anti-inflammatory mediators counter-balance this condition, however, in obesity, the phenotypic switch to M1 macrophages further contributes to inflammation (116, 181). Furthermore, CLS that form at the site of adipocyte death tend to express greater levels of TNF-α and IL-6, suggesting
that dead adipocytes are a primary location for macrophage induced expression of pro-inflammatory mediators (181). Therefore, cell death due to hypertrophy in obesity appears to be another contributor of adipocyte dysfunction.

Greater amounts of macrophages have been identified in visceral adipose tissue compared to subcutaneous adipose tissue in lean and obese subjects. Specifically, obese subjects with predominant visceral fat had a greater association with increased macrophage infiltration. Increased macrophage infiltration into visceral adipose tissue appears to be a trait of this specific depot. Therefore, those with obesity have greater adipose tissue macrophage infiltration, particularly if they have more visceral than subcutaneous fat (83). Increased levels of oxidative stress due to greater production of ROS and adipokines in WAT may also be greater in visceral adipose tissue. Adipocyte dysfunction, therefore, seems to be a direct result of infiltrating macrophages due to increased production of MCP-1 and other inflammatory adipokines, and increased fatty acids stimulating ROS production via NADPH oxidase (73).

**Obesity and Endothelial Function**

The condition of inflammation can be destructive when chronic, as is the case with obesity. It has been suggested that this state of inflammation in children with obesity is responsible for early endothelial damage and arterial abnormalities, setting the stage for CVD in adulthood (48, 75, 102, 157). The effects of childhood obesity on CVD have been assessed by endothelial response to internal and external stimuli. Risk factors for CVD are associated with endothelial dysfunction and include
elevated CRP, decreased adiponectin, and physical inactivity (33, 85). This suggests that any degree of overweight and obesity can attribute to arterial abnormalities at a surprisingly young age.

Endothelial dysfunction is one of the first signs of atherosclerosis and subsequent CVD. The process of atherosclerosis and the development of fibrous plaque lesions have been identified in the arteries of children as young as 2 years old (20). Atherosclerosis is specifically initiated by injury to the endothelium including that caused by the inflammatory condition of obesity. Endothelial dysfunction, the impairment of the endothelia’s ability to vasodilate, precedes plaque formation and is used as a tool to determine cardiovascular risk in children and adults (7).

Vascular endothelium secretes many factors that are capable of regulating vascular tone, including nitric oxide (NO) (43). NO is a liable gas created in the endothelium via a combination of L-arginine and endothelial nitric oxide synthase (eNOS), and is responsible for improved vasomotor tone. L-Arginine is compartmentalized within the endothelial cells, one of which is accessible to NOS. eNOS activity can be modulated by phosphorylating the Ser1179 site through post-translational modifications (36). When inactive, eNOS is bound to caveolin within the membrane. However, activation of the endothelial acetylcholine receptors activates phospholipase C (PLC) to initiate the dissociation of eNOS from the caveolin. PLC then catalyzes the production of inositol 1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG) from phosphatidylinositol 4,5-biphosphate (PIP$_2$). The
released IP₃ induces increased calcium release within the endothelium, activating calmodulin that binds to eNOS and allows for translocation to the cytoplasm. The enzyme then combines with L-arginine to produce NO and L-citrulline. Protein kinase A (PKA) can then phosphorylate eNOS to inactivate the enzyme, after which it returns to the membrane caveolin. Once formed, NO can diffuse into the smooth muscle from the endothelial cells and activate soluble guanylyl cyclase, which stimulates cyclic guanosin monophosphate (cGMP) resulting in vasorelaxation. Greater relaxation of the vascular smooth muscle cell allows for greater blood flow and reduced blood pressure. Inflammation due to injury, illness, or obesity may hinder endothelial function and contribute to the aggregation of platelets and cell adhesion molecules on the vascular walls resulting in endothelial dysfunction.

One proposed cause of endothelial dysfunction is a decreased bioavailability of NO in the vessel wall. NO is a cell-signaling molecule responsible for vasodilation in response to shear stress associated with increased blood flow in the endothelium. Increased shear stress contributes to the opening of specialized ion channels in the endothelial cell membrane, allowing for increased calcium flow into the cell and subsequent generation of NO (126). Formation of plaque and accumulation of adhesion molecules tends to occur in vessels with low shear stress (< 6 dyne/cm²) and subsequent diminished NO. Likewise, areas of extremely high shear (>70 dyne/cm²) demonstrate erosion of the endothelium and platelet aggregation, resulting in damage to the endothelial cell. However, physiological levels of shear
stress, considered to be between 6-70 dyne/cm², exert a protective effect on the endothelium (119).

Fluid shear stress alters the shape of endothelial cells and contributes to the decreased apoptosis and proliferation of endothelial cells, with concomitant increases in the production of vasodilators, growth inhibitors, and anti-oxidants (119). Impaired NO bioavailability may be due to the exposure of endothelial cells to subsequent pro-inflammatory cytokines, such as TNF-α and CRP, associated with increased adiposity and damage to the cells. These inflammatory mediators initiate monocyte adhesion and platelet aggregation, leading to plaque buildup on the vessel wall (123, 125). In conjunction with the increase in pro-inflammatory markers, ROS generation induced by obesity also contributes to decreased availability of NO and results in endothelial dysfunction.

Other comorbidities of obesity further contribute to adipocyte dysfunction and a subsequent cascade of inflammatory markers and ROS generation. Insulin resistance, often found in obesity, can also attribute to reductions in NO synthesis and endothelial dysfunction. Following the same activation path as adiponectin, insulin also phosphorylates eNOS at Ser^{1179} to increase production of NO (36). Insulin-stimulated NO synthesis occurs via activation of post-receptor pathways that involve PI3K and Akt. Impaired insulin signaling, however, impairs the vascular effects of NO and ultimately leads to a decrease in NO production, resulting in endothelial dysfunction (33, 36, 59). Therefore, the comorbidities associated with obesity further contribute to vascular damage and dysfunction.
Inflammatory mediators at the endothelium of the vasculature also contribute to inflammation in obesity and represent the link between obesity and CVD. Endothelial dysfunction is the first sign of atherosclerosis and is characterized by the reduced bioavailability of NO, increased inflammation, and the loss of vasomotor tone. Normal functioning of the endothelium relies on the conversion of L-arginine to L-citrulline and NO, catalyzed by eNOS. Adiponectin normally phosphorylates eNOS at Ser\textsuperscript{1179}, increasing eNOS and NO production. Yet, during the inflammatory cascade, cytokines like TNF-\(\alpha\) antagonize adiponectin and lead to reduced NO production (36).

*Endothelin-1 (ET-1)*

The effects of obesity on endothelial function are measured via multiple mechanisms, both invasive and non-invasive. Monitoring plasma levels of endothelin-1 (ET-1) is a non-invasive technique for identifying changes in endothelial function. ET-1, identified as the most potent vasoconstrictor peptide, is derived from a precursor peptide, preproendothelin, and cleaved to a mature 21 amino acid peptide (149, 216). The effects of ET-1 depend upon the receptor and location of the receptor to which the protein binds. Binding to endothelin A (ET\(_A\)) receptors on vascular smooth muscle results in vasoconstriction, while endothelin B (ET\(_B\)) receptors on endothelial cells, when bound by ET-1, initiate a signal cascade resulting in increased NO production (121, 134). When bound to ET\(_A\) and ET\(_B\) receptors on vascular smooth muscle cells, ET-1 activates the phospholipase C-inositol triphosphate (IP\(_3\)) pathway. This pathway leads to a surge of intracellular
calcium and results in the phosphorylation of myosin kinase, subsequently leading to smooth muscle contraction (108).

With respect to the effects of ET\textsubscript{A} receptors on vasomotor control, ET-1 and NO are mutual antagonists, each limiting production of the other. While NO is an anti-atherosclerotic factor, ET-1 functions as a pro-atherosclerotic protein contributing to platelet activation, lipid oxidation, and growth and proliferation of vascular smooth muscle cells. Ultimately, this can be deleterious when identified in aging and disease states such as hypertension, hyperinsulinemia, and insulin resistance, resulting in alterations in the vascular bed of smooth muscle and contributing to the pathology of vascular disorders including endothelial dysfunction (121, 134, 202). Expressed by endothelial cells, plasma levels of ET-1 are often elevated in aging, obesity, T2DM, and hypertension. When administered an agent to block ET\textsubscript{A} receptors, subjects with obesity showed improved basal vascular tone and endothelial-dependent vasodilation implicating the role of ET-1 in endothelial dysfunction (121, 149). Individuals with CVD often demonstrate both dysfunction of the endothelium and ET-1 signaling, ultimately a result of reduced levels of NO.

Increased ET-1 and reduced NO bioavailability was identified in patients with coronary syndrome X. Following administration of \textit{L}-arginine, the subjects demonstrated increased FBF and forearm cGMP levels, both of which are indicators of improved endothelial function. ET-1 levels also improved, dropping by 29.8% and reaching near normal values (149). Subjects with hyperinsulinemia and/or insulin
resistance also demonstrate greater secretory rates of ET-1, which may contribute to hypertension in some. More specifically, hyperinsulinemia has been found to promote greater release of ET-1 from vascular endothelial cells and is often implicated in the pathogenesis of atherosclerosis and hypertension (171). As insulin levels return to normal physiological values, ET-1 often decrease as the insulin-induced NO release is restored (149, 171).

Exercise and habitual participation in physical activity have been found to decrease plasma concentrations of ET-1 (134, 202). A study by Nyberg et al. (134) examined the effects of exercise on ET-1, specifically in subjects with essential hypertension and in older adults. Subjects with essential hypertension displayed increased levels of ET-1 compared to normotensive subjects, yet after an 8-week high intensity exercise program, plasma ET-1 decreased, resulting in values similar to normotensive subjects. Aging is another factor that contributes to higher levels of ET-1. Yet, older adults who were involved in regular physical activity demonstrated significantly lower levels of ET-1 when compared to their sedentary peers (1.93 pg mL vs. 3.54 pg mL) (134, 202). By helping to decrease chronic elevated levels of ET-1 in disease states, habitual physical activity may not only oppose the effects of aging, but may also reduce ET-1 levels in diabetes and obesity.

Plasma ET-1 activates ET<sub>A</sub> receptors, resulting in suppression of acetylcholine-mediated vasodilation, particularly in aging. Yet, participation in exercise and regular physical activity contributes to a significant reduction in plasma ET-1, subsequently increasing vasodilator response to acetylcholine. The
increased NO bioavailability resulting from physical activity has been shown to inhibit transcription and release of ET-1 (134). Therefore, the increase in vascular response to acetylcholine found in hypertensive subjects may be a direct effect of increased NO bioavailability.

*Flow Mediated Dilation (FMD)*

Recently, the use of flow mediated dilation (FMD) as a non-invasive tool to assess endothelial function has been gaining popularity in children and adults. In children, FMD measurements have been used to identify changes to the vascular structure due to many risk factors including familial hypercholesterolemia, diabetes, hypertension, and obesity (123, 157, 219). Specifically, in children with obesity, studies have found impaired FMD independent of any other risk factors (205, 215). These values have been found to strongly reflect coronary endothelial function and may often predict CVD.

By measuring FBF, FMD is able to identify the development of atherosclerosis, even in children and adolescents. Children with obesity, in particular, demonstrated significantly impaired FMD compared to their lean counterparts. This significant negative relationship between obesity and FMD was further exemplified by a strong correlation between FMD and waist circumference (217). This population of children, therefore, is already experiencing the development of atherosclerosis without any other signs or symptoms.
Some studies that measure FMD have also utilized intima media thickness (IMT) as a marker of atherosclerosis. IMT assesses the diameter of the vessel lumen in either the carotid or abdominal artery. Specifically, one study suggests a link between cardiovascular risk factors (ie. hypertension, impaired glucose metabolism, and chronic inflammation) and increased IMT in obese children compared to non-obese controls. This group also found an association between IMT and blood pressure that mimics the relationship between hypertension and coronary artery disease in adults (157). The decreased FMD in children with obesity have frightening future implications. In a study by Iannuzzi et al. (90), the data indicated early manifestations of vascular structural and mechanical changes in 6-14 year old children with obesity.

Zhu et al. (219) confirmed these early structural changes to the vessel wall, finding increased elastic modulus of the arterial wall in children with obesity, suggesting a deformation of the vessel due to stress. These vascular changes were also evident in a study on cardiovascular risk factors in obese children following an exercise program. Children with elevated IMT were found to have significantly higher BMI and lower physical activity levels. Increased amounts of physical activity appear to lead to improved IMT and vascular structure (123).

However, there has been some debate regarding the use of IMT in determining development of atherosclerosis in children with obesity. Changes in IMT have not been found in many studies, even when significant changes in FMD are
present (193). IMT is also more difficult to measure and, due to the small site of measurement, significant changes are not often detected. Many studies now rely solely on FMD or venous occlusion plethysmography (VOP) in children and adults to assess endothelial function.

*Venous Occlusion Plethysmography (VOP)*

VOP was designed on the basis of plethysmography, a measurement of variations in volume of liquid or undissolved gas in biological tissues. Specifically, VOP studies peripheral circulation by recording blood content in the forearm, calf, or thigh. When measuring FBF, a blood pressure cuff placed at the upper arm is first inflated to sub-occlusion pressures (40 mmHg) to interrupt venous drainage, but allow for continued arterial blood flow to the area. To account for the larger proportion of arterio-venous shunts in the hand and differing rate of skin blood flow due to temperature regulation, a small cuff is also placed around the wrist to occlude blood flow to the hand. At rest, nearly 70% of FBF occurs through skeletal muscle, while the remaining 30% is skin blood flow. Changes in limb volume are measured using a strain gauge to assess the relationship between limb volume and changes in vessel resistance (210).

Used to assess endothelial function, VOP is able to infer about the availability of biologically active mediators such as NO and ET-1. These mediators are responsible for regulating vessel tone and modulating platelet aggregation and adhesion, smooth muscle cell growth, and leukocyte adhesion. The endothelium is also responsible for synthesizing and releasing other substances such as
plasminogen activator, an enzyme involved with the fibrinolytic pathway (13). The presence of these enzymes suggests that vascular endothelium regulates vessel tone and blood pressure, while also preventing platelet activation, thrombus formation, and the development of atheromas.

Recent literature has examined the effects of childhood obesity on CVD by assessing endothelial response to internal and external stimuli. As blood flow in the vessels increases due to physical or chemical stimuli, the endothelium is responsible for self-regulating vasomotor tone to adjust the flow and distribution of blood through the lumen, a process that can be measured as forearm vascular resistance (FVR). FVR, as measured via VOP, is a non-invasive tool to assess endothelial function. Vascular resistance \( r \) is determined in the equation \( r = p/q \), where \( p \) is mean arterial pressure (MAP) and \( q \) is FBF. Previous studies have used VOP in children to identify changes to the vascular structure due to many risk factors including diabetes and obesity (130, 160).

VOP is specifically used to evaluate structural changes in circulation, particularly the microvasculature at rest and following occlusion (187). Post occlusion reactive hyperemia, the increase in blood flow following relief of ischemia, is based on the ability of the resistance vessels to dilate. The dilation of blood vessels is accompanied by a local release of mediators and metabolites, such as adenosine, prostaglandins, and NO (187). Impaired vasodilation, therefore, is the initial indicator of dysfunction and, ultimately, atherosclerosis.
Individuals with obesity have not only demonstrated endothelial dysfunction at rest, but also in response to exercise. Compared to lean controls, adults with obesity exhibited significantly lower forearm vascular conductance (FVC) during increasing workloads of handgrip dynamometry. In fact, as the workload increased an exaggerated reduction in FVC was noted in the group with obesity. Likewise, peak vasodilatory response was also blunted at workloads corresponding to 15-50% of maximal voluntary contraction (22). These results further highlight the negative impact of obesity on endothelial function and demonstrate the attenuated response of the peripheral vasculature to brief bursts of activity. Ultimately, these results speak to the reduced response of the vasculature in transition from rest to exercise in individuals with obesity, and may contribute to reduced ability to perform activities of daily living (ADL).

In children and adolescents, FMD and the elastic properties of the blood vessels are reduced in obesity (7, 66, 76, 124, 147). Children with obesity were found to have similar degrees of dysfunction in the endothelia and smooth muscles as children with type 1 diabetes (147). Consequently, platelet activation occurs at an astonishingly early age, further contributing to increased CVD risk in early adulthood. The reduced FMD was noted in children 6-11 years old with obesity compared to lean controls (7). Even prior to puberty, these children demonstrate the initial marker of atherosclerosis with impaired smooth muscle function as a result of obesity. Furthermore, a relationship between inflammation and endothelial function exists, specifically in children with obesity. Specifically, a correlation was
found between levels of hsCRP and endothelial function, with higher levels of hsCRP associated with impaired FMD (76). E-selectin, an endothelial cell-specific adhesion molecule, found at increased levels in children with obesity, was also associated with markers of inflammation, specifically CRP. 9-16 year old children with obesity demonstrated greater levels of E-selectin, further contributing to CVD (124). The endothelial dysfunction may be a result of inflammation caused by increased adiposity.

*Physical Activity*

The dearth of physical activity among children and adolescents may contribute to the rise in endothelial dysfunction within this age group. In fact, physical inactivity has recently been considered an independent risk factor for CVD in adults and may even be so for children. Reduced participation with physical activity was evident in a group of children 9-16 years old, as were the signs of early stage vascular changes, implicating sedentary behavior as a predictor of endothelial dysfunction (124). Similar findings were identified in a study comparing sedentary and active mice. Sedentary mice were found to have a significant upregulation of vascular lipid peroxides compared to the active group of mice. More specifically, physically inactive mice demonstrated increased release of superoxide radicals and NADPH oxidase with a concomitant rise in the translocation of cytosolic regulatory proteins, responsible for the activation of NADPH oxidase, to the plasma membrane. These components all contribute to the increased ROS production that contributes to endothelial dysfunction. The voluntary running group of mice showed significant
improvements to endothelial function while the sedentary group was found to have significantly impaired endothelial-dependent vasodilation (109).

To further identify changes in endothelial function between active and sedentary mice, the experiment was repeated using an apolipoprotein E-deficient (apoE\(^{-/-}\)) mice group fed a high-cholesterol diet. The apoE\(^{-/-}\) mice showed accelerated atherosclerotic formation and significantly elevated superoxide production and NADPH oxidase activity. However, after 6 weeks of either sedentary behavior or voluntary running, the running group experienced an upregulation of vascular eNOS mRNA and protein expression, along with increased NOS activity compared to the sedentary group (109). This study specifically highlights that, while sedentary behavior further contributes to increased oxidative stress and endothelial dysfunction, regular participation in moderate physical activity is capable of improving endothelial function, even when it is in a compromised state.

Even less vigorous activity may result in changes to the structure and function of the vascular endothelia. Leisure time physical activity (LTPA) was measured via self-administered questionnaires in 13, 15, and 17-year old adolescents. At baseline, endothelial function was positively and IMT negatively associated with LTPA. Even after adjusting for BMI, IMT was significantly lower in those who spent greater time with LTPA. Furthermore, those sedentary adolescents who moderately increased their physical activity from less than 5 MET h/d to more than 5 MET h/d demonstrated a decreased progression of IMT and increase in FMD compared to those who remained sedentary. The beneficial effect of LTPA on IMT
was present regardless of body weight, demonstrating the improvements of physical activity on the vasculature for normal weight, overweight, and obese individuals (141). This increased physical activity contributes to an increase in blood flow and shear stress, resulting in greater NO production. Ultimately, this is what alters arterial structure by increasing the vessel lumen and subsequently reversing arterial stiffness often seen in obesity.

**Arterial Stiffness**

Arterial stiffness is another complication often associated with obesity and has been identified as an independent predictor of cardiovascular morbidity and mortality over the last decade (6, 201). Even in children, obesity is associated with increased arterial stiffness and subsequent endothelial dysfunction, both of which act as independent risk factors for CVD later in adulthood (193). The associated arterial hypertension results in modifications to the vascular walls including both functional and morphological changes that disrupt the main functions of the blood vessels (6). Severely obese children demonstrated decreased cross-sectional compliance and distensibility of the blood vessels, along with impaired FMD. Consequently, these children also experienced an increase in diastolic wall stress, contributing to an alteration in the function of the vasculature (193). The shock absorbing function, for example, is disrupted with increased arterial wall stiffness, contributing to an increase in blood pressure and an acceleration and premature return of the reflective pulse wave at the ascending aorta, leading to increased afterload during systole (201). Ultimately, this leads to an increase in left ventricular
afterload, impaired left ventricular diastolic function, and increase in central arterial pressure.

*Pulse Wave Velocity (PWV)*

Central arterial pressure often differs from peripheral pressure and may be altered by factors such as aging, disease, and even exercise. Therefore, peripheral systolic pressure may not be an appropriate measure of central arterial pressure and, in fact, has been found to overestimate central arterial pressure, in some cases by more than 15 mmHg (105). The forward traveling pressure wave from central arterial pressure is amplified as it travels from central to peripheral arteries due to the summation of forward traveling and retrograde reflective pulse waves. The earlier arrival of the reflective wave, along with the increased magnitude of the wave due to greater arterial stiffening, results in the augmentation of systolic blood pressure.

Pulse wave velocity (PWV) is utilized to measure arterial wall distensibility. During the systolic portion of the pulsatile cardiac cycle, arteries with greater elasticity absorb a greater amount of energy and decrease the cardiac workload. With increasing arterial stiffness, the arteries become less elastic and contribute to a greater velocity and faster return of the pulse wave (11). Arterial wall thickness and diameter of the lumen are significant factors contributing to PWV, highlighting the inverse relationship between PWV and the distensibility of the arterial walls.

Obesity is not only an independent risk factor for CVD, but has also been found to be associated with arterial stiffness. Abdominal obesity, in particular, is
known to increase peripheral arterial stiffness and, therefore, increase PWV. In fact, measures of total and abdominal adiposity, including BMI, waist circumference, and hip circumference were positively correlated with PWV in African-American children with obesity (150). While exercise is believed to contribute to improved arterial elasticity and decreased PWV, 12 weeks of low-intensity resistance exercise training in sedentary, post-menopausal women with obesity had no significant effects on PWV (68).

**Augmentation Index (Alx)**

Augmentation index (Alx) is a measure of arterial stiffness calculated as the ratio of central pulse pressure and peripheral pulse pressure, and often used to assess central arterial pressure (209). More specifically, Alx measures the amplitude and timing of the reflective pulse waves at the ascending aorta. Waves that return earlier due to stiffer arteries augment left ventricular afterload and contribute to greater central pressure. Alx is influenced by many factors including heart rate and arterial stiffness. When the heart rate increases, the duration of systole decreases, shifting the reflective wave into diastole and decreasing Alx. Therefore, Alx is often corrected for heart rate by standardizing to 75 beats per minute (Alx@75 bpm) (142).

Regular participation in aerobic exercise can attenuate and/or improve arterial stiffening. Individuals with higher aerobic fitness have more favorable measures of central PWV and Alx compared to their sedentary peers (200). However, the data concerning Alx in children with obesity has yet to be elucidated.
While carotid-femoral PWV was significantly greater (7%) in overweight and obese African-American children compared to their normal weight peers, there were no differences between the two groups in Alx (150). Lurbe et al. (117) also found no differences in Alx in children with obesity. Therefore, children with obesity may demonstrate an increase in aortic stiffness without concomitant changes to pressure wave reflection, as measured via Alx. Yet, in a study comparing children with obesity related T2DM, to obese and lean controls noted increased Alx from lean to obese to obese diabetic (199). These results highlight significant vascular dysfunction in obese diabetic children compared to obesity alone as well as to lean controls.

**Aerobic Capacity in Children with Obesity**

Aerobic capacity, also referred to as cardiorespiratory fitness, aerobic power, and maximal volume of oxygen consumption (VO\(_{2\text{max}}\)), is a measure of physical fitness. In adults, VO\(_{2\text{max}}\) has been identified as an independent predictor of all-cause mortality, such that low levels of fitness are associated with increased risk of T2DM, cancers, and death (34, 56, 98, 179). However, there is a dearth of literature in the subject relating to the relationship between low aerobic capacity and morbidity and mortality in children. Children with obesity often demonstrate reduced aerobic capacity and subsequent increased risk of CVD due to several mechanisms resulting from obesity. Inflammation, endothelial dysfunction, and lack of physical activity are the most significant contributors to this reduction in aerobic capacity in children with obesity.
Normative data in children vary based on activity level, age, and demographics. Healthy, untrained children in North America have relative VO2max values of approximately 47 ml/kg/min for males and 42 ml/kg/min for females (204). NHANES data reported similar values for 12-18 year old children following a submaximal treadmill test. Girls exhibited average VO2max values of 39.1-41.1 ml/kg/min and boys demonstrated average values of 43.5-49.4 ml/kg/min (207). While maximal heart rates were found to be higher in females, males had higher VO2max values and demonstrated faster rates of recovery. Yet, when expressed as absolute values, increases in body surface area resulted in higher absolute values. Therefore, it is common practice to express VO2max in relative values when working with this population (204). This data was further used to develop standards for the evaluation of aerobic capacity in adolescents and the threshold for detecting cardiovascular risk. Subsequently, this data can be utilized to differentiate between adolescents with and without risk for metabolic syndrome (207).

A specific negative relationship has been found between measures of adiposity and aerobic capacity in children (27, 92, 97, 137, 138, 143, 212). Children within normal limits for healthy body composition have demonstrated higher levels of physical fitness including aerobic capacity, with much of the research identifying a significant inverse correlation between measures of adiposity and VO2max (137, 138, 212). For instance, children classified as having moderate to high levels of cardiorespiratory fitness had significantly lower abdominal and visceral subcutaneous adipose tissue than those identified as having low cardiorespiratory
fitness. This specific benefit was reflected by waist circumference, implicating higher waist measurements in the reduction of aerobic fitness (112, 137). Likewise, body fat percentage was found to be significantly and negatively related to VO\textsubscript{2peak} in 14 and 15 year old boys and girls, even after scaling for body size (62).

Recent literature has further highlighted the inverse relationship between adiposity and aerobic capacity. In fact, normal weight children were 3.5 times more likely to achieve a healthy fitness score during the an assessment of aerobic capacity (PACER) compared to children with obesity (92). The overweight status was related to impaired performance in those tests requiring balance, endurance, muscular strength and endurance, speed and agility, and aerobic capacity (27, 92, 97). This negative association between physical fitness and body composition was not only significant, but the correlation was also found to increase with age (27). Measures of physical activity were also found to be significantly associated with greater performance in physical fitness in normal weight boys (97). Not only did normal-weight boys perform better on a series of physical fitness tests compared to their overweight peers, but they were also found to participate in more habitual physical activity.

While obesity plays a role in the reduction of aerobic capacity, time spent with physical activity is positively associated with levels of cardiorespiratory fitness independent of measures of fatness. Therefore, increasing time spent with physical activity can improve cardiorespiratory fitness in children, regardless of weight status. Studies have shown that time spent with MVPA and even light physical
activity is positively associated with cardiorespiratory fitness (62, 143). More specifically, active energy expenditure, calculated as total energy expenditure minus sedentary time, was significantly and positively related to VO_{2peak} in 14 and 15 year old females (62). Yet, other studies show a positive association only between mean daily physical activity and VO_{2peak}, with no relationship between moderate or vigorous physical activity and aerobic capacity (51). Conversely, cardiorespiratory fitness was found to be inversely associated with time spent with sedentary behavior. Therefore, greater time spent with total physical activity may contribute to improved levels of cardiorespiratory fitness in children (61, 62).

On the contrary, children who do not participate in physical activity and who are physically unfit demonstrate increased risk of morbidity and mortality in adulthood. Unfit children have increased odds of obesity and insulin resistance as children and adults, while children with higher aerobic capacities are more likely to be physically fit adults. In fact, each one-unit increase in childhood aerobic capacity resulted in a .21 unit increase in adult fitness. Consequently, there are significant disadvantages for individuals with decreasing levels of aerobic capacity from childhood to adulthood, demonstrating that decreasing fitness over time is a stronger predictor of adult CVD risk than the degree of childhood fitness (60). Even after removing children with obesity from the analyses, Dwyer et al. (60) found that lower childhood fitness and decreasing fitness from childhood to adulthood were still associated with adult obesity. It is, therefore, important to establish healthy
physical activity habits early in life and work to maintain these habits into adulthood.

Aerobic fitness may be an independent indicator of overall health in children. Metabolic syndrome (MetS) is a clustering of risk factors including central adiposity, elevated blood pressure, dyslipidemia, and impaired glucose tolerance. Increased cardiorespiratory fitness has been found to be inversely associated with MetS score. In addition, within BMI categories of normal weight, at risk for overweight, and overweight, higher fitness levels can attenuate MetS score, such that those in the overweight category with higher aerobic fitness have lower MetS scores than those of lower aerobic fitness (58). Higher levels of aerobic fitness are, therefore capable of improving overall health, regardless of fatness.

Recently, an association has also been identified between aerobic capacity and inflammation. In fact, improved cardiorespiratory fitness has been found to protect against inflammation. Children with decreased levels of cardiorespiratory fitness not only demonstrated increased BMI, waist circumference, and metabolic syndrome risk scores, but also had significantly higher concentrations of CRP (38, 143, 153). In a study by Parrett et al. (143) cardiorespiratory fitness was inversely associated with CRP such that those children with lower measures of fitness and higher adiposity had greater levels of CRP compared to those with lower measures of adiposity. Even after controlling for body fat percentage, there was still a significant inverse relationship between participation in physical activity and CRP. Similar results were identified specifically in children with obesity, highlighting
reduced values of aerobic capacity and increased CRP concentrations compared to overweight and normal-weight children (131). Subsequently, poor cardiorespiratory fitness combined with obesity are independent predictors of increased CRP concentration and secretion.

Consequently, aerobic capacity is related to endothelial function and subsequent arterial compliance. Large conduit arteries are composed of collagen and elastin, with changes in collagen contributing to arterial stiffening. The increase of arterial pressure and heart rate during exercise contributes to the stretching of these large arteries, and ultimately working against the cross-linking of the connective tissue that naturally occurs with aging (201). There is also a nonlinear relationship between pressure and volume within the large arteries, contributing to an increased sensitivity to pressure. Subsequently, systolic blood pressure is a significant predictor of large artery compliance (155). During exercise, systolic blood pressure is elevated, contributing to greater large artery compliance and decreased arterial stiffening.

Smaller arteries, like the brachial and radial arteries, are also composed of collagen and elastin, but have a greater amount of smooth muscle and, therefore, compliance is more dependent on the release of NO. During exercise, shear stress on the vessels allows for an increased release of flow-mediated NO, resulting in greater arterial compliance. A study examining the effects of physical fitness and physical activity on arterial compliance in 9-11 year old children identified an association between aerobic fitness and arterial compliance of both the large and small arteries.
The relationship was much stronger in large arteries, however, most likely due to the difference in the structure and composition between the large and small vessels. When divided into fitness quartiles, large and small artery compliance was significantly greater in the highest fitness group compared to the lowest fitness quartile. Even though physical fitness was positively related to arterial compliance, no significant relationship was identified between physical activity, measured via self-report, and arterial compliance (155). However, another study of 5-10 year old children found that habitual physical activity, assessed via doubly labeled water, was the strongest predictor of FMD. The differences may be due to the method of reporting physical activity levels, with self-report being much less accurate than the doubly labeled water method (1). Therefore, increasing aerobic fitness and habitual physical activity are essential to aid in the prevention and treatment of comorbidities due to obesity in childhood.

**Aerobic Capacity Testing**

Aerobic capacity can be measured via maximal exercise tests or extrapolated using data from submaximal exercise testing. Often times, submaximal exercise tests are used with children to estimate aerobic capacity. One such test, the 20 meter shuttle run test (20 MST), has been validated with adults (113) and children (203). During this test, the subject runs back and forth between two lines set-up 20 meters apart. The pace is set by a prerecorded audio signal, starting at 8 km/h and increasing by 0.5 km/h each minute. The test is terminated when the subject is unable to reach the line before the audio signal, two consecutive times. VO$\text{2}_{\text{max}}$
values can then be ascertained using the following equation: \( \text{VO}_{2\text{max}} = -3.24 \times \text{maximum speed} - 3.25 \times \text{age} + 0.154 \times \text{maximum speed} \times \text{age} + 31.03 \times (107) \). Many researchers prefer this measure for predicting aerobic capacity in children. The test is generally performed indoors, is easy to set-up, and is most similar to maximal exercise running test due to the increasing workloads (203). Studies using this test have shown increases in aerobic capacity throughout the 9-month school year in 4\textsuperscript{th}-8\textsuperscript{th} graders. The significant increases in aerobic capacity were diminished over the summer, and then returned to normal projected rates after the first half of the school year (30). While this shows changes in aerobic capacity over time, this progression is not typical in the absence of an exercise intervention. These results may demonstrate changes due to familiarity with the submaximal test protocol rather than true increases in aerobic fitness.

Physical working capacity (PWC) tests are also common submaximal tests used on children and adults. This test assumes a linear relationship between heart rate and workload, with the PWC representing the workload at which a given heart rate is achieved. In the PWC\textsubscript{170} there are multiple stages, with intensity increasing based on heart rate response until the predetermined heart rate of 170 beats per minute (bpm) is reached. When compared to maximal testing on both a treadmill and cycle ergometer, the PWC\textsubscript{170} was only weakly to moderately correlated (23). Therefore, this test may not be appropriate for accurate extrapolation of maximal aerobic capacity values from submaximal data.
Cycle ergometry is often used with subjects who are elderly, diseased, and/or obese. The Åstrand 6-minute cycle test is a submaximal exercise test used to estimate aerobic capacity. The subjects complete 6-8 minutes on a cycle ergometer at a moderate resistance (125-150 W), with heart rate recorded at the completion of each minute. At minute 3, resistance is adjusted based on the subject’s heart rate, increasing resistance by 25 W if below 140 bpm and decreasing resistance if heart rate is above 149 bpm. If steady state heart rate is achieved by minute 6 (defined as less than 10 bpm difference between heart rates at minute 5 and minute 6), the test is terminated. Otherwise, subjects continue to cycle for another 2 minutes. The average heart rate for minutes 5 and 6 (or 7 and 8, if necessary) are used as the submaximal heart rate in the equation to estimate VO$_{2\text{max}}$. Åstrand and Ryhming (12) determined that VO$_{2\text{max}}$ can be calculated from heart rate and workload reached during this submaximal test in young adults. When reproduced in 18-33 year olds, Cink et al. (39) reevaluated the prediction of VO$_{2\text{max}}$ from the Åstrand and Ryhming nomogram using submaximal cycling. They found that maximal and submaximal cycle tests are significantly and positively correlated ($r = 0.83$) such that VO$_{2\text{max}}$ measurements will be within 5.6 ml/kg/min of VO$_{2\text{max}}$ predicted from the Åstrand and Ryhming nomogram (39).

**High Intensity Interval Exercise (HIIE)**

Recent literature has highlighted the beneficial effects of and urgent need for regular physical activity to treat obesity and improve cardiovascular function and the associated inflammation (185). Regular participation in physical activity has
been shown to increase anti-inflammatory mediators and decrease pro-inflammatory markers. Specifically, studies have demonstrated an inverse relationship between regular physical activity and IL-6 in children (122, 151). IL-6 however, has recently been identified as both a pro- and anti-inflammatory mediator. It is believed that working skeletal muscle can also act as a source of IL-6 release, thereby increasing circulating amounts during exercise. This form of IL-6 is thought to inhibit TNF-α release and decrease inflammation. In fact, one study noticed a significant increase in the IL-6 to TNF-α ratio in children following a bout of intense exercise (122).

There are several mechanisms responsible for the anthropometric, inflammatory, and vascular changes that often occur with more vigorous exercise. Most obviously is the increase in caloric expenditure that accompanies participation in vigorous exercise. Therefore, when comparing vigorous to moderate continuous exercise, researchers must create an isocaloric environment. Many studies comparing isocaloric exercise interventions have highlighted that varying intensities of exercise does not yield significant weight differences between groups (40, 80, 82, 190). Instead, other physiological mechanisms may be responsible for beneficial changes in overall health, including decreased adipogenesis, reduced inflammation, and improved vasomotor tone, following interventions of greater exercise intensities.

Greater intensities of exercise may affect the rate of adipogenesis compared to light and moderate physical activity. Young mice were administered low
magnitude mechanical signals (LMMS) via vertical whole body vibration. When compared to the control group, LMMS mice demonstrated a 27.4% decrease in body fat percentage, without significant differences in body mass. Furthermore, these changes were not strongly correlated with food intake, verifying that the decreased adiposity in the LMMS group was not due to food intake differences between the two groups (162). This process is believed to be a result of inhibited adipogenesis due to changes in stem cell differentiation with exercise. The mechanical stimulation of tissues during exercise affects the differentiation of marrow derived mesenchymal stem cells, the common progenitor of adipocytes, osteoblasts, and myocytes. LMMS contributes to anabolism of the musculoskeletal system, resulting in stem cells preferentially differentiating into osteoblasts and myocytes rather than adipocytes, and subsequently, curbing adipogenesis (162, 189). Higher intensities of exercise may therefore result in greater stimulation to the tissues, and contribute to reductions in adipogenesis (189).

A significant number of studies have shown that regular physical activity also improves endothelial function (100, 123, 192, 205) and that continuous training is necessary to maintain vascular and anthropometric benefits (205). In as little as 12 weeks, children participating in regular aerobic physical activity can decrease total body weight and fat mass, and increase fat free mass. In fact, 20-30 minutes of aerobic exercise performed at anaerobic threshold elicited significant improvements to body composition, aerobic capacity, and time spent with physical activity in boys with obesity (53). Improvements to aerobic capacity may be
indicative of exercise-induced changes to the vasculature and vasomotor tone. Exercise causes an increase in blood flow across the endothelium, which leads to shear stress mediated upregulation of NO-synthase expression. The increased NO bioavailability thus allows for more efficient vasodilation and improved blood flow (100). In a study comparing aerobic interval training to a multidisciplinary program with moderate physical activity, HDL, blood glucose, and insulin were all improved, all of which directly influence NO bioavailability (192). It, therefore, seems crucial to use physical fitness as a tool to improve endothelial function in children with obesity.

Multidisciplinary interventions have become popular methods for improving the health of children with obesity. Woo et al. (215) compared the effects of a dietary intervention to a dietary plus exercise treatment group for 6 weeks. While the dietary intervention helped improve FMD in overweight and obese subjects with initially impaired FMD, the effects were even greater in the group receiving diet and exercise treatment. In addition, those subjects in the diet plus exercise group who continued to exercise for an additional year after the 6 week program, continued to improve FMD by 8.6% from baseline as compared to those who discontinued the exercise program and concentrated solely on dietary modifications (215).

Watts et al. (205) also demonstrated an improvement of endothelial function in children with obesity without dietary modification and independent of changes in BMI. This study showed improved FMD (5.3% to 8.8%) in children with obesity following an 8-week circuit-training program, compared to the non-exercise group.
These subjects also demonstrated normalized vessel function following the exercise program (205). While the research has demonstrated the effectiveness of physical activity in reducing inflammation and cardiovascular risk in children with obesity, many debate the appropriate intensity required to elicit these changes.

With the overwhelming dangers of obesity many children now face, determining effective frequency, intensity, and duration of exercise has become vital. While many studies include exercise in their interventions, there is a lack of clarity concerning the proper intensity to produce the greatest benefits. It has been found, that the association between physical activity and inflammation, for example, depends on the mode and intensity of the activity (122, 151). However, there are only a few studies that have utilized vigorous levels of physical activity to determine effects on obesity, inflammation, or endothelial function.

Children rarely accumulate the recommended 60 minutes of daily moderate-to-vigorous physical activity (MVPA). A study examining the 2003-2004 National Health and Nutrition Examination Surveys (NHANES) found that less than 7% of children 8-17 years old participated in 60 minutes or more of MVPA. In fact, 69% participated in less than 30 minutes of physical activity per day or less and 62.6% accumulated 5 minutes or less of vigorous activity (120). The effects of more vigorous physical activity on children have yet to be elucidated, with many recent studies yielding conflicting results. Mark et al. (120) identified a dose-response inverse relationship between varying intensities of physical activity and adiposity. Total and trunk fat were found to be higher in children that spend less time
participating in physical activity. However, no significant benefit to total and/or trunk fat was found in those who participated in more vigorous physical activity (120).

On the contrary, a study of 9-10 year old children demonstrated that vigorous physical activity was significantly associated with lower body fat, while no correlation was found between moderate physical activity and body fat. This relationship was also found to be duration-dependent, with greater effects on body fat for those participating in more than 40 minutes of daily vigorous activity compared to those who spent 10-18 minutes daily with vigorous activity (163). Similar results were found when comparing 12 weeks of lower-intensity continuous exercise and high intensity sprint training in 8-12 year old sedentary children with obesity. While BMI was significantly reduced in both groups, the higher intensity exercise group also experienced a significant weight loss, while the continuous group did not. These results are particularly surprising seeing as the continuous exercise group expended a greater amount of energy than the high intensity group (268.1 Kcal vs. 84.0 Kcal) (44).

High intensity interval exercise (HIIE) is generally defined as activity at 80-95% of VO$_{2\text{max}}$. HIIE has been studied in adults and children to determine the effects on inflammation compared to moderate exercise. While both moderate and vigorous physical activity can produce significant cardiovascular benefits, the improvements appear to be greater with higher intensity activities. A study comparing moderate and vigorous exercise groups plus education to a solely
education control group, found the only significant change in cardiovascular fitness occurred in the vigorous exercise group. They also found that at baseline, cardiovascular fitness was significantly correlated with time spent participating in vigorous free-time activity (80).

**HIIE and Arterial Stiffness**

The effects of HIIE on insulin sensitivity, PWV, cardiorespiratory fitness, and endothelial function are often found to be greater than those demonstrated in continuous moderate exercise (40, 44, 82, 190, 192). For young women with familial hypertension, 16-weeks of HIIE and moderate exercise resulted in similar increases in insulin sensitivity and VO$_{2\text{max}},$ however, those in the HIIE group demonstrated significantly greater reductions in PWV. In fact PWV and biomarkers for endothelial dysfunction, including ET-1, were reduced to levels similar to those without familial hypertension (40). The higher intensity exercises may contribute to reductions in arterial stiffness, particularly in populations at risk for stiffer vessels, via mechanisms responsible for improving endothelial function in the vessels. By increasing vasomotor tone in the arterial smooth muscle cells, the arteries become less rigid and demonstrate reduced PWV.

**HIIE and Inflammation**

While low to moderate intensity exercise may elicit overall health benefits, some studies report no significant changes in levels of adiponectin following lower intensity exercise programs (103). However, one study demonstrated increased
adiponectin mRNA in subcutaneous adipose tissue in sedentary adults with obesity following 12 weeks of HIIE. The associated increase in plasma adiponectin was also found to be elevated following 1 week of detraining. Along with this rise in the anti-inflammatory marker, the acute phase inflammatory protein, hsCRP, was significantly reduced following HIIE compared to the control group, which did not participate in any physical activity (127). Often times the increase in anti-inflammatory proteins contributes to the reduction in inflammation through various mechanisms. Adiponectin, specifically, increases fat oxidation, resulting in decreased adipocyte size and potentially reducing the production and secretion of pro-inflammatory markers.

**HIIE and Endothelial Dysfunction**

Intense physical activity not only improves cardiovascular fitness, but also contributes to improved endothelial function. Hopkins et al. (85) found a significant correlation between participation with intense physical activity and vascular function, specifically in subjects with lower FMD at baseline. Interestingly, the study found no significant correlation between FMD tertiles (low, medium, and high) and measures of fitness or fatness. Participation in physical activity, therefore, is associated with endothelial function more so than body fat percentage.

HIIE has been found to elicit changes in inflammation and endothelial function, often times more significantly than moderate or lower intensity activities. For example, Tyldum et al. (198) examined the effects of moderate and high intensity exercise on FMD following a high fat meal. They found that not only did
HIIE attenuate the normal decrease in FMD following a high fat meal, it actually improved FMD from the initial baseline level (198). The protective effects of exercise appear to be intensity related, possible due to the effects of shear stress. While higher intensity exercise is believed to contribute to greater free radical concentration, there seems to be a concurrent increased antioxidant capacity, allowing for the continued production of NO (198). The effects of exercise on increased NO bioavailability are a result of increased adiponectin seen with higher intensity exercises (192).

Increased NO production following HIIE training also results in improved endothelial function. Three months of HIIE in children with obesity led to significant improvements in NO bioavailability compared to the multidisciplinary group receiving moderate exercise. The HIIE group also demonstrated reduced fasting blood glucose and increased levels of adiponectin (192). These two components help contribute to increasing the amount of available NO, which subsequently improves FMD and endothelial function. Following the 3-month exercise intervention, the HIIE group continued to exercise at least twice per week with 1 session under supervision of the study staff for an additional 9 months. At the 12-month follow-up, FMD in the HIIE remained above baseline values while the moderate exercise group values returned to baseline (192). Therefore, this study demonstrated that the improvements to FMD are more robust following HIIE than those seen with moderate exercise.
On the contrary, some short-term studies have identified improved endothelial function following both moderate and HIIE, without a significant difference between the two groups (154, 191). One 6-week study examining moderate exercise and HIIE, found improved FMD following the training program, however these improvements were not accompanied by significant changes in arterial structure, IMT, or carotid artery distensibility. This lack of difference between groups may be a result of the population used. This study was performed on healthy, physically active subjects, a population generally exhibiting low baseline IMT and normal-to-high endothelial function. While the change may not have been significant between the two intensities, HIIE yielded similar results in less time, making HIIE a potentially more attractive exercise protocol for subjects and practitioners alike (154).

HIIE is specifically superior to moderate exercise in improving endothelial function and NO bioavailability, even following acute exercise. For untrained subjects with metabolic syndrome, an acute bout of HIIE resulted in decreased weight, waist circumference, and fasting blood glucose and increased FMD and NO bioavailability. The improvements in endothelial function following an acute bout of exercise were significantly greater in the HIIE group than in the continuous moderate exercise group (191). The moderate group also demonstrated a significant increase in FMD, however, the change lasted less than 24 hours compared to the HIIE group, where increased FMD lasted more than 72 hours.
Improvements in endothelial function are largely due to increases in NO bioavailability, allowing for greater vasomotor tone. HIIE increases NO bioavailability via multiple mechanisms, including the reduction of ROS and the effects of increased shear stress. HIIE could potentially reduce fasting blood glucose, thereby reducing the activation of superoxide producing enzymes, which are responsible for uncoupling eNOS and ultimately inhibiting NO expression. Therefore, a reduction in fasting blood glucose helps increase NO bioavailability and subsequently improves endothelial function.

**HIIE and Aerobic Capacity**

Combined with the improvements in endothelial dysfunction, blood pressure, and LDL, HIIE demonstrates greater reductions in cardiovascular risk factors when compared to moderate exercise. In a case study regarding an individual following an MI, scar tissue was significantly reduced and aerobic capacity significantly improved following 14 weeks of HIIE. However, this individual had more than 30 years experience with intensive exercise, which may have contributed to the positive effects of HIIE (77). However, HIIE has also been found to partially or even fully reverse many cardiovascular risk factors found in children with obesity, with the effects often lasting longer than those seen in moderate exercise (28, 40, 79, 82, 127, 190-192, 214).

The short, high intensity periods alternating with lower intensity recovery periods exhibited in HIIE also contribute to greater challenges to the pumping ability of the heart, resulting in improved stroke volume and subsequent increased
Even in as little as 12 weeks, HIIE was able to improve aerobic capacity in sedentary children with obesity. However, like many other studies, these improvements were not significantly different than those seen in the lower intensity exercise group (15, 44). Often times, these improvements to aerobic capacity are seen independent of weight loss, suggesting a stronger link between aerobic capacity and all cause mortality than obesity and cardiovascular mortality (40, 192). Increased aerobic capacity may be more important than reduced weight in regards to decreasing cardiovascular risk factors. Therefore, it seems that rather than focus on weight loss, interventions should aim to implement and/or increase high intensity physical activity to improve vascular function (85).

One mechanism that may contribute to improved vascular function, aerobic capacity, and, subsequently, cardiovascular health is the increased expression and content of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α). PGC-1α is a transcriptional coactivator that is capable of inducing conversion of fast twitch (type II) to slow twitch (type I) muscle fibers, resulting in increased mitochondrial enzyme expression and time to fatigue. PGC-1α is regulated and suppressed by p160myb, but, once phosphorylated, the suppression is released, allowing for PGC-1α to recruit histone acetyltransferase enzymes to the promoter regions of specific genes, altering chromosome structure to allow for transcription. More specifically, PGC-1α is often phosphorylated by p38 mitogen-activated protein kinase (MAPK), which is activated by cellular stresses, particularly, aerobic-type
exercise. Studies have shown similar increases in PGC-1α in both HIIE and moderate exercise even at reduced volumes of HIIE (9, 29, 114).

The changes elicited by HIIE, while similar to those seen with moderate intensity exercise, occur over a shorter duration and, therefore, may be more attractive when promoting exercise programs. Compared to moderate endurance training protocols, HIIE can elicit similar adaptations in exercise performance, muscle buffering capacity, and oxidative capacity of skeletal muscle in significantly less time (29, 74). After 6 sessions of moderate or HIIE, active, adult males demonstrated similar improvements in short distance and long distance time trials, with a concomitant increases in mitochondrial enzyme activity and content and muscle glycogen content. While both groups completed the same frequency and duration of exercise (6 sessions, 3 days per week for 2 weeks), the exercise volume was approximately 90% less in the HIIE group compared to the moderate exercise group (2.5 hours for HIIE group vs. 10.5 hours for moderate group) (74). HIIE may also be attractive to children due to the nature of protocol, containing short bursts of activity interspersed with low recovery or rest periods. These high intensity protocols are very similar to the habitual non-structured physical activity patterns seen in children, characterized by multiple, short, intermittent bursts of vigorous activity throughout the day. Therefore, HIIE may be a more logical exercise protocol for weight management and overall health in children and adolescents.
**Physical Activity Behavior in Children**

Participation with physical activity should be an essential component of every child’s daily routine. Yet, research has shown that nearly 70% of children do not achieve the recommended 60 daily minutes of MVPA. After 3 days of wearing heart rate monitors, Al-Nakeeb et al. (8) found that 52% of 9-11 year old children did not even achieve a 15-minute bout of physical activity. In fact, 46% did not accumulate a 10-minute bout and 9% did not even acquire a 5-minute bout of moderate physical activity. Children and adolescents have been found to spend a greater percentage of their time with sedentary activities. In fact, during waking hours, boys and girls spend an average of 9.3 and 9.6 hours per day with sedentary activities, respectively (62). Time spent with physical activity has been found to be negatively associated with indicators of insulin resistance, hyperglycemia, and hyperlipidemia, independent of adiposity (61). Therefore, it is apparent that increased time spent with physical activity is beneficial to overall health regardless of fatness. However, it has become more apparent that children and adolescents do not spend the required amount of time participating in physical activity to reap these benefits.

*Gender Differences*

Physical activity behaviors differ based on gender, age, fitness, and adiposity. Many studies have identified a greater amount of time spent with physical activity in young males compared to females (17, 46, 61, 104, 140, 167, 173, 197). In general, pre-pubescent children have been found to participate in less than 20 minutes per
day of vigorous physical activity (104, 196). Specifically, boys were not only found to spend more time with MVPA than girls (both total time and 5-minute bouts), but they also spent a greater proportion of their time with MVPA (17, 61, 89, 104, 208). On the contrary, girls accumulated less total daily physical activity and less minutes of MVPA (140) and spent a significantly greater proportion of their time with sedentary (17) and light activities (104).

Predictors of time spent with MVPA also differed by gender. Time spent with MVPA for males was associated with family support, peer support, and self-efficacy, while the association for girls was with family support and self-efficacy. Physical activity enjoyment, assessed by the Physical Activity Enjoyment Scale (PACES) was also found to be an independent predictor of physical activity in boys, but not girls (208). Family support appears to be the strongest and most consistent predictor of MVPA in both males and females.

*Physical Activity Enjoyment*

PACES is a questionnaire consisting of 18 bipolar statements with a 7 point continuum beginning with the stem “When I am physically active...” It is specifically designed to assess the level of enjoyment for physical activity, with higher scores representing greater enjoyment during physical activity. PACES demonstrated high internal consistency ($\alpha = 0.93$) and high test-retest reliability for jogging and cycling (101). When used to assess physical activity enjoyment in children with a mean age of 14.4 years, average reported PACES scores were 100.5 ± 16.4 and 99.7 ± 15.1 in males and females, respectively (47).
Physical activity enjoyment may play a significant role in time spent with MVPA and, ultimately, measures of fitness, anthropometry, and overall health. Few studies have identified a relationship between physical activity enjoyment and time spent with MVPA in children and adolescents (54, 55, 167, 170). One particular study analyzed children randomized to 1 of 4 experimental intervention groups. Behavior modification, including increasing MVPA and decreasing time spent watching television, fundamental motor skills, and a combined intervention resulted in high levels of physical activity enjoyment among 10 year old children (170).

DiLorenzo et al. (54) also identified a relationship between enjoyment and participation with physical activity. The study found that physical activity enjoyment was the most important predictor of physical activity levels, suggesting that participating in activities that provide enjoyment, may aid in increasing time spent with MVPA (54). Similar results were identified in a study analyzing the influences of physical education and physical activity enjoyment on self-efficacy in high school girls. A modified PACES scale identified a positive relationship between enjoyment of physical activity and increased time spent with MVPA (55). Therefore, enjoyment of physical activity appears to be an important determinant of participation in MVPA.

*Tracking into Adulthood*

Even weekend and weekday physical activity habits differ based on age and gender. While one study revealed that 9-11 year old children achieve more MVPA on weekdays compared to weekends (8), another study showed that younger boys and
girls (grades 1-3) demonstrated significantly greater time spent with MVPA on weekends versus weekdays (197). The inconsistencies in the research may occur due to an identified change in behavior with age, such that as children age, they spend more time with MVPA during the week than on weekends. This decrease in time spent with MVPA on the weekends occurred at a younger age in females (197). Decreased participation in physical activity with age not only continues into adolescence, but has also been found to extend into adulthood. In fact, a longitudinal study of 12-18 year old children, reassessed as 34-46 year old adults, found that physical activity in adolescence was a significant predictor of physical activity behavior in adulthood. Those who participated in greater amounts of physical activity as children had a significantly reduced risk of being inactive adults (88).

Studies have identified an inverse relationship between age and time spent with MVPA, such that, as children get older, they spend less time participating in physical activity (25, 61, 197). This factor alone plays an important role in the tracking of obesity into adulthood. The decreased time spent with physical activity is a behavior that carries over into adulthood and contributes to further obesity with aging. This is particularly dangerous in children and adolescents with obesity, due to the tracking of risk factors developed in childhood, as a result of obesity, into adulthood. The strong tracking of weight status from childhood into adulthood contributes to the development of CVD earlier in adulthood, specifically due to the cumulative deleterious effects of obesity (72, 111). The duration of obesity, for example has been found to be an important predictor of T2DM. In fact, the risk of
developing T2DM increased when the duration of extreme obesity was greater than 1 year (165).

**Measurement**

Physical activity behaviors can be captured via self-report questionnaires and/or measurement devices, including pedometers, heart rate monitors, and accelerometers. While both subjective and objective methods have been validated in children with obesity, many studies show significant differences in physical activity behavior between different measures, specifically time spent with and intensity of the activity (25, 63, 140). In a comparison of self-reported physical activity and accelerometry in children with obesity, self-report data showed 41.2% greater time spent with physical activity versus accelerometry data recorded during the same time (63). Epstein et al. (63) further identified that determinants of physical activity were dependent upon the method of measurement, with objective measures relating mostly to socioeconomic status and parental physical activity behaviors, and subjective measures relating more to levels of fitness.

Accurate measurement of physical activity is critical for the assessment of physical activity behavior in both children and adults. Direct observation, doubly labeled water, subjective measure (ie. diaries, recalls, and questionnaires), and activity monitoring are the most common methods for tracking frequency, intensity, and time spent with physical activity. However, some of these methods are not plausible when working with a larger number of individuals, especially over a longer period of time. Subjective measures are often favored to capture physical
activity behaviors due to the low cost and easy administration (104). Yet, may of these measures are often difficult for subjects to complete and can result in significant overestimation. Validation and reliability studies have been performed on a multitude of questionnaires and, while many have been validated, only some correlate to other objective measures of physical activity (46).

*Self-Administered Physical Activity Checklist (SAPAC)*

The Self-Administered Physical Activity Checklist (SAPAC) was originally created for the Children and Adolescent Trial for Cardiovascular Health (CATCH) study, a multi-center study investigating school-based cardiovascular health promotion interventions for 3rd-5th graders. Specifically, the SAPAC was created to capture the amount of time spent with MVPA in 5th graders. The SAPAC consists of a list of 21 physical activities, with space to add additional activities. The children are asked to indicate if they did a specific activity (5 minutes or more at one time) during the previous school day on the sheet by writing in the amount of time spent with the activity in the appropriate time slot (before, during, or after school). Next to the time spent with activity, they are also asked to indicate if the activity made them breathe hard or feel tired none, some, or most of the time. There is also a section for them to record time spent with TV and movies, and computer, video games, and hand-held devices, both before and after school. The children are oriented to the SAPAC with an introduction period during which physical activity is defined (bodily movement in which you move your arms and legs) and time estimation is taught (169).
Accelerometry

Accelerometry data has been used in research to ascertain physical activity behaviors in adults and children. The advantages to using accelerometry in lieu of, or in conjunction with, subjective measures include ease of use and accuracy in deciphering between sedentary, light, moderate, and vigorous activities. Likewise, when compared to subjective measures, accelerometry is often more accurate at assessing sedentary behaviors, which are often under-reported in questionnaires and diaries (25, 168). Some studies have identified correlations between accelerometry and subjective measures of physical activity. When measuring the physical activity patterns of pre-pubescent children in India, accelerometry data correlated strongly with physical activity diaries for average time ($r = 0.73$), sedentary time ($r = 0.48$), and light time ($r = 0.70$). While there was a small, but significant correlation between the two measures for time spent with moderate physical activity ($r = 0.29$), there was no significant correlation for vigorous activity (104).

The reliability of accelerometry, specifically in children, differs based on age, gender, duration of measurement, and weekday versus weekend (17, 197). Children in grades 1-12 wore an accelerometer for 7 days to assess number of days required to characterize habitual physical activity, weekday versus weekend behavior, and differences in behavior based on time of day. The reliability coefficient was significantly higher in younger children compared to adolescents. The study also found greater reliability for MVPA scores after 7 days of monitoring versus a single
day of measurement. In fact, for younger children, 4-5 days and 9-11 days of monitoring were required for a reliability coefficient of 0.80 and 0.90, respectively. Adolescents in grades 7-12 required 8-9 days and 18-20 days for a reliability coefficient of 0.80 and 0.90, respectively. 7 days of monitoring was found to be an acceptable estimation of MVPA in children, with a reliability coefficient range of 0.76-0.86 (197).

Obesity and Physical Activity in Children

Significant differences have been identified in the physical activity patterns of children with obesity when compared to their non-obese peers. Accelerometry data from children show that those with obesity are significantly less active compared to non-obese children (140, 196), while other research from heart rate monitors showed no significant relationship between adiposity and time spent with MVPA (8). Boys with obesity were 15% less active with MVPA on school days, and 29% less active on weekend days compared to non-obese boys. Girls with obesity demonstrated even greater differences, participating in 20% less MVPA on schooldays and 36% less on weekend days versus non-obese girls. This difference in activity levels between children with obesity and their non-obese peers translates into an average energy expenditure difference of 296 kcal/day, or 2,072 kcal/week (140). Trost et al. (196) found that children with obesity spent significantly less time with daily moderate (62.6 vs 78.2 minutes) and vigorous physical activity (7.1 vs. 13.5 minutes) compared to non-obese children. Non-obese children also spent more time with continuous bouts of physical activity (5, 10, and 20 minute bouts)
compared to children with obesity (196). The reduced energy expenditure for children with obesity may be implicated with obesity and other associated comorbidities.

Children with obesity often avoid physical activity for many reasons, including perceived and actual physical abilities, enjoyment, and other perceived barriers. Yet, time spent with physical activity may be associated with improved measures of fitness and fatness, including BMI, body weight, and body fat percentage. Creating physical activity programs that enhance enjoyment and self-perception may contribute to increased time spent with MVPA, subsequently resulting in improved overall health (129). A longitudinal study examining the association between physical activity and BMI in 5-10 year old children considered to be overweight or obese, found that an increase in physical activity of 100 counts per minute (cpm) corresponded to a 0.11 decrease in BMI z-score from baseline to the end of study at 3 years. This trend was also evident from baseline to the 6-month time point and 6 months to the 3-year end point. More specifically, the improvements in BMI were most strongly associated with changes in MVPA and not light or sedentary activity (194).

A study by Chaput et al. (35) further reviewed the association between physical activity, sedentary behavior, and cardiometabolic risk factors. Only 25% of the 8-10 year old children were found to accumulate the recommended amount of daily physical activity (at least 60 minutes of MVPA). When analyzing levels of activity among children participating in an after-school physical activity program,
children spent the majority of their time engaging in light physical activity (47.4%) and the least amount of time with vigorous physical activity (6.7%). More specifically, those who were overweight and/or obese spent less time with vigorous activity and more time with sedentary activity compared to their normal weight peers. However, even during an exercise program designed to promote MVPA, children still did not spend a significant amount of time with MVPA (173).

Research shows that increased levels of MVPA are significantly associated with decreased waist circumference, triglycerides, and diastolic blood pressure along with an increase in HDL (132). Total physical activity, measured as counts per minute (cpm), was also found to be inversely associated with waist circumference and diastolic blood pressure, and positively associated with HDL. Sedentary time, however, was unrelated to cardiometabolic health, suggesting that participation in MVPA is more beneficial to optimal health in children than sedentary time (35). It is therefore essential to focus on increasing MVPA in children to improve cardiovascular health.

*Physical Activity Interventions*

With the increased prevalence of childhood obesity rates worldwide, it has become increasingly more important to implement and promote regular physical activity. Yet, over the last 10 years, countless physical activity and multidisciplinary interventions have tried and failed to improve physical activity behavior among children and their families. Practitioners continue to search for the most effective methods, venues, and delivery techniques to incorporate habitual physical activity
into the lives of all children. However, while some interventions demonstrate significant changes in physical activity behavior (5, 18, 21, 42, 132), including time spent with MVPA, others show little or no change (69, 172).

Weight management interventions should be designed to not only promote healthy weight, but also to instill healthy behaviors. Often times, these programs teach children and their families about healthy nutrition, habitual physical activity, and many behavioral issues including overcoming barriers and boundaries. Even short-term multi-disciplinary interventions have been shown to be effective in reducing weight and promoting regular physical activity. After 3 months of an exercise-diet-behavior intervention, children experienced a decrease in weight, body fat percentage, and BMI along with an increase in habitual physical activity compared to a control group. Even 1 year following the intervention, the children in the exercise group maintained these favorable effects, including significant increases in habitual physical activity (132). Exercise interventions, therefore, may be an effective method for increasing habitual physical activity in children with obesity.

Exercise interventions should be designed to increase habitual physical activity. In a group of school children in Latin America, a school-based exercise intervention contributed to increased participation with physical activity. While the control group received one, 39-minute physical education (PE) class per week, the ‘basic’ intervention offered a longer duration PE class (50 minutes) and added a daily, 15-minute recess. Another intervention group, the ‘plus’ intervention, offered
2, 50 minute PE classes per week, a daily 15-minute recess, plus a 20 minute exercise session before class. The children in the two intervention groups increased the number of steps per day during school while the control group demonstrated decreased number of steps from baseline to follow-up (5).

Yet, another school-based study designed to improve physical activity behaviors, failed to show increases in time spent with physical activity outside of school. The Sports, Play, and Active Recreation for Kids (SPARK) study was designed to increase time spent with physical activity in PE classes for elementary school children. However, accelerometry data indicated that, even though the children spent more time with physical activity during PE, there was no significant increase in time spent with physical activity outside of school. The increased time with physical activity in PE class, however, did improve fitness measures in girls, potentially due to the lower baseline values, compared to boys (166).

Given the trend of decreasing physical activity with increasing age, the effects of an exercise intervention may be particularly beneficial to very young children including toddlers and preschoolers. The prevalence of obesity among US preschoolers has been increasing at a startling rate. It is estimated that the rates of overweight and obesity in this age group have doubled over the past 30 years and almost one in four preschoolers (aged 2 to 5) is either overweight or obese (135). According to the National Health and Nutrition Examination Survey 2003-2006, the prevalence of obesity among children aged 2 to 5 years was 12.4% (136). Childcare
experiences during the preschool years may have an important influence in weight status in childhood.

Of the nation’s 21 million preschool children, 13 million spend a substantial part of their day in childcare facilities (180). Engaging in physical activity on a daily basis is an essential element for the long-term, overall health of children, particularly in their early years. Given this situation it is imperative to consider the opportunities that these childcare facilities have to address the problem of obesity by influencing childrens' physical activity and sedentary activity behaviors to prevent excess weight gain. Preschools and daycare centers are particularly influential in offering the opportunity for healthy living to all children. For example, a teacher-delivered, school-based weight control intervention administered to 3-5 year old children resulted in significant improvements in MVPA, measured by accelerometry, and decreased screen time, compared to a control group (70). The effects on exercise interventions may have a greater impact on physical activity habits when children are younger and still developing opinions and behaviors.

Childhood obesity is particularly dangerous, as “obese-years” have now been identified as a method to assess risk factors. Just as in “pack-years” for cigarette smoking, “obese-years” refer to the duration of obesity as it tracks into adulthood. The longer children with obesity experience these effects of chronic inflammation, increased ROS production, and cell dysfunction, the earlier the onset of diseases, originally termed “adult-onset (3, 4).” Even more alarming is that the duration of obesity not only increases the risk of being diagnosed with one of these diseases, but
the severity of the disease increases with “obese-years.” It is therefore essential to engage these children in an aerobic exercise program at the very least. Coupled with nutrition behavior, a lifestyle program can be extremely effective in reducing obesity and, more specifically, the associated chronic inflammation and subsequent cell dysfunction.
Chapter 3: Changes in Aerobic Capacity and Physical Activity Behaviors in Sedentary, Pubescent Adolescents with Obesity Following a 6-Week High Intensity Interval Exercise Intervention

Abstract

Introduction: Children with obesity demonstrate reduced aerobic capacity (VO$_{2\text{max}}$) and subsequent increased risk of cardiovascular disease (CVD). While obesity contributes to the reduction of aerobic capacity, time spent with physical activity (PA) may improve levels of aerobic fitness, independent of measures of fatness. Therefore, the aim of this study was to assess changes in VO$_{2\text{max}}$ in obese, pubescent, sedentary adolescents between moderate intensity (MOD) and high intensity interval exercise (HIIE) groups following a 6-week exercise program, independent of weight loss. Methods: Sedentary, pubescent adolescents with obesity were randomized to moderate intensity (MOD) or high intensity interval exercise (HIIE) groups for a 6-week exercise intervention, 3 days per week for 40 minutes. VO$_{2\text{max}}$, body composition, and physical activity behaviors were assessed prior to, and immediately following, the exercise intervention. Results: 27 subjects (17 females and 10 males; mean age 14.7 ± 1.5 years) completed the exercise intervention. Baseline VO$_{2\text{max}}$ for all subjects was negatively correlated with measures of body composition, including baseline waist circumference ($r = -0.407; p = 0.044$) and
body fat percentage ($r = -0.561; p = 0.004$). Waist to hip ratio decreased significantly from pre- to post-intervention in the entire sample ($p = 0.020$). $\text{VO}_2\text{max}$ increased significantly following the exercise intervention in the entire sample ($p = 0.027$). The increase was also significant in the HIIE group, but not the MOD group ($p = 0.015$). The HIIE group also demonstrated an increase in physical activity enjoyment, which was significantly and positively correlated with the increase in time spent with PA ($p = 0.022; r = 0.606$). The increased physical activity enjoyment in the HIIE group was positively correlated with the average percent of age predicted maximal heart rate sustained during the intervention ($p = 0.043; r = 0.547$).

**Conclusion:** A 6-week exercise intervention elicited improvements to aerobic capacity in sedentary, pubescent adolescents with obesity. These improvements were further identified in the HIIE group, but not the MOD exercise group. The HIIE group also experienced a relationship between increased time spent with PA and increased physical activity enjoyment. Therefore, enjoyment of physical activity appears to be an important determinant of participation in PA, contributing to improved aerobic capacity, specifically following higher intensity activities.

**Introduction**

Aerobic capacity, also referred to as cardiiorespiratory fitness, aerobic power, and maximal volume of oxygen consumption ($\text{VO}_2\text{max}$), is a measure of physical fitness. In adults, $\text{VO}_2\text{max}$ has been identified as an independent predictor of all-cause mortality, such that low levels of fitness are associated with increased risk of Type II
Diabetes Mellitus (T2DM), cancers, and death (34, 56, 98, 179). Similar associations have been identified in children, specifically, those with obesity. In fact, a significant negative relationship has been found between measures of adiposity and aerobic capacity in children (27, 92, 97, 137, 138, 143, 212). On the contrary, children within normal limits for body composition have demonstrated higher levels of physical fitness including aerobic capacity (137, 138, 212).

While obesity contributes to the reduction of aerobic capacity, time spent with physical activity may improve levels of cardiorespiratory fitness, independent of measures of fatness. Therefore, increasing time spent with physical activity can improve cardiorespiratory fitness in children, regardless of weight status. Studies have shown that time spent with physical activity (PA) is positively associated with cardiorespiratory fitness (62, 143). Conversely, cardiorespiratory fitness was found to be inversely associated with time spent with sedentary behavior. Therefore, greater time spent with total PA may contribute to improved levels of cardiorespiratory fitness in children (61, 62).

High intensity interval exercise (HIIE) often results in greater reductions in cardiovascular risk factors when compared to moderate exercise (MOD). HIIE has been found to partially or even fully reverse many cardiovascular risk factors found in children with obesity, with the effects often lasting longer than those seen in moderate exercise (28, 40, 79, 82, 127, 190-192, 214). The short, high intensity periods alternating with lower intensity recovery periods contribute to greater challenges to the pumping ability of the heart, resulting in improved stroke volume
and subsequent increased VO$_{2\text{max}}$ (40, 192). Often times, these improvements are seen independent of weight loss, suggesting a stronger link between aerobic capacity and all cause mortality than obesity and cardiovascular mortality (40, 192). Increasing aerobic capacity may be more important than reducing weight in regards to decreasing cardiovascular risk factors. Rather than focus on weight loss, interventions should aim to implement and/or increase high intensity PA to improve vascular function (85).

Therefore, the primary aim of this study was to assess changes in aerobic capacity in obese, pubescent, sedentary adolescents between moderate intensity level exercise (MOD) and high intensity interval exercise (HIIE) following a 6-week exercise program, independent of weight loss. In addition, this study examined changes in reported PA, sedentary activities, and level of physical activity enjoyment following the exercise intervention.

**Methods**

**Subjects**

Healthy, sedentary, pubescent adolescents with obesity were recruited from clinics at a Midwest children’s hospital. Obesity was defined as BMI ≥ 95$^{\text{th}}$ percentile for age and sex as defined by the Centers for Disease Control (CDC) (106). Subjects were excluded from the study if they were participating in ≥30 minutes of vigorous exercise more than 2 days per week, in an organized combined diet/exercise weight loss intervention, reported an acute inflammatory disease or febrile illness, recent
trauma or injury, asthma requiring steroid use or hospitalization within the prior 3 months, inflammatory/immune disorders (e.g. lupus), and any renal, cardiac, or liver disease.

All subjects and legal guardians provided written informed assent and consent, respectively. The study protocol was approved by the Institutional Review Board of the participating institutions. The study trial has been registered at ClinicalTrials.gov (http://clinicaltrials.gov) under the trial number NCT01821313.

A total of 34 adolescents, 13-17 years old with obesity agreed to participate in the study. Subjects were randomized to MOD or HIIE via random number generator. Completion of the intervention was defined as having attended 14 of 18 exercise sessions and follow-up testing.

**Experimental Design**

The study was a pretest/posttest true experimental design conducted at NCH and The Ohio State University (OSU) in Columbus, Ohio. Study participants underwent a series of lab visits at NCH and OSU pre- and post-intervention.

**Intervention**

Subjects were randomized to receive either moderate intensity (MOD) or high intensity interval exercise (HIIE). For both intervention groups, activity sessions were on 3 non-consecutive days per week over 6 weeks at OSU’s Physical Activity and Educational Services laboratory. Each intervention was performed on a cycle ergometer (Lode, The Netherlands). Intervention started with a five-minute
warm-up at 50-55% of the subject’s age predicted maximal heart rate (APMHR) as determined by the following equation: APMHR= 220-age. Following the warm-up, the MOD group cycled continuously for 30 minutes at 65-70% of APMHR. The HIIE group performed 10, two-minute cycling bouts at 90-95% of APMHR, with one minute of active recovery at 55% of APMHR between each interval for a total of 30 minutes. Both MOD and HIIE ended with a 5-minute cool-down at 50-55% of APMHR. Heart rate was measured with heart rate monitors (Polar Electro Inc, Lake Success, NY).

*Study Measures*

All study measures were obtained at baseline, prior to randomization and on completion of the 6-week exercise intervention.

*Anthropometric Measurements*

Height and body weight were measured in minimal clothing to the nearest 0.5 cm and 0.1 kg, respectively, with a stadiometer and scale, respectively. Body mass index (BMI) was calculated as weight (kg)/height (m²). Waist and hip circumference (WC and HC) were measured to the nearest 0.5 cm using standard anthropometric tape midway between the lowest rib and iliac crest and at the point of greatest protrusion, respectively. Body fat percentage (BF%) was measured via Bod Pod air-displacement plethysmography (Life Measurements Instruments, Concord, CA). Prior to body fat percentage measurement, the Bod Pod was calibrated for volume and mass. Subjects entered the Bod Pod in a fasted state,
wearing minimal, tight fitting clothing and a swim cap. Density models were used based on age and gender (115).

**Fitness Assessment**

Aerobic capacity (VO$_{2\text{max}}$) was assessed using the Åstrand Cycle Test, a submaximal exercise test on a cycle ergometer. Subjects completed 6-8 minutes on an electronically braked cycle ergometer at a moderate resistance (125 W). Heart rate was recorded at the completion of each minute using a Polar heart rate monitor (Polar Electro, Lake Success, NY). After 3 minutes, resistance on the bike was adjusted based on subject’s heart rate. At heart rates below 140 beats per minute (bpm), resistance is increased by 25 W and decreased by 25 W for heart rates above 149 bpm. If steady state heart rate was achieved by minute 6 (defined as less than 10 bpm difference between heart rates at minute 5 and minute 6), the test was terminated. Otherwise, subjects continued to cycle for another 2 minutes. The average heart rate for minutes 5 and 6 (or 7 and 8, if necessary) was used as the submaximal heart rate in the equation to estimate VO$_{2\text{max}}$.

**Activity Monitoring**

PA was assessed via the Self-Administered Physical Activity Checklist (SAPAC) (169). Briefly, the children are asked to indicate if they participated in specific activities during the previous day by writing in the amount of time spent with the activity (before, during, or after school). To specify intensity of the
activities, they were also asked to indicate if the activity made them breathe hard or feel tired none, some, or most of the time. The subjects also recorded time spent with TV and movies, and computer, video games, and hand-held devices, both before and after school.

Physical activity enjoyment was measured via the Physical Activity Enjoyment Scale (PACES). PACES is a questionnaire consisting of 18 bipolar statements with a 7 point continuum beginning with the stem “When I am physically active...” It is specifically designed to assess the level of enjoyment while participating in physical activity (101). Higher scores represent greater enjoyment of physical activity.

Statistical Analyses

The primary outcome variable was VO$_{2\text{max}}$. Repeated factor condition of pre-intervention and post-intervention for aerobic capacity, anthropometric measurements, and physical activity behavior data was analyzed by using paired samples $t$-tests. Change in variables from pre- to post-intervention between treatment groups (MOD versus HIIE) was analyzed using independent samples $t$-tests. Reported p-values are two-sided and < 0.05 are considered statistically significant. All data are presented as means ± SD. IBM SPSS Statistics 20.0 (IBM Corp, Armonk, NY) was employed for all analysis.
Results

Baseline Characteristics

Thirty-four subjects were randomized to MOD (n=16; 6 males and 10 females) and HIIE (n=18; 8 males and 10 females). Of the 34 subjects, 27 (17 females and 10 males; mean age 14.7 ± 1.5 years) completed the exercise intervention and carried out 87.3 ± 7.7% of the scheduled training sessions. There were no significant differences in baseline characteristics between the MOD versus HIIE treatment groups as shown in Table 1. Mean baseline VO₂max for males and females was 21.2 ± 7.7 ml/kg/min and 18.8 ± 5.3 ml/kg/min respectively. Baseline VO₂max for all subjects was negatively correlated with measures of body composition, including baseline WC (r = -0.407; p = 0.044) and BF% (r = -0.561; p = 0.004).

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics at baseline</th>
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<tr>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<td>BMIZ</td>
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<tr>
<td>BF (%)</td>
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<tr>
<td>WHR</td>
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<tr>
<td>VO₂max (ml/kg/min)</td>
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</tbody>
</table>

BMI= Body mass index; BMIZ= BMI z score; BF= body fat percentage; WHR= waist-to-hip ratio; VO₂max= Maximal volume of oxygen consumed; *n = 25, #n = 11; All data is presented as means ± SD.
**Within Group Body Composition Changes**

WHR decreased significantly from pre- to post-intervention in the entire sample (p = 0.020) (Table 2). No other measure of body composition changed significantly from pre- to post-intervention in either group.

**Table 2. Changes in body composition**

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 27)</th>
<th>MOD (n = 13)</th>
<th>HIIE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.82 ± 2.57</td>
<td>1.45 ± 3.23</td>
<td>0.24 ± 1.58</td>
</tr>
<tr>
<td>BF (%)</td>
<td>-0.47 ± 1.95</td>
<td>-0.22 ± 2.08</td>
<td>-0.71 ± 1.87</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>-1.39 ± 4.61</td>
<td>-0.62 ± 3.90</td>
<td>-2.11 ± 5.24</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.02 ± 0.03*</td>
<td>-0.01 ± 0.04</td>
<td>-0.02 ± 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.16 ± 0.85</td>
<td>0.19 ± 1.08</td>
<td>0.14 ± 0.60</td>
</tr>
<tr>
<td>BMIz</td>
<td>-0.001 ± 0.057</td>
<td>-0.002 ± 0.069</td>
<td>0.000 ± 0.047</td>
</tr>
</tbody>
</table>

*significant change from pre- to post-intervention, p = 0.020.

**Between Group Body Composition Changes**

The HIIE group demonstrated a greater decrease in BF%, WC, WHR, and less of an increase in weight, BMI, and BMIz compared to the MOD group following the intervention (Table 2). However, the differences between groups were not significant.
Within Group Changes in Aerobic Capacity

There was a significant increase in \( \text{VO}_{2\text{max}} \) from 19.7 ± 1.3 ml/kg/min to 21.5 ± 1.4 ml/kg/min following the exercise intervention in the entire sample (\( p = 0.027 \)) (Table 3). The increase in \( \text{VO}_{2\text{max}} \) following the intervention from 20.0 ± 1.5 ml/kg/min to 22.7 ± 1.7 ml/kg/min was also significant in the HIIE group, but not the MOD group (19.4 ± 2.2 ml/kg/min to 19.9 ± 2.5 ml/kg/min) (\( p = 0.015 \)).

<table>
<thead>
<tr>
<th></th>
<th>MOD</th>
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<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
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<td></td>
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<tr>
<td>( \text{VO}_{2\text{max}} ) (ml/kg/min)</td>
<td>19.4 ± 7.3</td>
<td>19.9 ± 8.2</td>
<td>2.6</td>
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<tr>
<td>PACES</td>
<td>85.6 ± 18.5</td>
<td>89.9 ± 21.9</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PA (min/day)</td>
<td>56.2 ± 61.3</td>
<td>81.2 ± 105.2</td>
<td>44.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary (min/day)</td>
<td>139.6 ± 102.7</td>
<td>194.2 ± 173.0</td>
<td>39.1</td>
<td></td>
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</table>

|                      | HIIE           |                  |                  |                  |                  |                  |                  |
|                      | Pretest        | Posttest         |                  |                  |                  |                  |                  |
| \( \text{VO}_{2\text{max}} \) (ml/kg/min) | 20.0 ± 5.7     | 22.7 ± 6.5       | 13.5*            |                  |                  |                  |                  |
| PACES                | 78.1 ± 16.3    | 85.2 ± 13.9      | 9.1              |                  |                  |                  |                  |
| PA (min/day)         | 53.1 ± 44.9    | 85.0 ± 82.2      | 60.1             |                  |                  |                  |                  |
| Sedentary (min/day)  | 202.5 ± 151.9  | 179.6 ± 110.9    | -11.3            |                  |                  |                  |                  |

\( \% \Delta \) = percent change from pre to post exercise intervention.

\(*\) = significant pre- to post-intervention, \( p = 0.015 \).
Between Group Changes in Aerobic Capacity

While the HIIE group experienced a greater increase in VO$_{2\text{max}}$ following the intervention compared to the MOD group (2.69 ± 3.59 vs. 0.46 ± 3.41 ml/kg/min vs., respectively), the difference was non-significant (p > 0.05).

Within Group Changes in Physical Activity Behavior

At baseline, subjects spent an average of 54.6 ± 52.4 min/day participating in PA. Conversely, an average of 172.2 ± 132 min/day were spent with sedentary activities. Time spent with daily PA increased non-significantly by 52% after the exercise intervention in the entire sample following the intervention. Overall time spent with sedentary activities increased slightly in the entire sample (8.4%). Time spent with sedentary activities decreased by 11% in the HIIE group and increased by 39% in the MOD group (Table 3). Enjoyment of physical activity increased non-significantly following the exercise intervention in the entire sample (7.0%) and in both MOD and HIIE groups (4.9% vs. 9.0%, respectively) (Table 3). The increase in physical activity enjoyment in the HIIE group was significantly and positively correlated with the increase in time spent with PA (p = 0.022; r = 0.606). A positive correlation was also identified between average percent of age predicted maximal heart rate sustained during exercise intervention and change in physical activity enjoyment in the HIIE group (p = 0.043; r = 0.547) (Figure 1).
Figure 1. Correlation between average percent of APMHR achieved during exercise and change in PACES score.

**Between Group Changes in Physical Activity Behavior**

The HIIE group demonstrated a greater increase in daily PA than the MOD group (60% vs. 45%) (Figure 2). The increase in time spent with daily PA was non-significant between groups (p > 0.05). While the HIIE group demonstrated a decrease in time spent with sedentary activities compared to the increase noted in the MOD group, the difference between the groups was not significant.

Physical activity enjoyment increased at a greater rate in the HIIE group compared to the MOD group, however, the differences between the two experimental groups were non-significant (Table 3).
Discussion

Children who do not participate in PA and/or who are physically unfit demonstrate increased risk of morbidity and mortality in adulthood. The children in this study demonstrated improved aerobic capacity following a 6-week exercise intervention without significant changes to weight or body fat percentage. Increases in cardiac output and central oxygen delivery, accompanied by peripheral oxygen uptake by skeletal muscle are the mechanisms most likely responsible for this improvement in VO2max. The effects of increasing aerobic capacity in childhood have been shown to track into adulthood. Specifically, studies show that children with
higher aerobic capacities are more likely to become physically fit adults, with each one-unit increase in childhood aerobic capacity resulting in a .21 unit increase in adult fitness (60). Consequently, reduced physical fitness from childhood to adulthood is a stronger predictor of adult CVD risk than the level of fitness during childhood. Even after removing children with obesity from the analyses, lower overall childhood fitness and decreased fitness level from childhood to adulthood were still associated with adult obesity (60). It is, therefore, important to establish healthy physical activity habits early in life and work to maintain these habits into adulthood.

Aerobic capacity not only increased significantly in the entire sample from pre- to post-intervention, but the improvement was also statistically significant within the HIIE group. While the change in VO$_{2\text{max}}$ between the MOD and HIIE groups was not statistically significant, the increase in the HIIE compared to the MOD group (13.5% versus 2.6%, respectively) may be clinically relevant for this population. A 13.5% increase in aerobic capacity following 6 weeks of exercise (120 minutes/week) demonstrates potential adaptation of the vasculature and skeletal muscle following HIIE. This study shows that the same duration of exercise (40 minutes) can elicit different responses based on exercise intensity, with HIIE resulting in greater aerobic capacity.

Compared to moderate endurance training protocols, HIIE can elicit similar adaptations in exercise performance, muscle buffering capacity, and oxidative capacity of skeletal muscle in significantly less time (29, 74). Active, adult males
randomized to sprint-interval training and endurance training exercise groups for 6 sessions demonstrated similar improvements in short distance and long distance time trials, mitochondrial enzyme activity and content and muscle glycogen content. However, the sprint-interval training group performed higher intensity exercises but at 90% less volume than the endurance training group (74). HIIE, therefore, produces similar results, but in a significantly shorter period of time.

Wisloff et al. (214) also showed the greater improvements in aerobic capacity following higher intensity exercise compared to moderate intensity exercise. Those in the higher intensity group experienced a 46% greater increase in VO$_{2\text{max}}$ compared to the moderate group in less time (38 versus 47 minutes, respectively). Similar results were identified in adolescents with obesity, with higher intensity exercises eliciting greater improvements in aerobic capacity when compared to moderate exercise (192). While the current study did not show statistically significant differences in changes to aerobic capacity between the two exercise groups, the 13.5% increase in the HIIE group is clinically relevant and highlights the benefits of HIIE.

While all of the subjects were still well below normal VO$_{2\text{max}}$ values following the intervention, the improvement occurred in only 6-weeks, implicating the role of vigorous exercise in enhancing cardiorespiratory health and fitness. Adolescents in the current study demonstrate significantly lower VO$_{2\text{max}}$ values than the normal values for 12-18 year old boys (47.3 ± 0.6 ml/kg/min) and girls (39.6 ± 0.4 ml/kg/min) determined by an NHANES study. Furthermore, the adolescents in the
current study were still below the ‘at risk’ zones for age and gender, following the intervention, considered to be 38.6-41.2 ml/kg/min and 39.7-38.8 ml/kg/min for 13-17 year old boys and girls, respectively (207).

While our subjects demonstrated a significantly lower baseline and follow up \( VO_{2\text{max}} \) than normal values, aerobic capacity did improve significantly following 6 weeks of exercise, independent of changes to weight and body fat percentage. The increased aerobic capacity exhibited in the current subjects may be due to the concurrent increased time spent with PA. Total energy expenditure has been found to be significantly and positively related to \( VO_{2\text{peak}} \) in children of a similar age (62). Therefore, the improved aerobic capacity identified in the HIIE group may be related to the increase in time spent with PA and decreased time spent with sedentary activities.

This introduction to vigorous physical activity may have also contributed to increased enjoyment, ultimately resulting in the increased participation in daily PA. Physical activity enjoyment, measured by the PACES, was found to be an independent predictor of PA in boys (208). Physical activity enjoyment may play a significant role in time spent with PA and, ultimately, measures of fitness, anthropometry, and overall health. A positive relationship has been identified between physical activity enjoyment and time spent with PA in children and adolescents (54, 55, 167, 170). One particular study found that increasing PA and decreasing time spent watching television, and improving fundamental motor skills, resulted in high levels of physical activity enjoyment among 10 year old children.
The increase in time spent with PA and decrease in sedentary behavior, along with improved fundamental motor skills may have contributed to increased self-esteem in regards to physical activity, resulting in greater enjoyment of physical activity. Similar studies have found that physical activity enjoyment was the most important predictor of physical activity levels, suggesting that participating in activities that provide enjoyment, may aid in increasing time spent with PA (54).

Some limitations to the study may have contributed to a lack of statistically significant results between the MOD and HIIE groups. While the HIIE protocol included exercise at 90-95% of APMHR, some of the subjects were unable to reach their target heart rate during the exercise sessions. In fact, in the HIIE group the percent of APMHR achieved was 88.8 ± 4.1%. Perhaps if all of the subjects were able to sustain the prescribed exercise intensity, we may have seen a greater difference in aerobic capacity between the MOD and HIIE groups. A larger study sample size may also have helped identify the greater improvements in the HIIE versus MOD exercise groups. HIIE may ultimately provide an exercise protocol capable of eliciting greater changes in aerobic capacity than moderate intensity exercise.

In the current study, we found that a 6-week exercise intervention elicited significant improvements to aerobic capacity in sedentary, pubescent adolescents with obesity. Further, the change in aerobic capacity was found to be significant in the HIIE group, but not the MOD exercise group. The HIIE group also experienced significant positive correlations between changes in time spent with PA and changes in physical activity enjoyment, such that greater time spent with PA resulted in
improved enjoyment of physical activity. Therefore, enjoyment of physical activity appears to be an important determinant of participation in PA and ultimately plays a significant role in improving aerobic capacity, specifically following higher intensity activities.
Chapter 4: The Effects of High Intensity Interval Exercise on Inflammation and Endothelial Function in Pubescent, Sedentary Adolescents with Obesity

Abstract

Introduction: Children and adolescents often fail to attain the recommended dosage of daily moderate to vigorous physical activity (MVPA). Consequently, decreased time spent with MVPA contributes to the risk of future health complications. Those who are physically inactive and obese may further contribute to cardiovascular disease and inflammation. The purpose of this study was to compare the effects of exercise of differing intensity (HIIE vs. moderate) on improving endothelial function and inflammation in sedentary, pubescent adolescents with obesity. Methods: Sedentary, pubescent adolescents with obesity were randomized to moderate intensity (MOD) or high intensity interval exercise (HIIE) groups for a 6-week exercise intervention, 3 days per week for 40 minutes. All measures were obtained prior to and immediately following the intervention. Fasted blood draw was obtained to measure markers of inflammation including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP), and adiponectin. Endothelial function was measured as serum levels of endothelin-1 (ET-1) and percent change in forearm vascular resistance (%ΔFVR) via venous occlusion plethysmography (VOP). Measures of body composition included weight,
BMI, BMI z score (BMlz), waist circumference (WC), waist to hip ratio (WHR), and body fat percentage (BF%). Results: hsCRP was positively associated with BMlz at baseline for all subjects. No significant change in inflammatory markers was noted following the intervention. %ΔFVR experienced a non-significant decrease in the MOD group, but was maintained in the HIIE group. The HIIE group also experienced a non-significant decrease in ET-1 while ET-1 increased slightly and non-significantly in the MOD group. The decrease in ET-1 in the HIIE group was associated with greater percentages of age predicted max heart rate (%APMHR) achieved during the intervention (p = 0.035, r = -0.567). Conclusion: Adolescents with obesity exhibited elevated levels of the inflammatory marker hsCRP. Likewise, ET-1, a blood marker of endothelial dysfunction was elevated at baseline, but decreased in those who exercised at higher intensities. Higher intensity exercises also attenuated the decline in endothelial function noted in the MOD group. Higher intensity exercises may, therefore, elicit greater improvements in endothelial function than moderate exercise.

Introduction

Childhood obesity rates among children and adolescents remain elevated in most industrialized countries. While many contributing factors are responsible for this epidemic, positive energy balance is ultimately the culprit. Currently, The American College of Sports Medicine (ACSM) recommends children 6-18 years old accumulate at least 60 minutes, and up to several hours, of moderate to vigorous
daily physical activity (MVPA). Yet, some research finds that 58% of children (ages 6-11) fail to attain the recommended amount of physical activity (PA), with rates of participation further declining through adolescence (195).

While inactivity is present in both healthy weight and obese children alike, the dangers of future health complications are greater in the obese population. Physical inactivity not only contributes to obesity and associated comorbidities in childhood, but also sets the stage for a host of diseases in adulthood, including hypertension, type II diabetes mellitus (T2DM), cardiovascular disease (CVD), and even premature death (71, 177, 185). Many of these risk factors are accompanied by an inflammatory response, but childhood obesity is also independently associated with systemic low-grade inflammation, contributing to CVD (75). However, whether this inflammatory state is initiated solely by obesity or if physical inactivity also plays a role is not well understood (85, 123).

Increased inflammation in children with obesity may cause early endothelial damage and arterial abnormalities, which sets the stage for CVD in adulthood (48, 75, 102, 157). Other risk factors for CVD associated with endothelial dysfunction and physical inactivity include elevated CRP and decreased adiponectin (33, 85). This suggests that any degree of overweight and obesity can attribute to arterial abnormalities at a surprisingly young age. Endothelial dysfunction is one of the first signs of atherosclerosis and subsequent CVD. The process of atherosclerosis and the development of fibrous plaque lesions have been identified in the arteries of children as young as 2 years old (20).
With the overwhelming dangers of obesity many children now face, determining effective frequency, intensity, and duration of exercise has become vital. Extremely vigorous PA, generally defined as activity at 80-95% of VO$_2$max, is often referred to as high intensity interval exercise (HIIE). HIIE has been studied in adults and children to determine the effects on inflammation compared to moderate exercise. While both moderate and vigorous PA can produce significant cardiovascular benefits, the improvements appear to be greater with higher intensity activities (80). Three months of HIIE in children with obesity led to a significant increase in nitric oxide (NO) bioavailability, reduction in fasting blood glucose, and increase in adiponectin compared to the moderate exercise group, subsequently improving endothelial function (192).

The amount of time required to elicit adaptations to HIIE, including increased NO bioavailability due to increased shear stress has not yet been elucidated, but may be visible in as little as 6 weeks. Further, previous research on the effects of exercise on endothelial function has relied on flow mediated dilation (FMD) or intima-media thickness (IMT) as tools for measurement (2, 85, 96, 100, 123, 141, 191, 198, 205). Few studies have utilized venous occlusion plethysmography to assess the changes in microvasculature following an exercise intervention and none have analyzed the effects in pubescent, sedentary adolescents with obesity.

The purpose of this study was to compare the effects of exercise of differing intensity (HIIE vs. moderate) on improving endothelial function and inflammation in sedentary, pubescent adolescents with obesity. Our hypothesis was that HIIE
would result in greater improvements in both endothelial function and inflammation.

**Materials and Methods**

**Subjects**

Healthy, sedentary, pubescent adolescents with obesity were recruited from clinics at a Midwest children’s hospital. Obesity was defined as BMI ≥ 95th percentile for age and sex as defined by the Centers for Disease Control (CDC) (106). Subjects were excluded from the study if they were participating in ≥30 minutes of vigorous exercise more than 2 days per week, in an organized combined diet/exercise weight loss intervention, reported an acute inflammatory disease or febrile illness, recent trauma or injury, asthma requiring steroid use or hospitalization within the prior 3 months, inflammatory/immune disorders (e.g. lupus), and any renal, cardiac, or liver disease.

All subjects and legal guardians provided written informed assent and consent, respectively. The study protocol was approved by the Institutional Review Board of the participating institutions. The study trial has been registered at ClinicalTrials.gov ([http://clinicaltrials.gov](http://clinicaltrials.gov)) under the trial number NCT01821313.

A total of 34 adolescents, 13-17 years old with obesity agreed to participate in the study. Subjects were randomized to MOD or HIIE via random number generator. Completion of the intervention was defined as having attended 14 of 18 exercise sessions and follow-up testing.
Experimental Design

The study was a pretest/posttest true experimental design conducted at NCH and The Ohio State University (OSU) in Columbus, Ohio. Study participants underwent a series of lab visits at NCH and OSU pre- and post-intervention.

Intervention

Subjects were randomized to receive either moderate intensity (MOD) or high intensity interval exercise (HIIE). For both intervention groups, activity sessions were on 3 non-consecutive days per week over 6 weeks at OSU’s Physical Activity and Educational Services laboratory. Each intervention was performed on a cycle ergometer (Lode, The Netherlands). Intervention started with a five-minute warm-up at 50-55% of the subject’s age predicted maximal heart rate (APMHR) as determined by the following equation: APMHR= 220-age. Following the warm-up, the MOD group cycled continuously for 30 minutes at 65-70% of APMHR. The HIIE group performed 10, two-minute cycling bouts at 90-95% of APMHR, with one minute of active recovery at 55% of APMHR between each interval for a total of 30 minutes. Both MOD and HIIE ended with a 5-minute cool-down at 50-55% of APMHR. Heart rate was measured with heart rate monitors (Polar Electro Inc, Lake Success, NY).

Study Measures

All study measures were obtained at baseline, prior to randomization and on completion of the 6-week exercise intervention.
**Anthropometric Measurements**

Height and body weight were measured in minimal clothing to the nearest 0.5 cm and 0.1 kg, respectively, with a stadiometer and scale, respectively. BMI was calculated as weight (kg)/height (m²). Waist and hip circumference were measured to the nearest 0.5 cm using standard anthropometric tape midway between the lowest rib and iliac crest and at the point of greatest protrusion, respectively. Body fat percentage was measured via Bod Pod air-displacement plethysmography (Life Measurements Instruments, Concord, CA). Prior to body fat percentage measurement, the Bod Pod was calibrated for volume and mass. Subjects entered the Bod Pod in a fasted state, wearing minimal, tight fitting clothing and a swim cap. Density models were used based on age and gender (115).

**Fitness Assessment**

Aerobic capacity was assessed using the Åstrand Cycle Test, a submaximal exercise test on a cycle ergometer. Subjects completed 6-8 minutes on an electronically braked cycle ergometer at a moderate resistance (125 W). Heart rate was recorded at the completion of each minute using a Polar heart rate monitor (Polar Electro, Lake Success, NY). After 3 minutes, resistance on the bike was adjusted based on subject’s heart rate. At heart rates below 140 bpm, resistance is increased by 25 W and decreased by 25 W for heart rates above 149 bpm. If steady state heart rate was achieved by minute 6 (defined as less than 10 bpm difference between heart rates at minute 5 and minute 6), the test was terminated. Otherwise, subjects continued to cycle for another 2 minutes. The average heart rate for
minutes 5 and 6 (or 7 and 8, if necessary) was used as the submaximal heart rate in the equation to estimate VO$_{2\text{max}}$.

**Laboratory Assessment**

Within 1 week of the fitness assessment and prior to participation in the exercise intervention, subjects reported to Clinical Research Services at NCH in the morning for a fasted blood draw and assessment of endothelial function and arterial stiffness. The blood draw was performed after a 12-hour overnight fast, to obtain high sensitivity C-reactive protein (hsCRP), adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and endothelin-1 (ET-1). hsCRP was assayed via Immulite 2000XPi (Siemens, Tarrytown, NY). Adiponectin was measured in serum using the Human Adiponectin ELISA Kit (Otsuka Pharmaceuticals, Japan). TNF-α and IL-6 were also measured in serum via Luminex 100/200 analyzer (ARUP Laboratories, Salt Lake City, Utah). ET-1 was also assayed via serum collection using a 1-25 Radiogamma counter (Inter Science Institute, Inglewood, CA).

**Venous Occlusion Plethysmography (VOP)**

Prior to the blood draw, endothelial function was measured as forearm vascular resistance (FVR) via venous occlusion plethysmography (VOP) as described by Higashi et al. (84), using a Hokanson A16 plethysmograph (DE Hokanson Inc, Bellevue, WA). Briefly, the subjects rested in a supine position for 5 minutes. A sphygmomanometric cuff was then placed around the wrist and inflated to 200 mmHg to occlude blood flow to and from the hands. Another cuff was placed
around the upper arm and, during forearm blood flow (FBF) measurement, was inflated to 40 mmHg for 10 seconds to occlude venous blood flow from the forearm, while not changing the rate of arterial inflow. Each subject had two minutes of baseline flow recorded, followed by inflation of the upper cuff to 200 mmHg for five minutes to occlude arterial flow. The cuff was then released, resulting in hyperemia and increased shear stress. Changes in forearm volume were measured using a mercury-in-silastic strain gauge plethysmograph. FBF was then measured for the next minute and transmitted to the computer and FBF was expressed as mL per minute per 100 mL of forearm tissue volume. FVR was calculated by mean arterial pressure (MAP) divided by FBF. All measurements were scored by a single experienced investigator (RPH) and the reactive hyperemic change in FVR from pre-to post-occlusion was used to assess endothelial function.

Power Analysis

The sample size calculation for this study was based on the previously reported data comparing the percent changes in forearm vascular resistance (%ΔFVR) in lean and obese children and adolescents. With a 2-sided, 0.05 significance level and percent change in FVR as the primary variable, 13 subjects in each group would allows us to detect a significant difference between exercise groups at 80% power.
**Statistical Analyses**

The primary outcome variables were the percent change in forearm vascular resistance (%ΔFVR) and inflammatory markers (hsCRP, IL-6, TNF-α, and adiponectin) from pre- to post-intervention in both groups. TNF-α and IL-6 were not present at elevated levels in most subjects at baseline and were, therefore, excluded from analysis. Secondary outcomes include percent difference in these changes between MOD and HIIE groups. All analyses were conducted using IBM SPSS Statistics 20.0 (IBM Corp, Armonk, NY). Descriptive statistics (means, medians, percentiles, ranges, etc.) were calculated and provided for all outcome variables and demographics. Paired t tests were performed to compare pre- and post-intervention mean levels of inflammatory markers and endothelial function. Initial comparisons of measures between the two intervention groups were assessed using t tests. In final modeling, a two-way ANOVA with repeated measures was performed to test the differences between subjects in the moderate and high intensity interval groups.

**Results**

**Baseline Characteristics**

At baseline, the two experimental groups demonstrated comparable height, weight, body mass index (BMI), BMI z score (BMIz), body fat percentage (BF%), waist-to-hip-ratio (WHR), systolic blood pressure (SBP), and %ΔFVR (Table 4).
<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 27)</th>
<th>MOD (n = 13)</th>
<th>HIIE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.7 ± 1.5</td>
<td>14.5 ± 1.4</td>
<td>14.9 ± 1.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.2 ± 9.4</td>
<td>166.8 ± 9.9</td>
<td>167.4 ± 9.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>105.4 ± 20.7</td>
<td>108.3 ± 23.2</td>
<td>102.7 ± 18.5</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>37.6 ± 6.0</td>
<td>38.7 ± 6.7</td>
<td>36.5 ± 5.4</td>
</tr>
<tr>
<td>BMIZ</td>
<td>2.38 ± 0.35</td>
<td>2.42 ± 0.37</td>
<td>2.34 ± 0.34</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>43.8 ± 7.1</td>
<td>44.3 ± 8.1</td>
<td>43.4 ± 6.2</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.06</td>
<td>0.87 ± 0.06</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>%ΔFVR</td>
<td>78.7 ± 9.3*</td>
<td>82.4 ± 5.8</td>
<td>75.1 ± 10.8#</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>128.3 ± 12.9</td>
<td>129.7 ± 12.5</td>
<td>127.1 ± 13.5</td>
</tr>
</tbody>
</table>

BMI= body mass index; BMIZ= body mass index z-score; WHR= waist-to-hip ratio; VO_{2max}= maximal oxygen consumption; %ΔFVR = % change in forearm vascular resistance; SBP= systolic blood pressure. All data is presented as mean ± SD. *n = 26; #n = 13.

In total, 27 of the 34 subjects carried out 87.3 ± 7.7% of the scheduled exercise sessions (Figure 3). Subjects in the MOD group maintained the prescribed exercise intensity during 89.5 ± 14.4% of the attended sessions, while the HIIE subjects stayed in the prescribed heart rate zone for 50.9 ± 40.7% of attended sessions. The HIIE group attained an average heart rate of 88.8 ± 4.1% of age predicted maximum heart rate during exercise. There were no significant changes in weight or BF% between or within groups following the intervention.
Figure 3. CASH consort diagram.

Endothelial Function

Mean baseline SBP did not change significantly between or within groups following the exercise intervention. Greater changes in %ΔFVR indicate increased vasodilation and improved endothelial function. For all subjects, mean baseline %ΔFVR and ET-1 were 78.7 ± 9.3% and 4.1 ± 2.5 pg/mL, respectively. Follow up means of %ΔFVR and ET-1 were 77.3 ± 10.4% and 4.2 ± 2.5 pg/mL, respectively. Paired samples t-tests revealed no significant changes in %ΔFVR or ET-1 from pre-
to post-intervention. No statistical differences were detected in %ΔFVR or ET-1 values between HIIE and MOD groups at baseline or following the exercise intervention. %ΔFVR remained constant in the HIIE group from pre- to post-intervention (78.3 ± 9.7% to 78.1 ± 10.1%), and decreased in the MOD group (81.4 ± 4.4% to 77.9 ± 3.4) (Figure 4). However, these changes were non-significant within and between groups. ET-1 decreased slightly and non-significantly in the HIIE group and increased slightly in the MOD group. In the HIIE group, the change in ET-1 was significantly and negatively associated with percent of APMHR achieved during the intervention (p = 0.035; r = -0.567) (Figure 5).

![Figure 4. % change FVR pre- to post-intervention between MOD and HIIE groups.](image)
Figure 5. Relationship of change in ET-1 and average exercise intensity in HIIE group.

Inflammatory Markers

Mean baseline hsCRP and adiponectin were 3.8 ± 5.1 mg/dL and 7.9 ± 3.6 μg/mL, respectively. hsCRP was strongly and positively associated with BMIz score at baseline for all subjects (p = 0.006; r= 0.511). hsCRP and adiponectin did not change significantly between or within groups following the exercise intervention (Table 5).
Table 5. Changes in markers of endothelial function and inflammation from pre- to post-intervention

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 27)</th>
<th>MOD (n = 13)</th>
<th>HIIE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%ΔFVR (%)</td>
<td>-1.86 ± 12.17</td>
<td>-3.43 ± 12.64</td>
<td>-0.29 ± 12.13</td>
</tr>
<tr>
<td>ET-1 (pg/mL)</td>
<td>0.10 ± 3.21</td>
<td>0.59 ± 3.56</td>
<td>-0.70 ± 2.88</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>0.47 ± 4.90</td>
<td>1.62 ± 5.20</td>
<td>-0.60 ± 4.42</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>0.41 ± 3.21</td>
<td>0.39 ± 1.45</td>
<td>-0.02 ± 0.03</td>
</tr>
</tbody>
</table>

%ΔFVR= % change in FVR; ET-1= endothelin-1; hsCRP= high sensitivity C-reactive protein. All data presented as mean ± SD.

Discussion

HIIE has been found to elicit changes in inflammation and endothelial function, often times more significantly than moderate or lower intensity activities. However, in the current study, no statistical changes in endothelial function or inflammation were identified between or within groups. However, the HIIE group did appear to attenuate the continued increase in endothelial dysfunction experienced in the MOD group following 6 weeks of exercise. This was further highlighted by the significant association of decreased ET-1 with higher percentages of APMHR in the HIIE group. Subjects in the HIIE group that exercised at greater intensities experienced a greater reduction in ET-1.

At baseline, our subjects demonstrated elevated hsCRP levels, and specifically, those with greater BMI z scores were found to have higher hsCRP levels. CRP values are positively associated with measures of fatness showing significantly greater levels in children with obesity compared to normal weight children (133). This linear relationship between obesity and CRP is often due to the actions of TNF-
α and IL-6 on the liver (75). However, our subjects’ TNF-α, IL-6, and adiponectin values, were within normal limits at baseline. This is contrary to many studies identifying low-grade chronic inflammation in children with obesity. However, while the majority of these studies found the levels to be elevated in comparison to lean children, they may not have been clinically elevated (24, 75, 158, 175). Compared to lean children, those with obesity have significantly higher values of the pro-inflammatory markers hsCRP and TNF-α, and lower values of the anti-inflammatory marker adiponectin (75). Other studies have also shown this link between increased pro-inflammatory and decreased anti-inflammatory markers in childhood obesity (67, 100, 102, 133, 158, 176). However, due to the lack of elevated inflammatory markers at baseline, no significant changes in inflammation occurred following exercise in our subjects. The young age of the subjects and lower number of years the subjects have been obese may be the reason for non-elevated levels of TNF-α and IL-6 at baseline.

ET-1, a potent vasoconstrictor expressed by endothelial cells, is also elevated in adults with obesity, contributing to endothelial dysfunction. Participation in exercise and regular physical activity contributes to a significant reduction in plasma ET-1, subsequently increasing vasodilator response. In agreement with these studies, those subjects in the current study who maintained higher exercise intensities in the HIIE group also experienced greater reductions in ET-1 from pre- to post-intervention. The increased nitric oxide bioavailability resulting from participation in PA has been shown to inhibit transcription and release of ET-1
(134). By decreasing chronic elevated levels of ET-1 present in obesity, habitual PA may oppose the vasoconstriction associated with obesity, independent of changes in body composition.

The vascular endothelium regulates vessel tone and blood pressure through changes in NO and ET-1. Other endothelial functions include preventing platelet activation, thrombus formation, and the development of atheromas (187). VOP was employed to non-invasively assess endothelial function in adolescents with obesity by assessing the reactive hyperemic response to shear stress, which is mediated by NO. Impaired vasodilation is a result of increased ET-1 and decreased NO bioavailability, and is subsequently the initial indicator of dysfunction and, ultimately, atherosclerosis. Based on normative data from previous studies, mean baseline %ΔFVR was not impaired in our subjects and, therefore, did not experience significant changes following exercise (59). However, we did note a difference in the trends of %ΔFVR between the MOD and HIIE groups from pre- to post-intervention. The MOD group demonstrated a decline in %ΔFVR from pre- to post-intervention, while subjects in the HIIE group maintained %ΔFVR. While not statistically significant, the trend in %ΔFVR is clinically relevant in the realm of obesity and associated endothelial dysfunction. HIIE appears to have attenuated the increase in endothelial dysfunction measured via VOP, noted in the MOD group. Without vigorous exercise, the subjects in the HIIE may have experienced the continued decline in %ΔFVR that was identified in the MOD group. However, the trajectory
was diminished, most likely as a result of increased shear stress, decreased ET-1, and increased NO bioavailability.

Many other studies have identified improved endothelial function following HIIE. For instance, HIIE was found to attenuate the normal decrease in endothelial function, following consumption of a single high-fat meal. In fact, HIIE contributed to endothelial function improving beyond baseline values (198). Further, the protective effects of exercise appear to be intensity related, possibly due to the effects of shear stress. Increased physical activity intensity contributes to greater blood flow in and shear stress mediated upregulation of NO-synthase expression. The increased NO bioavailability thus allows for more efficient vasodilation and improved blood flow (100). In agreement with the findings of Hopkins et al. (85) we demonstrated that higher intensity exercise was associated with greater reductions in ET-1 and, thus, improvements in endothelial function. Participation in physical activity, therefore, is associated with improved endothelial function more so than body fat percentage.

Our study was comprised solely of a 6-week exercise intervention, without nutrition or behavior modifications. It is, therefore, plausible that a similar protocol carried out over a longer period of time, may further amplify these findings. The lack of adherence to the prescribed exercise protocol for some subjects in the HIIE may have impacted the results of this study. Had all subjects maintained an APMHR of 90-95% for the majority of the sessions, we may have seen greater changes to %ΔFVR, ET-1, and hsCRP. Likewise, difficulty with FBF measurements meant pre-
and post-intervention %ΔFVR data was only obtained for 20 of the 27 completed subjects, potentially contributing to the lack of statistically significant changes in %ΔFVR following the exercise intervention. The difference in trends identified in %ΔFVR between the MOD and HIIE group may approach significance with the addition of more subjects.

In conclusion, our study found that adolescents with obesity exhibited elevated levels of hsCRP, but no other elevations in inflammatory markers. Furthermore, endothelial function measured via VOP may not have been significantly impaired at baseline, however, mean ET-1 values were significantly elevated in both groups. The exercise intervention did not significantly improve the elevated hsCRP or ET-1 levels, yet those who exercised at higher intensities in the HIIE group experienced a greater decrease in ET-1. The HIIE group also experienced an attenuation of decreased endothelial function measured via VOP as identified in the MOD group. HIIE may elicit greater improvements in endothelial function than moderate exercise. Future research with larger sample sizes and longer intervention periods is needed.
Chapter 5: Conclusion

Comorbidities associated with obesity appear to accelerate atherosclerosis, accentuating the need to control these risk factors for treatment and long-term prevention during childhood. While weight loss may seem like the most reasonable outcome for children with obesity, exercise interventions on their own may offer additional health benefits independent of weight loss. For example, the adaptations to increased physical activity, both in duration and intensity, have been shown to attenuate the inflammatory effects of obesity and its comorbidities (80). It may therefore be beneficial to introduce children with obesity to programs that aim to improve health and cardiovascular function through exercise, rather than emphasize extreme weight loss.

For children and adolescents with obesity, time is of the essence. The identification of adult diseases in early childhood should be evidence enough of the threat to overall health in young children and adolescents (93). Furthermore, the tracking of obesity and associated comorbidities from childhood into adulthood, highlights the dangers of poor health habits in children. The longer children with obesity experience these effects of chronic inflammation, increased ROS production, and cell dysfunction, the earlier the onset of diseases, originally termed “adult-
onset.” Even more alarming is that the duration of obesity not only increases the risk of being diagnosed with one of these diseases, but the severity of the disease increases with the number of years the child has had obesity (4).

Exercise alone can be instrumental in reducing obesity-associated comorbidities in children and adolescents. By implementing physical activity programs in day care centers and elementary school, children may be spared from lifelong physical and emotional pain. Rather than focus on weight loss, interventions should aim to implement and/or increase high intensity physical activity to improve vascular function (85). High intensity or vigorous exercise is capable of increasing NO availability along with decreasing levels of TNF-α, IL-6, and CRP, and increasing adiponectin. Subsequently, improved insulin sensitivity and endothelial function occur with exercise, independent of fat loss. HIIE may be the best method to reach these goals in schools and health-care facilities. Compared to moderate exercise, HIIE is able to achieve similar goals in a significantly shorter period of time, making it a much more attractive protocol for children and health professionals alike.

Recent literature has focused on the importance of exercise in improving inflammation in children with obesity. In this regard, the effects that accompany HIIE have been found to be of greater benefit than low or moderate exercise. Research on low to moderate exercise tends to focus on the importance of fat burning and weight loss. While these effects are certainly positive, cardiovascular improvements seen in HIIE are more beneficial with longer lasting outcomes.
References


26. Bruce CR, Mertz VA, Heigenhauser GJF, Dyck DJ. The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid


110. Le KA, Mahurkar S, Alderete TL et al. Subcutaneous adipose tissue macrophage infiltration is associated with hepatic and visceral fat deposition,


Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation. 2003;112(12):1796-808.


Appendix A: Grant Proposal
SIGNATURE PAGE

Principal Investigator Attestation:
1. I understand and agree that funds granted as a result of this application are to be expended for the purposes set forth herein and that the grant may be revoked in whole, or in part at any time, in the event that the funds are not so utilized.

2. I intend to publish as a result of this research project, to seek additional grants related to this clinical research topic and/or to seek external funding opportunities related to this clinical research area.

3. I agree to submit a progress report every 6 months and a full report within thirty (30) days after the close of the grant. Unexpended funds will revert to RI at the end of the project.

4. I signify that the application complies with the rules of submission for a RI internal grant.

Principal Investigator Signature & Date: ________________________________

Collaborative Projects may list a Clinical Co-PI and a Lab Co-PI:

Clinical Co-Principal Investigator Signature & Date: ________________________________

Lab Co-Principal Investigator Signature & Date: ________________________________

FACULTY APPLICANTS:

Department/Division Chair, Attestation:
I have reviewed the proposed clinical/translational research and am in agreement that the research should be conducted at Nationwide Children’s Hospital and/or The Research Institute at Nationwide Children’s Hospital.

Department/Division Chair Signature & Date: ________________________________

HEART CENTER APPLICANTS:

Steering Committee Member Attestation:
I have reviewed the proposed clinical/translational research and am in agreement that the research meets the expectations of the Heart Center Steering Committee and the research should be conducted at Nationwide Children’s Hospital and/or The Research Institute at Nationwide Children’s Hospital.

Steering Committee Member Signature & Date: ________________________________

FELLOW/RESIDENT APPLICANTS:

Chair, Scholarly Oversight Committee OR Program Director Attestation:
I have reviewed the proposed clinical/translational research and am in agreement that the research should be conducted at Nationwide Children’s Hospital and/or The Research Institute at Nationwide Children’s Hospital.

Signature & Date: ________________________________

Title of Research Project:
CASH- Children Active to Stay Healthy

**Proposed start and end date of project:**
April 1, 2012- February 1, 2013

**Principal Investigator: Dr. Ihuoma Eneli**
Position/Title: Associate Professor/Medical Director
Department: Ambulatory Pediatrics
Center (if applicable): Center for Healthy Weight and Nutrition

**Collaborative Projects may list a Clinical Co-PI and a Lab Co-PI:**
Clinical Co-PI: Dr. Andrea Bonny
Position/Title: Assistant Professor of Pediatrics
Department: Adolescent Health
Center (if applicable): CCTR

Lab Co-PI: Position/Title: Department:
Center (if applicable):

**Clinical Research Mentor:**

**Location where research will be conducted:** Nationwide Children’s Hospital Clinical Research Unit and Ohio State University's Exercise Science Laboratory

**Assurances REQUIRED:**

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**Biostatistician Review**
date: 10/20/11 Name: Mahmoud Abdel-Rasoul

If this proposal was not reviewed by a Biostatistician, please provide justification below:

**Sponsored Projects Office Review**
date: 10/27/11 Name: Heidi Hallas

Are there any conflicts of interest or issues related to confidentiality with this project?

**No** or **Yes**, ________________________________

**ABSTRACT:** State the project’s broad, long term objectives and specific aims. Describe concisely the research design and methods for achieving these objectives. Avoid summaries of previous accomplishments and use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed clinical research project. If this application is funded, this description, as is, will become public information. Therefore, do not include any proprietary/confidential information. **DO NOT EXCEED 500 words.**
Obesity, even in children, is generally accompanied by a state of chronic inflammation. To combat childhood obesity, clinicians and scientists recommend lifestyle interventions that include increased physical activity and exercise in an attempt to promote weight loss and, consequently, decrease comorbidities associated with excess adiposity. More importantly, it appears that the influence of regular exercise may offer children with obesity a multitude of health benefits, independent of weight loss. However, the intensity of exercise required to elicit significant health benefits is still unclear. Therefore, the aim of the present project is to study the influence of high intensity interval exercise (HIIE) on the existing inflammatory state found in obesity. Specifically, the proposed project will examine endothelial function and markers of inflammation in children with obesity before and after an exercise intervention. The data will then be used to determine if changes in these values differ in magnitude based on the intensity of exercise. Children with obesity will be randomized into either moderate exercise or HIIE groups, and attend sessions 3 times per week for 6 weeks. The moderate group will cycle continuously for 30 minutes at 65%-70% of maximal heart rate and the HIIE group will perform ten, 2-minute bouts at 90%-95% of maximal heart rate. Outcome measures of body composition, aerobic capacity, blood lipids, glucose metabolism, endothelial function, and inflammation will be measured pre- and post-intervention. Results may help in establishing exercise protocols not only for children with obesity, but also other inflammatory diseases such as diabetes, cancer, and arthritis.

**KEY PERSONNEL:** Those who participate in the scientific development and execution of the project. Include name, title, role on project, % effort

Brooke Starkoff, Ph.D. student- research assistant, WOC 25%- data collection, analysis and interpretation, exercise testing and intervention implementation (stipends agreement being addressed)

Eneli Ihuoma, MD, WOC PI- 5%- Project oversight, patient recruitment

Andrea Bonny, MD, WOC Co-PI- 5%- patient recruitment

**COLLABORATORS/OTHER STUDY STAFF:** Include their name, title and role on the project.

Steven T. Devor, Ph.D., FACSM, WOC, 10%Associate Professor, Advisor/Mentor- project oversight, manuscript preparation

**BUDGET:** List total amount requested per category, with total budget requested at the bottom. In addition, give justification for each budget item in each category:

*Collaborators/Other Study Staff- define their role and explain why they are qualified to do the work. Justify the time assigned to the project by explaining how that time will be spent. If support personnel are listed without compensation, list the source of funding. RI funds will not support any portion of resident/fellow salary.*

CRN hours – nursing time to run labs and VOP- ($21.63/half hour x 2 visits x 36)= $1557
Consortium/Contractual Costs/Consultants: Investigators or personnel from other institutions may be listed as a consultant. Any non-NCHI/RI investigator requires a subcontract with that person’s institution. Costs proposals from that institution will be required. Explain what role they will play, hourly rate and number of hours requested.
N/A

Statistician Time: ($95-$125 per hour for OSU; $75 per hour for on-site Biostats Core. It is suggested that an estimate is obtained prior to submission)

Initial: Free service through OSU’s Center for Clinical and Translational Science
Interim: Statistics independent study program (Stat 893)- 15 hours of consultation per quarter
Final: Statistics independent study program- 15 hours of consultation per quarter

Additional Space/Equipment: Approval of additional space must be obtained from administration. RI internal grants are not to be used to purchase major equipment (anything >$5000). Leases or lease to purchase agreements are not permitted. Standard rental agreements may be permitted on a case by case basis. It is strongly suggested that investigators consult with Grace Wentzei, Kathy Milem and/or Aaron Ufferman.
N/A

Supplies-
Heart rate monitors- 12 x $54= $648
Heart rate monitor shipping- $25

Travel- Internal funding can only be used for travel costs associated with consultants; not study personnel.
N/A

Subject Care Costs- List all lab tests, inpatient and/or outpatient procedures. All subject related expenses including parking, travel, lodging or incentives should be listed under other expenses.

Blood draw- Lipids, glucose, insulin, adiponectin, high sensitivity C-reactive protein, tumor necrosis factor-alpha, interleukin-6, von Willebrand antigen, endothelin-1 (pre and post x 36)= $11,785

Other Expenses-
Incentives for children- $5 Dick’s Sporting Goods Gift cards- 1 per week for 6 weeks x 36= $1,080
Incentives for parents- $175= $40 for completing first assessment, $60 for completing 6 week intervention, and $90 for completing second assessment x 36= $6,300
Parking at The Ohio State University- $4 parking pass x 20 visits x 36= $2,880
Parking at Nationwide Children’s Hospital- $2 x 2 x 36= $144

TOTAL AMOUNT OF BUDGET: $25,000
TOTAL AMOUNT REQUESTED: $24,959

If there is a difference between the amount of the budget and the amount requested, please explain how the difference will be covered.

For those proposals recruiting adult participants only, please make a link to how this project will have a direct implication for pediatric health.

For those applicants who are not required to complete the Research Release Time application, please provide information on your plans for future research as a result of this proposal, manuscript submission and/or plans for external funding.
Objective and Specific Aims

The objective of the proposed study is to examine the effects of high intensity interval exercise (HIIE) on the existing inflammatory state found in obesity. Our working hypothesis is that, compared with obese children prescribed moderate exercise, obese children prescribed HIIE will demonstrate greater improvements in endothelial function and inflammatory markers. There is a significant amount of literature highlighting the anti-inflammatory effects of exercise on children with obesity (100, 122, 123, 146, 160, 192, 205, 215). Specifically, we will examine endothelial function in children with obesity to assess the improvement in vasomotor capability of the vasculature following 6-weeks of exercise training to determine long-term effects of HIIE. Identification of the health benefits of specific exercise programs are essential in establishing protocol to provide clinical guidelines for children with other inflammatory diseases as well as to aid in implementing regular exercise programs during the school day for all children.

We plan to conduct a prospective study on a cohort of 36 children ages 8-18 which includes a 6-week intervention consisting of moderate and HIIE. Our specific aims include:

1. To assess endothelial function measured as forearm vascular resistance (FVR) via venous occlusion plethysmography (VOP) in children with obesity before and after exercise training;
2. To measure markers of inflammation (hsCRP, IL-6, TNF- α, and adiponectin) in children with obesity before and after exercise training;
3. To determine if changes in endothelial function and markers of inflammation (hsCRP, IL-6, TNF- α, and adiponectin), following exercise training, differ in magnitude based on the intensity of exercise (moderate intensity vs. high intensity interval).

To our knowledge, only one study has used VOP to assess endothelial function in children with obesity following an exercise intervention and none have measured the effects following HIIE. It is plausible that the increased shear stress associated with HIIE can induce even greater anti-inflammatory effects than what has been found with moderate exercise. We will test the specific hypotheses that, compared with children with obesity in the 6-week moderate exercise group, the children with obesity in the 6-week HIIE group will demonstrate: 1.) improved endothelial function; 2.) greater increases in the anti-inflammatory marker adiponectin; and 3.) greater decreases in the pro-inflammatory markers TNF- α, IL-6, and hsCRP.

Background and Significance

The dangers of obesity in childhood have become glaringly obvious. Recently, a plethora of research has recognized an increased risk of morbidity and mortality among children with obesity compared with their normal weight peers (48, 71, 75, 157, 184). Specifically, a recent study by Franks et al. (71) concluded that children who are obese are the first generation that will not live longer than their parents...
and, in fact, **are 2 times more likely to die before the age of 55.** While many contributing factors are responsible for this epidemic, positive energy balance is, without question, a key variable. The American College of Sports Medicine recommends children 6-18 years old accumulate at least 60 minutes, and up to several hours, of moderate to vigorous daily physical activity. Yet, some research indicates that 58% of children (ages 6-11) fail to attain the recommended amount of physical activity, a pattern that continues to decline rapidly through adolescence (195). While inactivity is not uncommon in healthy weight and obese children alike, the dangers of future health complications are greater in the obese population.

**Obesity & Inflammation**

Excess adipose tissue that accompanies obesity contributes to the amount of circulating concentrations of adipokines, which are responsible for a myriad of biological processes. Specifically, tumor necrosis factor-alpha (TNF-α), adiponectin, and interleukin-6 (IL-6) have been implicated as major contributors to the inflammatory state found in obesity (75, 102, 122). These inflammatory markers are capable of triggering other pro-thrombotic mediators, such as C-reactive protein (CRP), which may ultimately induce cardiovascular disease (CVD) (146).

The links between childhood obesity, CVD, and inflammation have been documented in recent research (19, 75, 100, 102, 146, 157). In a study comparing obese to healthy weight children, the obese group was found to have significantly higher values of the pro-inflammatory markers high-sensitivity C-reactive protein (hsCRP) and TNF-α, and lower values of the anti-inflammatory marker, adiponectin (75). Other studies have also shown this link between increased pro-inflammatory and decreased anti-inflammatory markers in childhood obesity (67, 100, 102, 133, 158, 176). However, whether this inflammatory state is initiated solely by obesity or if physical inactivity also plays a role is not well understood (85, 123).

The condition of inflammation can be destructive when chronic, as is the case with obesity. It has been suggested this state of inflammation in children with obesity is responsible for early endothelial damage and arterial abnormalities, setting the stage for CVD in adulthood (48, 75, 102, 157). The effects of childhood obesity on CVD have been assessed by endothelial response to internal and external stimuli. As blood flow in the vessels increases due to physical or chemical stimuli, the endothelium is responsible for self-regulating vasomotor tone to adjust the flow and distribution of blood through the lumen, a process that can be measured as forearm vascular resistance (FVR). FVR, as measured via venous occlusion plethysmography (VOP), is a non-invasive tool to assess endothelial function. Vascular resistance (r) is determined in the equation \( r = p/q \), where \( p \) is blood pressure and \( q \) is forearm blood flow. Previous studies have used VOP in children to identify changes to the vascular structure due to many risk factors including diabetes and obesity (130, 160).

**Exercise and Endothelial Function**
Regular participation in exercise has been shown to increase anti-inflammatory mediators and decrease pro-inflammatory markers. A significant number of studies also show that regular exercise improves endothelial function (100, 123, 192, 205) and that continuous training is necessary to maintain vascular and anthropometric benefits (205). Exercise causes an increase in blood flow across the endothelium, which leads to shear stress mediated upregulation of nitric oxide synthase expression. The increased nitric oxide (NO) bioavailability thus allows for more efficient vasodilation and improved blood flow (100). In fact, Tjonna et al. (192) demonstrated that aerobic exercise improved HDL, blood glucose, and insulin, all of which directly influence NO bioavailability (192). It, therefore, seems crucial to use exercise as a tool to improve endothelial function in children with obesity.

Multidisciplinary interventions are popular methods for improving the health of children with obesity. Woo et al. (215) compared the effects of a dietary intervention alone to a dietary intervention plus exercise treatment group for 6 weeks. While the dietary intervention helped improve vascular function in overweight and obese subjects with initially impaired endothelial function, the effects were even greater in the group receiving diet and exercise treatment. In addition, subjects in the diet plus exercise group who continued to exercise for up to one year, continued to improve endothelial function as compared to those who discontinued the exercise program and concentrated solely on dietary modifications (215).

Watts et al. (205) also demonstrated an improvement of endothelial function in children with obesity with exercise therapy alone and independent of changes in BMI (205). Their results demonstrated improved endothelial function in children with obesity following an 8-week circuit-training program, compared with the non-exercise group. These subjects also demonstrated normalized vessel function following the exercise program (205). While the research has demonstrated the effectiveness of exercise in reducing inflammation and cardiovascular risk in children with obesity, it is not clear what appropriate intensity is required to elicit these changes.

**Moderate and High Intensity Interval Exercise (HIIE)**

Due to the overwhelming dangers of obesity many children now face, determining effective frequency, intensity and duration of exercise has become increasingly important. While many interventions include an exercise component, there is a lack of clarity concerning the proper intensity of exercise required to produce the greatest health benefits. In adults, both moderate and HIIE have been shown to produce significant cardiovascular benefits, however, the improvements appear to be greater with higher intensity exercise. HIIE has been found to reduce inflammation and improve endothelial function in adult populations, often times more significantly than moderate or lower intensity exercise (192).

**Conclusion**
To date, there have been no studies that have investigated the effects of HIIE on inflammation and endothelial function in children with obesity. Much of the research has focused on interventions that include diet, exercise and/or behavior modification to treat childhood obesity (156, 177). The proposed investigation differs in that it seeks to find an appropriate exercise intensity that will help attain optimal cardiovascular benefits. The knowledge gained from this study can aid in the implementation of new physical activity and physical education policies for local schools.

Our team of pediatricians and exercise physiologists will work to identify the exercise intensity that elicits the greatest health benefits. These results can then be used to create an exercise protocol for other pediatric populations with inflammatory diseases such as diabetes, cancer, and other metabolic, pulmonary, cardiovascular, and renal disorders. All results will be written up in manuscript form in an attempt to educate those involved in the fight to protect children with obesity and other inflammatory diseases. Ultimately, we anticipate results that will elicit further funding to not only aid in implementing specific exercise programs within the school districts, but also establish protocols to utilize in clinical settings.

**Experimental Design and Methods**

**Study Subjects:** Subjects will be children (aged 8 – 18) with obesity (defined as BMI ≥ 95th percentile for age and sex as defined by the Centers for Disease Control). Using a mean percent change FVR from previous studies of 29.4% for moderate intensity exercise and estimating a 38.3% change with HIIE, we desire a final sample size of 12 subjects into each group for a total of 24 subjects. This will allow us to detect a 10% difference between moderate and HIIE groups with 80% power. Based on previous studies implementing a pediatric weight management intervention, we anticipate a 46% attrition rate and, therefore, plan to recruit 36 subjects to achieve our desired final sample size.

**Exclusion Criteria:** Subjects will be excluded for active participation in ≥30 minutes of vigorous exercise more than 2 days per week, participation in an organized combined diet/exercise weight loss intervention, chronic disease known to affect inflammation (e.g. lupus), and any renal, heart, or liver disease. Subjects will be randomized into moderate aerobic exercise and high intensity interval aerobic exercise groups.

**Recruitment Procedures:** Subjects will be recruited from 2 sites; Nationwide Children’s Hospital’s (NCH) Center for Healthy Weight and Nutrition and the Adolescent Medicine Clinic at NCH. Eligible subjects will be identified by their clinician during regularly scheduled clinical visits. Interested subjects will be provided with detailed study information and study protocol from a nurse recruiter or study investigator. Written informed consent will be obtained from one legal guardian and written informed assent will be obtained from all children.

**Study Methods:** Study participants will undergo a series of lab visits at Nationwide Children’s Hospital and Ohio State University both pre- and post-intervention.
Visit one will take place at the NCH Clinical Research Unit (CRU). Subjects will report to the CRU in the morning within 1 month prior to the exercise intervention for a fasted blood draw and assessment of endothelial function. Fasted blood draw will be performed to obtain lipids (total cholesterol, LDL, HDL, triglycerides), glucose, insulin, high-sensitivity C-reactive protein, adiponectin, tumor necrosis factor-α, interleukin-6, von Willebrand antigen, and endothelin-1. Endothelial function will be measured as forearm vascular resistance via venous occlusion plethysmography.

After completion of the baseline study at NCH CRU, subjects will report to the W. Michael Sherman Exercise Physiology Lab at the Ohio State University to have anthropometric measurements taken including height, weight, waist to hip ratio, and body composition measured via BodPod. Immediately following anthropometric measurements, an assessment of aerobic capacity will be performed using the Åstrand Cycle Test, a submaximal exercise test on a cycle ergometer. Briefly, the subjects will complete 6-8 minutes of cycling at a moderate resistance.

Following completion of the baseline NCH CRU visit and the exercise assessment, subjects will be randomized to the moderate intensity or HIIE group. Subjects will be randomized into one of two groups via random number generator. Both groups will participate in a 6-week exercise intervention, 3 days per week at the Ohio State University’s Physical Activity and Education Services building. For both groups, the intervention will be performed on a cycle ergometer and will consist of a five-minute warm-up at 50-55% of the subject’s maximal heart rate as determined by the initial fitness assessment. Following the warm-up, the moderate group will cycle for 30 minutes at 65-70% of maximal heart rate. The HIIE group will perform 10, two-minute bouts at 90-95% of maximal heart rate, with one minute of active recovery at 55% of maximal heart rate between each interval for a total of 30 minutes. Both groups will complete a 5-minute cool-down at 50-55% of maximal heart rate. Heart rate will be measured via individual heart rate monitors.

A repeat visit to the NCH CRU for fasting blood draw and assessment of endothelial function will occur within two weeks following the end of the exercise intervention. A repeat visit to OSU’s exercise physiology lab for a follow-up assessment of aerobic capacity and anthropometric measurements will also occur within the same time-frame. Subjects who attend <14 exercise visits (75%) will be considered dropouts and their data will not be included in the results.
**Statistical Analysis:** The primary outcomes are the percent change in FVR and in inflammatory markers (hsCRP, IL-6, TNF-α, and adiponectin) from pre- to post-intervention in both groups. Secondary outcomes include percent difference in these changes between the moderate and high intensity interval groups.

All analysis will be conducted using SPSS Version 19. Descriptive statistics (means, medians, percentiles, ranges, etc.) will be calculated and provided for all outcome variables and demographics. Paired t tests will be performed to compare pre- and post-intervention mean levels of inflammatory markers and endothelial function. Initial comparison of measures between the two intervention groups will be measured using t tests. In final modeling, a two-way ANOVA with repeated measures will be performed to test the differences between subjects in the moderate and high intensity interval groups.
Appendix B: Award Letter
March 12, 2012

Dr. Eneli
Department of Ambulatory Medicine
700 Children’s Drive
Columbus, Ohio 43205

Dear Dr. Eneli:

Congratulations! You have been awarded $24,959.00 from internal funds to support your proposal entitled, “Children Active to Stay Healthy (CASH)”. You have also been awarded 5% Research Release Time to complete this project. Clinical and Translational research continues to be a top priority at Nationwide Children’s. We are very encouraged to see a proposal like yours and to support not only your academic growth and development, but also the growth of the institution’s research programs.

Below you will find important administrative information that you will need in order to make your proposal successful. You are encouraged to read through this letter and ask any questions that you might have regarding implementation and execution of the funding.

The award period for this intramural grant is April 1, 2012-March 31, 2014.

A. These funds can be accessed through a grant # once it has been awarded in the eTRAC system. Your sponsored projects officer (SPO) will be able to give you this number. If you have not worked with a SPO before, one will be assigned to you for this proposal. In addition, you or someone from your staff, should contact Rhonda Francies at x22793 for access to Navision (the system you will need to use to request checks or purchase supplies etc.). The grant number should be used on all correspondence referring to this project. Please review your grant funds regularly to ensure the grant does not go into deficit.

B. There are some minor restrictions of the program that you should be aware of:

1) A PI may only hold one intramural grant at a time.
2) If you should receive external funds to support this work, they will offset your intramural award, and the unused dollars will return to the Research Institute.
3) If your project should have a major change in its scope of work (study population, additional procedures/visits, increase in risk, randomization procedures, etc.), a revision to your application should be sent to the Committee as what you are actually doing would differ from what is funded.
4) The funds are to be expended in accordance with the budget approved by the Research Institute Administrative Council.
5) All purchasing expenditures related to this project must comply with pertinent Nationwide Children’s Hospital, Inc. and Research Institute policies and procedures.
6) All personnel issues related to this project are subject to applicable Nationwide Children’s Hospital, Inc. policies and procedures.
7) Publications resulting from work funded by an intramural grant should acknowledge the Institute’s support by appropriate citation.
8) If this research project involves the use of human subjects, written approval of the protocol and associated consent forms must be obtained from the Institutional Review Board prior to expenditure of any project funds.

9) You must immediately inform the Research Institute of any potential patents emanating from this project. Patent rights resulting from this work will be retained in accordance with the Patents and Copyright Policy for Nationwide Children's Hospital, Inc. and All Subsidiaries and Affiliates.

10) You also are responsible for informing your staff members of the contents of this policy.

C. Extensions: The funds must be expended within the 2 year project period. Unfortunately, we can not allow extensions as it impacts the amount of funding we have available for new proposals. At the end of the grant period, any remaining funds will revert back to the Research Institute. Remaining funds may not be transferred to another investigator or institution.

D. Progress Reports: Progress reports are required every 6 months, in January and July, regardless of award date. A final written report on the research project must be submitted to the Research Institute no later than three months after the termination date.

E. Salary support for project staff: Please ensure that any staff you have working with you on this project, that are paid from project funds have found alternative employment or are aware of the possibility of job termination, two months prior to the end of the project.

F. Presentation at Research Institute Seminar: All recipients of intramural funding are expected to present their proposal and/or their findings at a Research Institute seminar within 9-18 months of receipt of award.

By accepting this award, you agree to abide by the terms of Nationwide Children's Hospital, Inc.'s Policy and Procedures for Reporting Misconduct in Scientific Research, and indicates compliance with all above conditions.

We want to offer our Congratulations again! Your research is an important part of our mission as an Innovative and Agile Institution. Our hope for you is that this award will lead to the publication of at least one manuscript and submission of an extramural grant application if appropriate.

Sincerely,

Grace Wentzel, CCRP

Director,

Clinical Research Services
Appendix C: IRB Approval
July 25, 2012

Ihuoma Eneli
Ambulatory Pediatrics

Study ID: IRB12-00197
Study Name: CASH: Children Active to Stay Healthy

Dear Dr. Eneli,

The response to modifications requested, submitted on 7/19/2012, for the above study has been reviewed by the Institutional Review Board on 7/20/2012- STUDY APPROVED.

Date of Approval: 6/1/2012
Date of Expiration: 5/31/2013

This approval is for one year only. A Continuing Review Report must be approved before this study can proceed beyond the date of expiration. Please be aware that all changes to the research protocol consent form, or any other aspect of this study must receive prospective IRB approval. IRB policy requires that provisions are made for assent of subjects age nine and older.

The Federalwide Assurance number assigned to the IRB at Nationwide Children's Hospital, Inc is FWA00002860.

If we can provide additional assistance, please do not hesitate to call this office at ext. 22708.

Sincerely,

Grant Morrow III, MD, Vice-Chair
Institutional Review Board

Important Warning: If the reader of this message is not the intended recipient you are hereby notified that any dissemination, distribution or copying of this information is STRICTLY PROHIBITED.
Appendix D: CASH Informed Consent
CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

STUDY TITLE: CASH- Children Active to Stay Healthy
PRINCIPAL INVESTIGATOR: Ihuoma Eneli, MD, MS
CONTACT TELEPHONE NUMBER: 614 722-3591

SUBJECT’S NAME: __________________ DATE OF BIRTH: ________________

NOTE: The words “you” and “your” are used in this consent form. These words refer to the study volunteer whether a child or an adult.

1) INTRODUCTION

We invite you to be in this research study because your child’s weight was found to be above the normal range for their age.

Participation is voluntary. Please learn enough about this research study, its risks and benefits, to decide whether you should agree to participate. We will explain the study to you, and give you a chance to ask questions about anything you do not understand. This process is called “informed consent”. It is up to you to choose if you want to be in this study. You may refuse to be in this study or quit this study at any time, and standard medical care will still be available here or at a doctor of your choice without a penalty or loss of benefits to you.

Before agreeing to participate, it is important to read and understand the study information in this consent form. By signing the consent form, you agree to be in this study.

If this study involves a child between 9 and 18 years of age, he/she must also agree to be in the study by signing an Assent form or on the assent line of this form.

You will be given a signed and dated copy of the consent and the assent form.

2) WHY ARE WE DOING THIS RESEARCH STUDY?

Many children who are obese have poorer heart health than non-obese children. Regular exercise has been found to improve heart health and reduce the risk of heart disease. The goal of this study is to see which intensity of exercise provides the greatest improvement in heart function.

We are doing this study to look at the effects of different exercise intensities on heart health. We are looking at 2 different intensities of exercise on a stationary bicycle. The first group will be moderate intensity exercise. The second group will be high intensity interval exercise.

3) WHERE WILL THE STUDY BE DONE AND HOW MANY SUBJECTS WILL TAKE PART?
This study will be done at Nationwide Children’s Hospital and at the W. Michael Sherman Exercise Physiology Laboratory at The Ohio State University. We hope to enroll about 54 subjects. There will be 27 subjects in each group.

4) **WHAT WILL HAPPEN DURING THE STUDY AND HOW LONG WILL IT LAST?**

The study will take place over a 6-month period. For this study you and your child will be asked to attend a total of 4 evaluation visits; 2 visits in the Clinical Research Unit (CRU) at Nationwide Children’s Hospital and 2 visits in the W. Michael Sherman Exercise Physiology Laboratory at The Ohio State University.

Your child will participate in an exercise program over 6 weeks.

Two evaluation visits will take place before the exercise program and the remaining two evaluation visits after the exercise program.

**Evaluation visit 1 and 4.**

You and your child will report to the W. Michael Sherman Exercise Physiology Laboratory at The Ohio State University. This visit will take approximately 2 hours and the following things will be done.

- **You and your child will fill out questionnaires about your child’s exercise habits.**
- **Your child will have their height, weight, waist and hip size measured.**
- **Your child will have a BodPod test. The BodPod is an egg shaped box that your child will sit in to measure muscle mass and body fat. Your child will sit quietly in the BodPod in fitting clothing, like a bathing suit and swim cap. The test will last no more than 5 minutes.**
- **Your child will be asked to participate in a fitness test on an indoor stationary bicycle. Your child will be given a heart rate monitor and will pedal for 6-8 minutes. The first 3 minutes will be at a moderate pace, and the pace may be slightly increased for the last 3-5 minutes. Your child should bring gym shoes and exercise clothes for this test.**
- **Your child will be given an accelerometer to wear for one week. An accelerometer is a small device that will be worn around your child’s waist like a belt. It will tell us how much your child moves during the week. These will be returned to the study staff after the week is over.**

Your child will complete the same procedures at Visit 4 within one month after the exercise intervention.

**Evaluation visit 2 and 3**

You and your child will spend about 2 hours at the Nationwide Children’s Hospital Clinical Research Unit. This visit will begin early in the morning and your child must not eat or drink anything except water after midnight the night before (about 8-9 hours of fasting). This fasting is needed for the blood tests that will be done to check for high cholesterol, diabetes, and inflammation.

- **About 10 ml (about 2 teaspoons) of blood will be drawn from a vein in your arm. The blood test will help the staff know your baseline levels of cholesterol, blood sugar, insulin, and other blood markers of inflammation so that they can check for potential health risks.**
• Your child will have his/her blood flow measured in their forearm. For this procedure your child will lie comfortably on their back in a bed. They will have a cuff (similar to a blood pressure cuff) wrapped around both their upper arm and wrist. An elastic wire will also be put around their lower arm. When the blood flow is being measured, the cuff on the wrist will be inflated to a high pressure to stop blood flow to their hand and the cuff on the upper arm will be inflated at a low pressure off and on every 15 seconds. This will be done for 2 minutes. The upper arm cuff will then be inflated to a high pressure to cut off blood flow to the arm for 5 minutes. The upper arm cuff will then be released and the cuffs will go up and down as before for 1 minute. This entire test will take 8 minutes and the upper arm cuff will stop blood flow to your child’s arm for 5 of these 8 minutes. The lower cuff will cut off blood supply to your child’s hand for 8 minutes. During the test another device called an “arterial tonometer” will be put on your child’s other wrist to sense their pulse and determine their blood pressure.

• Your child will have his/her arterial stiffness measured. This will involve holding a pencil-like device with the eraser end down against the artery in their wrist for approximately 1 minute. There is no pain associated with this measure.

Your child will complete the same procedure at Visit 3, within one month following the exercise intervention

**The 6-week Exercise Intervention**

Following visit 1 and 2, you and your child will attend exercise sessions at the W. Michael Sherman Exercise Physiology Laboratory at The Ohio State University 3 times per week for 6 weeks. Your child will be supervised at all times by a certified physical activity instructor.

Your child will be randomly selected (like drawing straws) to participate in one of 2 exercise groups. Neither you, your child, nor the study team will have any choice as to which group you are assigned. Each group uses a different style of exercise designed to improve your child’s health.

Your child will be given a heart rate monitor to wear during each session, which consists of a watch and a strap that goes around the chest. This will allow them and the study staff to read their heart rate and will help them stay within a safe and optimal range during the exercise.

**GROUP 1: Moderate Exercise:** Your child will begin the session with a 5-minute warm-up on an indoor stationary bicycle at a very light speed and no resistance. Your child will then pedal for 30 minutes at a moderate speed and resistance set by the study staff. Finally, your child will ride for another 5 minutes at a low intensity before completing the session.

**GROUP 2: HighIntensity Interval Exercise:** Your child will begin the exercise with a 5-minute warm-up on an indoor stationary bicycle at a very light speed and no
resistance. The high intensity cycling will involve your child pedaling for 2 minutes as fast as they can. After 2 minutes, your child can then pedal at a slow speed for 1 minute. Your child will perform a total of 10, two-minute intervals of high intensity cycling, with one minute of light cycling in between each interval. They will do this 10 times for a total of 30 minutes. Following the 30 minutes, your child will pedal at a slow rate again for 5 minutes to cool-down.

5) **WHAT ARE THE RISKS OF BEING IN THIS STUDY?**

Drawing blood by putting a needle in a vein may cause pain, lightheadedness and fainting, bleeding, bruising, or swelling at the puncture site. Infection is a rare possibility. Numbing cream may be used on the skin to decrease the discomfort, if needed. Although there are no known side effects from the numbing cream, skin irritation or an allergic reaction is possible. Using the numbing cream or spray may also increase the length of the procedure because of the time it takes the cream or spray to work.

The blood flow measurements may also cause discomfort in the arms, hands, and fingers, including tingling and numbness. The pressure your child will feel will be similar to the pressure felt during a routine blood pressure measurement. The feeling will go away when the pressure is released. If this becomes too uncomfortable, you or your child can ask the investigators to stop.

Your child may feel embarrassed wearing a bathing suit for the BodPod testing. The test is, however, done in a private room and your child will be given a robe to wear.

Your child may become tired and out of breath as a result of pedaling in the fitness test. He/she may also develop temporary soreness in the legs after exercising. This discomfort is generally reduced as time goes on. You will receive a guide sheet from the study staff about how to prevent and take care of these minor aches and pains.

If any of the symptoms listed above are severe, you must get medical help right away. If you are worried about anything while in this study, please call the Principal Investigator or study coordinator at 614-292-0458.

There may be other risks of being in this research study which are not known at this time.

6) **SPECIAL INFORMATION ABOUT PREGNANCY:**

If you are pregnant or become pregnant while taking part in this research, the study procedures may cause harm to your pregnancy or to your fetus that is currently unknown. Participation in this study will not be offered to females who are pregnant or breast-feeding. A pregnancy test will be done for any female who is sexually mature enough (started having periods), to become pregnant. The pregnancy test may be done using urine. You (and your parents) will be told the results of the pregnancy test.
Pregnancy should be avoided, and an effective method of birth control must be practiced during the whole study. The best way to avoid pregnancy is abstinence (not having sexual intercourse).

If at any time, there is a suspicion of pregnancy, you must call the Principal Investigator or study coordinator right away. In the case of pregnancy during this study, participation in the study will be terminated.

7) ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

Possible benefits to you might be weight loss, improved cholesterol, improved blood sugar and/or insulin levels, improved heart health and function, and decreased inflammation. And, we might learn something that could help others.

8) WHAT OTHER TREATMENTS OR OPTIONS ARE THERE?

Your participation in this study is voluntary. It is not necessary to be in this study to get care for this condition. Other treatments such as other interventions, or self-managed diet and exercise are available. If you decide not to be in this study, the Principal Investigator will talk to you about other treatments or refer you to your regular doctor for care.

9) WILL THERE BE ANY COSTS TO ME?

It will not cost you anything to be in this study. For your time and inconvenience, you will receive a total of $150.00 broken into three payments; $25 for completing visits 1 and 2, $50 for completing the 6-week intervention (must be at least 14 visits), and $75 for completing visits 4 and 5. Your child will also receive a $5 gift card to Dick’s Sporting Goods for each completed week of the intervention. We will also pay for your parking at each study-related visit to Nationwide Children’s Hospital and The Ohio State University while you are in this study.

If your child does not attend a lab visit, you will not receive payment for that visit. Likewise, if your child does not attend at least 14 of 18 the exercise sessions, you will not receive payment for the intervention portion.

10) WHAT HAPPENS IF BEING IN THIS STUDY CAUSES INJURIES?

If your child is hurt by the procedures that are part of the study, you should seek medical treatment for the injuries and tell the study doctor as soon as possible at the number on the first page of this form. If it is an emergency, call 911 or go to the nearest emergency department.

In most cases, this care will be billed to your health insurance company or whoever usually pays for your health care at the usual charges, but some insurance companies will not pay for care related to a study. If the care is provided at Nationwide Children's Hospital, we make no commitment to pay for the medical care provided to you. No funds have been set aside to compensate you in the event of injury. If no one else pays for your care, you may have to pay for the cost of this care. This does not mean that you give up any of your legal rights to seek compensation for your injuries.
11) WHAT WILL HAPPEN IF NEW INFORMATION IS FOUND OUT ABOUT THE DRUG OR TREATMENT?

If new information is found out during this study that might change your mind about participating or might affect your health, a study staff member will discuss it with you as soon as possible.

12) WHAT HAPPENS IF I DO NOT FINISH THIS STUDY?

Your child’s participation in this study is voluntary and you may stop your child’s participation at any time. If you decide to stop your child’s participation, you must call the study staff. There will not be a penalty or loss of benefits to which your child is otherwise entitled.

If at any time the study doctor believes participating in this study is not the best choice of care, the study may be stopped and other care prescribed. If the study instructions are not followed, participation in the study may also be stopped. If unexpected medical problems come up, the study doctor may decide to stop your child’s participation in the study.

13) OTHER IMPORTANT INFORMATION

It is important that health care providers know about all medicines that you are taking. This includes the medicine being tested in this research study. Because of this, we plan to tell your primary care doctor (if you have one) that you are in this research study. This is done so care can be taken in prescribing other medicines and looking at any unexplained symptoms that may occur. You cannot take part in this study if you do not want us to tell your primary care doctor.

Also, being in more than one research study at the same time may cause injury. Please tell us if you are in any other research study so a decision can be made about being in more than one study at the same time. We may need to notify the other study team to see if you can participate in this study.

If you are an employee of Nationwide Children’s Hospital or the Research Institute at Nationwide Children’s Hospital, your job or performance appraisal will not be affected in any way if you decline to participate or withdraw your consent to participate in this study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Web site at any time.

If you are interested, the final study results will be shared with you once they are available. Please provide us with an email or address where we can send these results.

Nationwide Children’s Hospital is a teaching hospital and we are committed to doing research. Doing research will enable us to learn and provide the best care for our patients and families. You may be asked to participate in other research studies in the future. You have the right to decide to participate or decline to participate in any
future studies. We will not share your contact information with researchers outside Nationwide Children’s Hospital.

14) HOW WILL MY STUDY INFORMATION BE KEPT PRIVATE?

Information collected for this study will be kept confidential to the extent allowed by law. Information used and/or disclosed (shared with someone outside of Nationwide Children’s Hospital) may include information that can identify your child. This is called “protected health information” or PHI. By agreeing to be in this study, you are giving permission or authorizing the study doctor and his study staff to collect, use, and disclose your child’s PHI for this research study. Information collected is the property of the study doctor and the study sponsor. In the event of any publication regarding this study, your child’s identity will not be revealed.

If your child has a bad outcome or adverse event from being in this study, the study doctor and staff or other health care providers may need to look at your child’s entire medical records.

During participation in this research study, the study doctor and staff may review, collect, create, and use, personal health information, such as:

- Demographic Information (Names, Addresses, Telephone/Fax numbers, E-mail Addresses)
- Dates (such as admission/discharge, birth/death)
- Identifying numbers, characteristics, or codes (for example medical records, health plan or beneficiary numbers, account numbers)

Demographic information such as name, address and birth date is collected for registration purposes and to determine if your child is eligible to be in the study.

Some of the people or companies that may be authorized to use, disclose, and receive PHI collected or created about you are:

- The study doctor and the study staff
- Representatives of the Office for Human Research Protections (OHRP and other regulatory agencies within and outside the United States
- Members of Nationwide Children’s Hospital Institutional Review Board (IRB), a committee that reviews all human subjects research for Nationwide Children’s Hospital.
- Nationwide Children’s Hospital internal auditors

Because of the need to give information to these people, absolute confidentiality cannot be guaranteed. Information given to these people may no longer be protected by federal privacy rules.

Every effort will be made to keep your child’s protected health information (PHI) private. Your child’s PHI will be removed or coded (de-identified) as much as possible to protect your child’s privacy.
The PHI collected or created under this research study will be used/disclosed for the purpose of conducting this study as needed until the end of the study. The records of this study will be kept for an indefinite period of time.

You may decide not to authorize the use and disclosure of your child’s PHI, however, if it is required for this study, your child will not be able to be in this study. If you agree to allow your child to be in this study and later decide to withdraw, you may also withdraw your authorization to use your child’s PHI. This request must be made in writing to the study doctor. Please address this request to:

Ihuoma Eneli, MD  
Nationwide Children’s Hospital  
c/o Clinical Research Services  
545 South 18th Street, TH485  
Columbus, OH 43205

If you withdraw your authorization, no new PHI may be collected and the PHI already collected may not be used unless it has already been used or is needed to complete the study analysis and reports.

Dr. Eneli keeps a database of all subjects who participate in a research study. This database is used to contact people about future studies. Only Dr. Eneli and her staff have access to this database. The database will not be disclosed or sold to others outside Nationwide Children’s Hospital.

Please initial:

____ I want to be contacted about future research studies.

____ I do not want to be contacted about future research studies.

15) OTHER IMPORTANT INFORMATION

It is important that health care providers know about all medicines being taken.

Being in more than one research study at the same time may cause injury. You should tell the study doctor if your child is in any other research studies. The study doctor will decide if it is OK to be in more than one study at the same time.

The study doctor is being paid by the sponsor for the time and knowledge needed to do this study.

16) WHOM SHOULD I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you have questions about anything while on this study, you have 24-hour access to talk to your child’s study doctor. Please call: M-F 8-4:30: 614-722-4824; After hours: 614-292-0458

If you have questions, concerns or complaints about the research, questions about your child’s rights as a research volunteer, cannot reach the study doctor or want to call someone else, please call The Nationwide Children’s Hospital Institutional Review Board, (IRB, a committee that reviews all human research) at (614) 722-2708.
Subject’s Name __________________________ Date of Birth __________________________

SUBJECT or SUBJECT’S PARENT OR PERSON AUTHORIZED TO CONSENT ON BEHALF OF THE CHILD (SUBJECT TO THE SUBJECT’S GENERAL MEDICAL CARE)

I have read this consent form and have had a chance to ask questions about this research study. These questions have been answered to my satisfaction. If I have more questions about participation in this study or a research-related injury, I may contact the Principal Investigator. By signing this consent form, I certify that all health information I have given is true and correct to the best of my knowledge.

I have been given a copy of the Nationwide Children's Hospital Notice of Privacy Practices. I understand that my right to my patient information that is created or collected by Nationwide Children's Hospital in the course of this research can be temporarily suspended for as long as the research is in progress. I also understand that my right to access will be reinstated upon completion of this research.

I agree to participate in this study/I give permission for my child to participate in this study. I will be given a copy of this consent form with all the signatures for my own records.

Printed name of subject or subject’s legal representative
Signature of subject or subject’s legal representative

Date and time AM/PM

CAREGIVER PARTICIPATION CONSENT

As the caregiver of a child in this study, you are being asked to participate as well. The caregiver will be expected to come to each of the child’s study visits. Caregiver participation will take about 15 minutes. You will be asked to complete a questionnaire. These will have to do with your child’s exercise habits.

Printed name of caregiver (subject’s legal representative)
Signature of caregiver (subject’s legal representative)

Date and time AM/PM

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I have explained the research to the participant before requesting the signature above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

<table>
<thead>
<tr>
<th>Printed name of person obtaining consent</th>
<th>Signature of person obtaining consent</th>
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Date and time ___________________________ AM/PM
Appendix E: CASH Informed Assent
ASSENT TO PARTICIPATE IN RESEARCH

(FOR SUBJECTS 9 YEARS UP TO 18 YEARS OF AGE)

Study Title: Children Active to Stay Healthy (CASH)
Study Doctor: Ihuoma Eneli, MD, MS
Contact Number: 614 722-3591

Subject’s Name: ______________________  Date of Birth: ___________

You are being asked to be in a research study. Studies are done to find better ways to treat people or to understand things better.

• This form will tell you about the study to help you decide whether or not you want to volunteer to participate.
• You should ask any questions you have before making up your mind. You can think about it and discuss it with your family or friends before you decide.
• It is okay to say "No" if you don’t want to be in the study. If you say "Yes" you can change your mind and stop being in the study at any time without getting in trouble.
• If you decide you want to be in the study, an adult (usually a parent) will also need to give permission for you to be in the study.

1. What is this study about?

This study will help us understand how exercise makes some children healthier. We will be enrolling children with obesity to learn about how your health changes following an exercise program. The study involves a 6-week period where you will be enrolled to take part in either moderate intensity exercise (activity that will make you sweat and your heart beat hard) or high intensity interval (several two-minute periods of activity that will make you sweat and your heart beat hard followed by one minute of less vigorous activity). This study will be done at Nationwide Children’s Hospital and The Ohio State University.

2. What will I need to do (what will be done to me) if I am in this study?

This study involves 2 parts: Evaluation and intervention.

You will have 4 evaluation visits.

Visit 1 will be at the exercise lab at The Ohio State University. We will look at how tall you are, how much you weigh, and the distance around your hips and waist. You will sit in a big
egg-shaped box called a BodPod for 2-3 minutes. You will be wearing a tight fitting bathing suit and a swim cap while sitting in the BodPod. This will tell us how much muscle and fat your body has. You will also be asked to fill out a brief questionnaire about your exercising interests and habits. These questions will ask what kinds of activities you are involved in and how much time you spend with them during the week. You will also answer questions about your age, gender, and ethnicity.

After these measurements, you will then ride an indoor bicycle for 6-8 minutes. We will give you a heart rate monitor to wear around your chest that tells us how fast your heart is beating. The pedaling will start off fairly easy and, after 3 minutes, we may make it slightly easier, harder, or leave it the same.

For one week after visit 1 you will wear an accelerometer around your hip. It is a very small box you will wear like a belt that can tell us how much you move during that week.

Visit 2 will be at Nationwide Children’s Hospital in the morning for 2 hours. We will need to take some blood from your arm. We will use a numbing cream, so the blood test will not be very painful. You will also have a test done to look at how the blood flows in your arm. For this test, you will be lying in a bed while we put a cuff around your lower arm and another around your upper arm. An elastic wire will be put around your lower arm. The wrist cuff will go up and down to stop blood flow to your hand for a total of 8 minutes and the upper arm cuff will stop flow to your arm for 5 of these 8 minutes. These will squeeze your arm. If this becomes too uncomfortable you can ask the researchers to stop. A machine will be put on the other wrist to measure blood pressure. We will also measure how stiff your blood vessels are. You will hold something that looks like a pencil, with the eraser end down on your wrist for 1 minute. You will not feel any pain during this test.

After evaluation visits 1 and 2, you will return to The Ohio State University for a 6-week intervention of moderate or high-intensity interval exercise on an indoor bicycle. If you are in the moderate group, you will ride the bike at one speed for 30 minutes. If you are in the high intensity interval group, you will ride the bike as fast as you can for two minutes, followed by one minute of slower pedaling. You will then ride as fast as you can again for two minutes, followed by another minute of slower pedaling. You will do this for a total of 30 minutes.

Following the 6-week exercise intervention, you will return to Nationwide Children’s Hospital to repeat the lab work you did at visit 2. You will then also return to the Ohio State University to repeat the lab work you did at visit 1 before the exercise intervention.

You will also complete 2 short surveys about your physical activity. Before, during, and after the exercise portion you will also be asked to rate how your are feeling on a scale of very bad to very good.

3. How long will I be in the study?

The activity intervention lasts for 6 weeks, though it may take you extra time to schedule the 4 evaluation visits. Therefore, your participation in the study may last up to 3 months.
4. **Can I stop being in the study?**

You may stop being in the study at any time without getting in trouble.

5. **What bad things might happen to me if I am in the study?**

You may feel uncomfortable from the blood draw, which may cause some bruising, swelling, bleeding, soreness, or dizziness.

When we measure the blood flow in your arm you may feel uncomfortable pressure, tingling in your fingers, or discomfort in your arms, hands, and fingers. The feeling will go away when the pressure is released.

You may also feel embarrassed when filling out questionnaires about yourself or when wearing your bathing suit in the BodPod. The exercise may make you feel tired and your legs might get sore.

If you become pregnant while taking part in this research, we will have to discontinue the study. Participation in this study will not be offered to females who are pregnant or breastfeeding. A pregnancy test will be done for any female who is sexually mature enough (started having periods), to become pregnant. The pregnancy test may be done using urine. You (and your parents) will be told the results of the pregnancy test.

6. **What good things might happen to me if I am in the study?**

You may feel healthier and stronger after being in this study. You may lose weight and we may find that your blood and heart are healthier.

7. **Will I be given anything for being in this study?**

You will receive a gift card to Dick’s Sporting Goods for each week of the intervention that you complete. Your parent will also be paid for completing each section of the testing.

8. **Who can I talk to about the study?**

For questions about the study you may contact Brooke Starkoff, the research coordinator or Dr Eneli, the principal investigator at 614 722-3591

To discuss other study-related questions with someone who is not part of the research team, you may contact the Institutional Review Board Office (the group that reviews all human subject research) at 614-722-2708.
**Signing the assent form**

I have read (or someone has read to me) this form. I have had a chance to ask questions before making up my mind. I want to be in this research study.

---

**Signature or printed name of subject**

---

**Date and time**

**AM/PM**

---

**Investigator/Research Staff**

I have explained the research to the participant before requesting the signature above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

---

**Printed name of person obtaining assent**

---

**Signature of person obtaining assent**

---

**Date and time**

**AM/PM**

---

This form must be accompanied by an IRB approved consent form signed by a parent/guardian.
Appendix F: CASH Flyer
Are you looking for ways to increase your EXERCISE & HEART HEALTH?

Researchers at Nationwide Children’s Hospital and The Ohio State University Medical Center are now recruiting for a new study called the Children Active to Stay Healthy (CASH) Study.

**You May Be Eligible If You Are:**
- 13-17 years of age.
- Above the normal weight range for your age (BMI ≥ 95th percentile for age and sex).
- Willing to participate in an exercise program, participate in 2 blood draws, and complete brief questionnaires.

*NOTE: Those who are pregnant or breast-feeding are not eligible to participate.*

Parents will receive free parking and will be compensated for their time. Participants will receive gift cards to Dick’s Sporting Goods for each completed week of the study.

If you are interested in hearing more about this study, please contact the study coordinator, Brooke Starkoff at (614) 722-3591 or by email at brooke.starkoff@nationwidechildrens.org.

Interested in research studies but don’t qualify for this one? Visit www.ResearchMatch.org OR Call (614) 293-HERO.

Nationwide Children’s
When your child needs a hospital, everything matters.

O”}

Wexner Medical Center
Appendix G: CASH Self-Administered Physical Activity Checklist (SAPAC)
### SELF-ADMINISTERED PHYSICAL ACTIVITY CHECKLIST (SAPAC)

#### SECTION A. GENERAL INFORMATION

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<table>
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<tr>
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<td>A 2. TODAY’S DATE:</td>
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<td>DAY OF WEEK:</td>
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<td>A 3a. ADMINISTRATOR’S INITIALS</td>
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</table>

A 4. DID YOU ATTEND SCHOOL YESTERDAY?

- NO………………….1
- YES……………….2

A 5. DID YOU PARTICIPATE IN PHYSICAL EDUCATION CLASS YESTERDAY?

- NO………………….1
- YES……………….2

A 6. IF YES, HOW MANY MINUTES LONG WAS PHYSICAL EDUCATION CLASS?

—————

A 7. DID YOU PARTICIPATE IN A BREAK YESTERDAY?

- NO………………….1
- YES……………….2

A 8. IF YES, HOW MANY MINUTES LONG WAS YOUR BREAK?

—————
### Section B. Activities

<table>
<thead>
<tr>
<th></th>
<th>A. Activity</th>
<th>B. Before School</th>
<th>C. None, Some, Most</th>
<th>D. During School</th>
<th>E. None, Some, Most</th>
<th>F. After School</th>
<th>G. None, Some, Most</th>
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<tbody>
<tr>
<td>1</td>
<td>Bicycling</td>
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<td>2</td>
<td>Swimming Laps</td>
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<td>Gymnastics: bars, beam, tumbling, trampoline</td>
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<td>4</td>
<td>Exercise: non-jumping - push-ups, sit-ups</td>
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<td>5</td>
<td>Exercise: jumping – jumping jacks, jump rope</td>
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<td>6</td>
<td>Weight lifting</td>
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<td>7</td>
<td>Basketball</td>
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<td>8</td>
<td>Baseball/Softball</td>
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<td>9</td>
<td>Football</td>
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<td>Soccer</td>
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<td>Volleyball</td>
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<tr>
<td>12</td>
<td>Skating: ice, roller, roller blade</td>
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<tr>
<td>13</td>
<td>Hockey: ice, floor, field</td>
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<td>14</td>
<td>Racket Sports: badminton, tennis, paddle ball</td>
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<td>15</td>
<td>Active Games: chase, tag, hopscotch</td>
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<tr>
<td>16</td>
<td>Outdoor Play: climbing trees, hide and seek</td>
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<td>17</td>
<td>Water Play: (in pool, ocean or lake) water skiing</td>
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<td>Combative: martial arts, competitive wrestling</td>
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<tr>
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<td>Dance</td>
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<td>Cheerleading</td>
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<td>21</td>
<td>Outdoor Chores: mowing, raking, gardening</td>
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<td>22</td>
<td>Indoor Chores: mopping, vacuuming, sweeping</td>
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<tr>
<td>23</td>
<td>Mixed Walking/Running</td>
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<tr>
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<td>Walking</td>
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<td>25</td>
<td>Running</td>
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<td>26</td>
<td>Other, Name of Activity</td>
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<td>27</td>
<td>Other, Name of Activity</td>
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<td>28</td>
<td>Other, Name of Activity</td>
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<table>
<thead>
<tr>
<th></th>
<th>Before School</th>
<th>After School</th>
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<tbody>
<tr>
<td>1</td>
<td>H. 1 Hours plus minutes</td>
<td>H. 2 Hours plus minutes</td>
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<tr>
<td>2</td>
<td>H. 3 Hours plus minutes</td>
<td>H. 4 Hours plus minutes</td>
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<tr>
<td>3</td>
<td>H. 3 Hours plus minutes</td>
<td>H. 4 Hours plus minutes</td>
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Appendix H: CASH Physical Activity Enjoyment Scale (PACES)
Please rate how you feel at the moment about the physical activity you have been doing.

**When I am physically active...**

<table>
<thead>
<tr>
<th>I enjoy it</th>
<th>1 2 3 4 5 6 7</th>
<th>I hate it</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel bored</td>
<td>1 2 3 4 5 6 7</td>
<td>I feel interested</td>
</tr>
<tr>
<td>I dislike it</td>
<td>1 2 3 4 5 6 7</td>
<td>I like it</td>
</tr>
<tr>
<td>I find it pleasurable</td>
<td>1 2 3 4 5 6 7</td>
<td>I find it unpleasurable</td>
</tr>
<tr>
<td>I am very absorbed in this activity</td>
<td>1 2 3 4 5 6 7</td>
<td>I am not at all absorbed in this activity</td>
</tr>
<tr>
<td>It's no fun at all</td>
<td>1 2 3 4 5 6 7</td>
<td>It's a lot of fun</td>
</tr>
<tr>
<td>I find it energizing</td>
<td>1 2 3 4 5 6 7</td>
<td>I find it tiring</td>
</tr>
<tr>
<td>It makes me depressed</td>
<td>1 2 3 4 5 6 7</td>
<td>It makes me happy</td>
</tr>
<tr>
<td>It's very pleasant</td>
<td>1 2 3 4 5 6 7</td>
<td>It's very unpleasant</td>
</tr>
<tr>
<td>I feel good physically while doing it</td>
<td>1 2 3 4 5 6 7</td>
<td>I feel bad physically while doing it</td>
</tr>
<tr>
<td>It's very invigorating</td>
<td>1 2 3 4 5 6 7</td>
<td>It's not at all invigorating</td>
</tr>
<tr>
<td>I am very frustrated by it</td>
<td>1 2 3 4 5 6 7</td>
<td>I am not at all frustrated by it</td>
</tr>
<tr>
<td>It's very gratifying</td>
<td>1 2 3 4 5 6 7</td>
<td>It's not at all gratifying</td>
</tr>
<tr>
<td>It's very exhilarating</td>
<td>1 2 3 4 5 6 7</td>
<td>It's not at all exhilarating</td>
</tr>
<tr>
<td>It's not at all stimulating</td>
<td>1 2 3 4 5 6 7</td>
<td>It's very stimulating</td>
</tr>
<tr>
<td>It gives me a strong sense of accomplishment</td>
<td>1 2 3 4 5 6 7</td>
<td>It does not give me a strong sense of accomplishment</td>
</tr>
<tr>
<td>It's very refreshing</td>
<td>1 2 3 4 5 6 7</td>
<td>It's not at all refreshing</td>
</tr>
<tr>
<td>I felt as though I would rather be doing something else</td>
<td>1 2 3 4 5 6 7</td>
<td>I felt as though there was nothing else I would rather be doing</td>
</tr>
</tbody>
</table>