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Time Course of Vascular Function changes Following an Acute Maximal Exercise Bout in Obese and Normal Weight Males

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TIME COURSE OF VASCULAR FUNCTION CHANGES FOLLOWING AN ACUTE MAXIMAL EXERCISE BOUT IN OBESE AND NORMAL WEIGHT MALES

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Abstract

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One of the earliest sub-clinical stages associated with atherosclerosis is endothelial dysfunction (ED), which has been shown to predict future cardiovascular events. Chronic exercise is thought to improve endothelium-dependent vasodilation; however, few studies have evaluated the effects of acute exercise on vascular function (VF). Moreover, studies evaluating ED following an exercise training program lack a standardized time frame in which to measure VF. Although most studies require subjects to abstain from exercise for 24 hours prior to any VF measure, no study to date has assessed VF longer than 24 hours after the cessation of exercise. Additionally, no studies have compared VF responses in

obese and non-obese individuals following acute exercise. **Purpose:** Therefore, the purpose of this study was to evaluate VF, as determined by the assessment of forearm blood flow (FBF) and vascular reactivity (VR) before and up to 48 hours after a single bout of maximal exercise in obese and non-obese males. **Methods:** Twelve obese (37.0 ± 1.1 kg/m^2) and twelve non-obese (21.9 ± 0.3 kg/m^2) males volunteered to participate. FBF was assessed before and during reactive hyperemia (RH). FBF measures were obtained prior to (PRE-E), immediately after (POST-E), and at 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after exercise. Total excess flow, calculated as the difference between baseline FBF and FBF during RH, was used as an indicator of VR. Blood samples were also obtained at each time point to evaluate the response of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), which are potential modifiers of VF. **Results:** Baseline FBF and FBF during RH were significantly ($P < 0.05$) increased in both groups POST-E before returning to baseline values by POST-1. VR was enhanced in both groups POST-E, although the magnitude of change was greater in non-obese males. VR was significantly ($P < 0.05$) increased in non-obese males POST-E and was not significantly ($P < 0.05$) reduced until POST-48. Concentrations of IL-6 and TNF-α were unchanged in response to exercise in non-obese and obese males. **Conclusions:** An acute bout of maximal exercise significantly increased forearm endothelium-dependent vasodilation in non-obese and obese males. Additionally, an increased reactive vasodilation was observed only in non-obese males following exercise. These results also suggest that in non-obese males, measurements used to verify improvements in VF following exercise training should be employed after a minimum of 48 hours following physical activity.

Introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death in the United States, and has a 50% prevalence risk after the age of 40 years. (Lloyd-Jones et al., 2004). Atherosclerosis is distinguished by increased thickening of the arterial wall, accounting for approximately three-fourths of all CVD deaths (Rosamond et al., 2007). The clinical signs of CVD are final expressions of a disease process that has been progressing for decades. One of the earliest sub-clinical stages in the atherosclerotic process is impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (ED) (Singhal, 2005). ED can be detected prior to the development of atherosclerotic plaques in both coronary and peripheral vessels and has been implicated in the pathogenesis of diseases such as hypertension, diabetes, coronary artery disease, peripheral artery disease, chronic heart disease, and chronic renal failure (Endemann & Schiffrin, 2004).

In healthy individuals, the quiescent state of the vascular system is maintained by vasoactive compounds produced from endothelial cells, most notably nitric oxide (NO). NO bioavailability is regulated by nitric oxide synthase (NOS), primarily located within caveolae of the cellular plasma membrane (Boo & Jo, 2003). Shear stress, known as the dragging force of blood on the endothelial cell wall generated by flow, is one of the most

important mechanical regulators of endothelial NOS (eNOS) (Fisslthaler, Dimmeler, Hermann, Busse, & Fleming, 2000). Both laminar and turbulent shear stresses induce phosphorylation of eNOS, although direct regulatory mechanisms of specific kinases and phosphorylases have yet to be elucidated.

During endothelial cell activation an early biochemical marker is the loss of NO, initiating a chronic inflammatory response of the arterial walls. The most notable disruption in vascular homeostasis is an imbalance in the release of vasoconstrictor and vasodilator compounds, initiating ED. The bioavailability of this potent vasodilator, NO, is attenuated in many cardiovascular diseases such as hypertension, obesity, and atherosclerosis (M. Charakida et al., 2006).

In 1988, researchers described the link between increased risk of cardiovascular mortality and observed risk factors such as obesity, hypertension, insulin resistance, and hypercholesterolemia as syndrome X, better known as the metabolic syndrome (MetS) (G. Reaven, 1988). As a central causative component of MetS, obesity has been associated with several metabolic alterations (Hutley & Prins, 2005). The World Health Organization defines obesity as a condition of excess body fat to the extent that health is impaired (Obesity: Preventing and managing the global epidemic. report of a WHO consultation.2000). Body mass index (BMI, weight(kg)/height(m)^2) has a close correlation to body fat and obesity-related diseases and is therefore often used to assess obesity (Y. Wang & Beydoun, 2007). BMI cutoff points of 25 and 30 kg/m^2 are used to define overweight and obesity, respectively.

In 1994, adipose tissue was recognized as a major site for secretion of protein signals with the discovery of leptin, helping to establish white adipose tissue (WAT) as an endocrine organ. Obesity, which is directly related to an individual's WAT, has recently been characterized as a state of chronic low-grade inflammation. This review primarily focuses on two of the most frequently measured pro-inflammatory adipokines, TNF-α and IL-6, and their impact on endothelial function (EF).

Although elevated levels of TNF-α play a role in numerous functions, a primary target is endothelial membrane receptors, which stimulate biological activity suggestive of ED (Ritchie et al., 2004). TNF-α levels are inversely correlated to NO bioavailability (P. Wang, Ba, & Chaudry, 1994; Bhagat & Vallance, 1997). IL-6, another adipokine often expressed as a pro-inflammatory marker, has recently received a great deal of attention, in particular with its release from skeletal muscle and its role as a myokine. IL-6 is thought to negatively affect EF through an indirect pathway mediated by the stimulation of C-reactive protein (CRP) released from the liver (Ritchie et al., 2004). However, it has been proposed that the role of IL-6 is dependent upon its origin of secretion, such that release from adipose and skeletal muscle tissue may interact and oppose one another (Pedersen & Fischer, 2007b). IL-6 secreted from contracting skeletal muscle results in the activation of anti-inflammatory pathways (Febbraio & Pedersen, 2005).

The pathophysiology of ED includes an impairment of vasodilation initiated by a pro-inflammatory state. Measurement of specific adipokines and other pro-inflammatory laboratory markers are considered a good complement to the direct assessment of EF. Due to a strong association between ED and the initiating stages of atherosclerosis, as well as

clinical symptoms such as unstable angina and myocardial infarction (MI), the assessment of EF is crucial in targeting individuals with impaired vascular reactivity (Vita & Keaney, 2002). Coronary ED has been shown to be an independent predictor of atherosclerotic disease progression and cardiovascular event rates in individuals with coronary artery disease (CAD), as well as those without CAD (Halcox et al., 2002; Schachinger, Britten, & Zeiher, 2000). Since EF can be assessed years before any cardiovascular event, the need for a non-invasive measure of EF that can be used on multiple occasions during one's lifespan to determine the presence and effectiveness of treatment of atherosclerosis is appealing. Forearm blood flow (FBF), as assessed by venous occlusion strain-gauge plethysmography can provide non-invasive assessment of EF in peripheral vessels. The relationship between ED in peripheral arteries is well established and peripheral ED has also been shown to predict future cardiovascular events (Anderson et al., 1995; Kitta et al., 2009; Neunteufl et al., 1997; Shechter et al., 2007; Takase, Hamabe, Satomura, Akima, Uehata, Matsui, Ohsuzu, Ishihara, & Kurita, 2006; Tentolouris et al., 2004; Vita & Keaney, 2002; Yeboah, Crouse, Hsu, Burke, & Herrington, 2007).

To date, there are no known studies that have evaluated the prognostic impact of FBF assessed by strain gauge plethysmography in predicting the long-term outcome of individuals with and without CAD. However, strain gauge plethysmography has been used to demonstrate relationships among FBF and several cardiovascular disease risk factors (Ishibashi et al., 2006). Chronic pro-inflammatory conditions, such as obesity can be significant in the initiation of atherosclerosis. Due to ED being an early event in the progression of CVD, EF assessment could be of prognostic value since it reflects vascular

biology. If ED is associated with the pathogenesis of CVD, it is often proposed that reversal of ED will reduce the risk for CVD.

The beneficial effects of exercise on reducing risk for CVD are well known. Studies that have assessed the effects of exercise on ED have strengthened decisions to employ exercise training as an important therapy treatment for ED. As previously described, increasing shear stress on endothelial cells stimulates secretion of NO leading to smooth muscle cell relaxation and subsequent vascular dilation. Data suggest that regular exercise using a large muscle mass can induce adaptations in non-exercising limbs, which enhance EF (Kingwell, Sherrard, Jennings, & Dart, 1997). Important findings such as this justify the evaluation of FBF with common aerobic exercise modalities such as cycling and walking.

Many studies have shown an exercise training effect on EF with subjects who were at higher risk for CVD (Watts, Beye, Siafarikas, O'Driscoll et al., 2004; Woo et al., 2004; Hamdy et al., 2003; Schjerve et al., 2008; Higashi et al., 1999; Maiorana et al., 2001; Walsh, Yong et al., 2003). Interestingly, Green et al. (2003) investigated whether EF improvements observed with exercise resulted from short term repetitive exercise bouts or exercise associated improvements in CVD risk factor profiles (Green et al., 2003). The study suggested that these changes in EF are not exclusively related to the effect that exercise has on CVD risk factors, further demonstrating the impact exercise training has on the vascular system, with its frequent, periodic increase in shear stress.

Although most exercise training studies have evaluated subjects with an increased risk for CVD disease, a limited number of studies have evaluated the effect of exercise

training on EF in healthy subjects (Clarkson et al., 1999; Goto et al., 2003; Higashi et al., 1999; Rakobowchuk et al., 2008; Bergholm et al., 1999; Goto et al., 2003; Rakobowchuk et al., 2005). Moreover, these studies are not in complete agreement that EF is enhanced following exercise training in healthy individuals. Currently, studies appear to support an improvement in EF in healthy individuals following moderate-intensity aerobic exercise training. These positive changes in EF with moderate-intensity training are not well supported in high-intensity aerobic exercise training or strength exercise training models.

While most studies examine EF following exercise training, EF following acute exercise has not been extensively researched. Only a few studies have evaluated the effect of a single bout of exercise on EF in healthy individuals (Baynard, Miller, & Fernhall, 2003; Bousquet-Santos, Soares, & Nobrega, 2005; Rognmo et al., 2008). Although all of the studies demonstrated an increased EF measure immediately post-exercise, Bousquet-Santos et al. suggested a return of EF measures to baseline levels within 2 hours of exercise (Bousquet-Santos, Soares, & Nobrega, 2005). Previous studies have presented important findings in further understanding the effects of acute exercise on EF. However, an extensive review of literature has produced no study in which the extended time-course for EF following a single bout of maximal exercise is shown, especially in obese individuals. This is of primary importance considering a previous study has demonstrated an exercise-induced effect up to 24 hours of inflammatory biomarkers that may have an effect on the EF (Louis, Raue, Yang, Jemiolo, & Trappe, 2007). The impact of inflammatory markers post-exercise could be crucial in assessing EF post-exercise, where

there does not appear to be a standardized optimal time before EF is assessed following a training program's last exercise bout.

The critical effect of endothelial wall activation and its associated role in initiating atherosclerosis warrants further research of the mediating receptors and circulating agents that allow for the control of vascular homeostasis. Assessing EF and the biomarkers associated with its control is vital in understanding interventions that may help not only slow the progression of CVD but negate specific CVD risk factors. The underlying mechanisms that control EF changes following exercise training have received a large amount of attention and continue to be evaluated. PA has been shown as an important mediator for improving EF through the generation of shear stress. This study aims to investigate an extended time course of acute exercise-induced adaptation on EF. The time course of exercise induced adaptations on EF has been evaluated in only a few studies giving rise to the need for a similar study in healthy and obese subjects. Exercise training study results are inconclusive as to whether the exercise interventions improve EF or whether improvements in EF are simply a reflection of the alterations from the last exercise bout performed. Findings may help establish an optimal time point to assess EF following an exercise training program and also allow researchers to further understand the impact an acute exercise bout can have on regulating the vasodilatory capacity of the arterial wall.

Review of Literature

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death in the United States, and has a 50% prevalence risk after the age of 40 years. (Lloyd-Jones et al., 2004). Atherosclerosis is distinguished by increased thickening of the arterial wall, accounting for approximately three-fourths of all CVD deaths (Rosamond et al., 2007). Pathological changes in arteries that occur in children and young adults prior to the onset of adult clinical symptoms have indicated a long preclinical phase of atherosclerosis. The clinical signs of CVD are final expressions of a disease process that has been progressing for decades. It is now known that early life events have a lasting impact on the development of atherosclerosis and risk for CVD (Napoli et al., 1999; Singhal, 2005; Skilton & Celermajer, 2006).

One of the earliest subclinical stages in the atherosclerotic process is impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (ED) (Singhal, 2005). ED can be detected prior to the development of atherosclerotic plaques in both coronary and peripheral vessels and has been implicated in the pathogenesis of diseases such as hypertension, diabetes, coronary artery disease, peripheral artery disease, chronic heart disease, and chronic renal failure (Endemann & Schiffrin, 2004). Vascular homeostasis is regulated by the endothelium, a single layered paracrine organ lining the

blood vessels with a pleiotropic role consisting of modulating vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation (Deanfield, Halcox, & Rabelink, 2007). The earliest studies on the significance of endothelial cells investigated the production and release of compounds effecting vascular tone. In 1980, experiments of Furchgott and Zawadzki were the first to demonstrate the action of acetylcholine on endothelial cells, which released a substance targeting smooth muscle and initiated relaxation (Furchgott & Zawadzki, 1980). The biological activity of this endothelial-derived relaxing factor (EDRF) was later compared to nitric oxide (NO), where the EDRF and NO relaxation of bioassay tissues were shown to be significantly similar (Palmer, Ferrige, & Moncada, 1987). It was also suggested that both EDRF and NO were inhibited by oxyhemoglobin and stimulated by superoxide dismutase (SOD), thus providing evidential support in proposing that EDRF and NO were the same (Ignarro, Buga, Wood, Byrns, & Chaudhuri, 1987; Palmer et al., 1987).

MECHANISMS REGULATING ENDOTHELIAL FUNCTION

In healthy individuals, the quiescent state of the vascular system is maintained by vasoactive compounds produced from endothelial cells, most notably NO. NO bioavailability is regulated by nitric oxide synthase (NOS), primarily located within caveolae of the cellular plasma membrane (Boo & Jo, 2003). When activated, NOS mobilizes, converting molecular oxygen and L-arginine to NO and L-citrulline. Three tissue specific NOS isoforms (neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS)) have been identified. NO derived from eNOS is essential in regulating cardiovascular homeostasis (M. Charakida, Deanfield, & Halcox, 2006;

Forstermann & Munzel, 2006). Endothelium-derived NO diffuses into adjacent smooth muscle cells activating soluble guanylyl cyclase (sGC), which synthesizes cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) (M. Charakida et al., 2006; Murad, 2006). cGMP is an intracellular second messenger with many roles, one of which includes the activation of intracellular protein kinases. Activated protein kinases decrease the concentration of cytosolic calcium, which leads to the dephosphorylation of smooth muscle myosin light chain, thus vascular relaxation (Murad, 2006).

There are many eNOS agonists, which include humoral, mechanical, metabolic, and pharmacological stimuli. Initially, the proposed stimulation of eNOS consisted of a simple calcium (Ca^{2+}) dependent model, in which activation of eNOS was controlled by Ca^{2+}/calmodulin (CaM) binding (Fleming & Busse, 2003; Moncada & Higgs, 1993; Mount, Kemp, & Power, 2007). However, it has since been shown that a wide variety of eNOS stimuli regulate NO production through more complex pathways including protein-protein interactions and site-specific phosphorylation involving kinases and phosphorylases (Boo et al., 2002; Fleming & Busse, 2003; Mount et al., 2007). Shear stress, known as the dragging force of blood on the endothelial cell wall generated by flow, is one of the most important mechanical regulators of eNOS (Fisslthaler, Dimmeler, Hermann, Busse, & Fleming, 2000). Both laminar and turbulent shear stresses induce phosphorylation of eNOS, although direct regulatory mechanisms of specific kinases and phosphorylases have yet to be elucidated.

In brief, shear stress activates guanine nucleotide binding (G) protein's-coupled transmembrane receptor, which induces activation of phosphoinositide 3-kinase (PI3K).

PI3K indirectly activates 3-phosphoinositide-dependent protein kinase 1 and 2 (PDK1 and PDK2, respectively) (Boo et al., 2002; Sessa, 2004). PDK1 and PDK2 phosphorylate protein kinases responsible for phosphorylation of eNOS at specific amino acid sites. Thus far, five eNOS phosphorylation sites have been identified, the most notable recognized as serine 1177 (Ser^{1177}) (Boo & Jo, 2003; Mount et al., 2007). Other phosphorylation sites stimulated by shear stress include serine 114 (Ser^{114}) and serine 633 (Ser^{633}) (Mount et al., 2007).

Although NO is widely known for its vasodilatory effect, there are other actions elicited by NO production that are beneficial such as regulation of smooth muscle cell proliferation and inhibition of vascular platelet adhesion (de Graaf et al., 1992; Freedman et al., 1997; Ignarro et al., 2001; Michelson et al., 1996; Stein, Fabry, Murphy, & Hart, 1995). Along with impaired endothelium-dependent vasodilation, increased vascular smooth muscle cell proliferation is a familiar characteristic of atherosclerosis. Growth factors necessary for cellular reproduction include polyamines, such as putrescine, spermidine, and spermine. NO has been shown to inhibit ornithine decarboxylase, an enzyme responsible for converting the amino acid ornithine to putrescine (Ignarro et al., 2001). Research has also suggested that NO prevents thrombus formation through inhibition of platelet aggregation (de Graaf et al., 1992; Freedman et al., 1997; Michelson et al., 1996). NO production has also been shown to inhibit surface platelet P selectin (Freedman et al., 1997; Michelson et al., 1996). P selectin, a cell adhesion molecule, is secreted from activated platelets and is responsible for increasing platelet adhesion to monocytes and stimulating tissue factor (Freedman et al., 1997). Monocytes and tissue

factor can both exacerbate atherosclerosis by increasing arterial plaque and coagulation of blood, respectively.

During endothelial cell activation an early biochemical marker is the loss of NO, initiating a chronic inflammatory response of the arterial walls. The most notable disruption in vascular homeostasis is an imbalance in the release of vasoconstrictor and vasodilator compounds, initiating ED. The bioavailability of this potent vasodilator, NO, is attenuated in many cardiovascular diseases such as hypertension, obesity, and atherosclerosis (M. Charakida et al., 2006).

This review will focus primarily on adipokines and the impact they have in regulating NO bioavailability and thus vascular reactivity. Although reactive oxygen species (ROS) are not discussed in this review, they do exert a significant influence on NO bioavailability and future research in this area will be critical in helping clarify the relationship between NO and endothelial function (EF).

METABOLIC SYNDROME AND OBESITY

In 1988, researchers described the link between increased risk of cardiovascular mortality and observed risk factors such as obesity, hypertension, insulin resistance, and hypercholesterolemia as syndrome X, better known as the metabolic syndrome (MetS) (G. Reaven, 1988). Individuals diagnosed with MetS were identified for an early intervention in an attempt to decrease the development of type 2 diabetes or CVD (G. M. Reaven, 2005). Today, MetS is identified as having three of five criteria (abdominal obesity, impaired fasting glucose, high triglyceride and low HDL-cholesterol concentrations, and increased blood pressure) determined by the Adult Treatment Panel III of the National

Cholesterol Education Program (G. M. Reaven, 2005). Current data used for extrapolative analysis project that nearly 40% of the world's population will be affected by MetS by 2020 (New developments in metabolic syndrome. proceedings of the 1st conference of the paul hamel institute. 7-8 june 2004, monaco.2005). As a central causative component of MetS, obesity has been associated with several metabolic alterations (Hutley & Prins, 2005). While obesity is the primary focus of this review, each component of MetS has the potential to independently affect EF.

The World Health Organization defines obesity as a condition of excess body fat to the extent that health is impaired (Obesity: Preventing and managing the global epidemic. report of a WHO consultation.2000). Body mass index (BMI, weight(kg)/height(m)^2) has a close correlation to body fat and obesity-related diseases and is therefore often used to assess obesity (Y. Wang & Beydoun, 2007). BMI cutoff points of 25 and 30 kg/m^2 are used to define overweight and obesity, respectively. The prevalence of obesity has more than doubled in the last thirty years, accounting for approximately 32.4% of obese adults in the United States (Y. Wang & Beydoun, 2007). Using linear regression models, Wang and Beydoun suggested that by 2015 the prevalence of obesity in adults and adolescents will reach 40.8 and 22.7 percent, respectively (Y. Wang & Beydoun, 2007).

Until recently, white adipose tissue's (WAT) primary role was thought to be energy storage. In 1994, adipose tissue was recognized as a major site for secretion of protein signals with the discovery of leptin, helping to establish WAT as an endocrine organ. Over 50 protein signals are now known to be secreted from WAT and various functional roles have led researchers to collectively name them 'adipokines' (Trayhurn & Wood, 2004).

The pleiotropic role of adipokines has helped establish WAT's elaborate communication system with other tissues and organs. Obesity, which is directly related to an individual's WAT, has recently been characterized as a state of chronic low-grade inflammation. Several mechanisms have been proposed to help explain this phenomenon. These include: a) the elevation of obesity-related adipokines with proinflammatory functional properties (Fantuzzi, 2005; Lau, Dhillon, Yan, Szmitko, & Verma, 2005; Ritchie, Ewart, Perry, Connell, & Salt, 2004; Wisse, 2004), b) the reduced anti-inflammatory function of insulin as a result of the increased obesity related adipokines (Bruun, Verdich, Toubro, Astrup, & Richelsen, 2003; Caballero, 2003; Fantuzzi, 2005; Fasshauer & Paschke, 2003; Lau et al., 2005; Ritchie et al., 2004; Wisse, 2004), c) the increased production of free radicals as a result of oxidative stress (Ritchie et al., 2004), and d) the hypoxic condition of adipocytes as WAT increases disproportional to its vascular network (Trayhurn & Wood, 2004).

ADIPOKINES AND NITRIC OXIDE REGULATION

During the last 15 years, many pro-inflammatory adipokines have been discovered including interleukin (IL) 1βeta, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), and leptin (Trayhurn & Wood, 2005). This review will primarily focus on two of the most frequently measured pro-inflammatory adipokines, TNF-α and IL-6, and their impact on EF.

TNF-α is biologically active as a 17 kDa protein involved in the escalation of a pro-inflammatory state (Ronti, Lupattelli, & Mannarino, 2006). BMI and percent body fat are both positively correlated to plasma TNF-α (Ritchie et al., 2004). Although elevated levels of TNF-α play a role in numerous functions, a primary target is endothelial membrane

receptors, which stimulate biological activity suggestive of ED (Ritchie et al., 2004). TNF-α levels are inversely correlated to NO bioavailability. Wang et al. revealed that 2 hours of in vivo administered TNF-α in male Sprague-Dawley rats significantly decreased NO synthesis and release, leading to impairment of endothelium-dependent vasodilation for up to 80 minutes (P. Wang, Ba, & Chaudry, 1994). Bhagat et al. infused TNF-α for one hour in healthy males and females 19 – 40 years of age (Bhagat & Vallance, 1997). The researchers suggested that endothelium-dependent vasodilation was attenuated up to six hours. However, in this study, it is important to note that prior to six hours only one post TNF-α measurement was taken, which was obtained at one hour. Therefore, it is not known if the return to baseline endothelium-dependent vasodilation occurred prior to six hours. These findings suggest that TNF-α effects endothelium-dependent vasodilation in a time-dependent manner. Possible explanations for the depression in endothelium-dependent vasodilation with TNF-α include inhibition of receptor-mediated release of NO from endothelial cells, degradation of NOS mRNA, and increases in endothelin (ET) (Bhagat & Vallance, 1997; Ritchie et al., 2004; P. Wang et al., 1994). TNF-α has also been shown to stimulate adhesion molecules such as intracellular cell-adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Ritchie et al., 2004; Ronti et al., 2006). These adhesion molecules enhance monocyte attachment to the endothelial wall (Ronti et al., 2006). The monocytes then migrate to the subendothelial space where they become foam cells. Foam cells contain oxidized low density lipoprotein (LDL) and can lead to the development and progression of atherosclerosis.

ET is released from endothelial cells and binds to two receptor subtypes, ET_A and ET_B, located on smooth muscle cells, adjacent to the endothelial cells (Bohm & Pernow, 2007). The receptors are coupled to a G-protein, which stimulates the production of intracellular inositol triphosphate (IP_3). Subsequently, Ca^{++} is released from the sarcoplasmic reticulum, leading to phosphorylation of smooth muscle myosin light chain, thus vascular constriction. ET_B receptors can also be found on endothelial cells and when stimulated result in the production of cGMP and secretion of NO, leading to vascular relaxation (Bohm & Pernow, 2007). G-proteins can have different functional roles depending on the stimulation of various subunits. G-proteins are heterodimers made of α, β, and γ subunits, each with several isoforms (Neer, 1994; Oldham & Hamm, 2006). The various G-protein types give rise to a wide range of specific cellular responses from external stimuli. It has been suggested that the $G\alpha_s$ and $G\alpha_q$ families are specific in production of cGMP and IP_3, respectively (Neer, 1994; Oldham & Hamm, 2006). In a healthy vessel, the net balance effect of ET_A and ET_B activation is vascular relaxation. In a disease condition such as ED, the sensitivity of ET is much more pronounced, resulting in an upregulation of the vasoconstrictor ET_B receptor located on smooth muscle cells (Bohm & Pernow, 2007). ET has also been shown to stimulate the production of both TNF-α and IL-6, which in turn exacerbate the production of ET.

IL-6, another adipokine often expressed as a pro-inflammatory marker, has recently received a great deal of attention, in particular with its release from skeletal muscle and its role as a myokine. Although IL-6 is positively correlated with obesity and significantly decreases with weight loss, secretion from adipose tissue only accounts for ~10% of total

concentration (Ronti et al., 2006). Plasma IL-6 concentration is positively correlated with the development of CVD (Avogaro & de Kreutzenberg, 2005). Although IL-6 is thought to negatively affect EF, it has been suggested that this occurs primarily through an indirect pathway mediated by the stimulation of C-reactive protein (CRP) released from the liver (Ritchie et al., 2004). Recent evidence has shown IL-6 to be the only cytokine secreted from skeletal muscle and be released into circulation following exercise (Pedersen & Fischer, 2007a). IL-6 had previously been recognized as a pro-inflammatory cytokine that contributed to insulin resistance. Mechanistically, an exercise-induced increase in IL-6 failed to explain the increase in post-exercise insulin action. Researchers then began to suggest that the pro-inflammatory association with IL-6 was primarily due to positive correlations in cohort studies, animal studies, and *in vitro* studies that had supraphysiological concentrations, none of which best represent *in vivo* human physiological studies (Pedersen & Fischer, 2007b). It is important to distinguish between acute versus chronic secretion of IL-6 and different functional properties that may be associated with each. It has been proposed that the role of IL-6 is dependent upon its origin of secretion, such that release from adipose and skeletal muscle tissue may interact and oppose one another (Pedersen & Fischer, 2007b). IL-6 secreted from contracting skeletal muscle results in the activation of anti-inflammatory pathways (Febbraio & Pedersen, 2005). Muscle derived IL-6 is linked to an increase in well-known anti-inflammatory cytokines (e.g. interleukin 10 (IL-10) and interleukin 1 receptor antagonist (IL-1ra)) as well as inhibition of TNF-α. Whereas, IL-6 released from adipose tissue is

associated with an increase in the pro-inflammatory marker CRP (Avogaro & de Kreutzenberg, 2005; Pedersen & Fischer, 2007b; Ritchie et al., 2004).

CRP is thought to be one of the strongest independent predictors of CHD and is significantly correlated to body fat, MetS, and type 2 diabetes (Lau et al., 2005; Verma et al., 2002). CRP directly alters EF through augmented expression of adhesion molecules VCAM-1 and ICAM-1, as well as increasing secretion of ET (Lau et al., 2005; Pasceri, Willerson, & Yeh, 2000). CRP further exacerbates its production through a positive feedback mechanism in which it increases expression of IL-6. CRP has also been shown to down regulate eNOS mRNA, attenuating NO production (Verma et al., 2002). This impact is mediated through a reduction in eNOS mRNA half-life, which has been suggested to significantly decrease from 24 to 14 hours in plate grown endothelial cells treated with CRP.

ASSESSMENT OF ENDOTHELIAL FUNCTION

As previously discussed, the pathophysiology of ED includes an impairment of vasodilation initiated by a pro-inflammatory state. Measurement of specific adipokines, cellular adhesion molecules, and other pro-inflammatory laboratory markers are considered a good complement to the direct assessment of EF. Currently, there are three primary methods of EF assessment. These include coronary angiography, high-resolution vascular ultrasonography, and venous occlusion strain-gauge plethysmography.

Coronary Angiography

Direct measurement through coronary angiography is an invasive *in situ* test that poses high risk for cardiovascular events such as myocardial infarction or stroke

(Kasprzak, Klosinska, & Drozdz, 2006). Researchers infuse acetylcholine into the coronary artery before attempting to detect diameter change. Acetylcholine increases the release of NO by activating muscarinic receptors located on the endothelial membrane. An increased risk for atherosclerosis is often designated in areas where vasodilation does not occur (Kasprzak et al., 2006).

High Resolution Vascular Ultrasonography

ED can also occur in peripheral conduit arteries, which allows for the non-invasive *in vivo* assessment of a more accessible vessel, such as the brachial artery. It is presumed that ED through brachial artery assessment is closely related to ED in coronary arteries (Vita & Keaney, 2002). Flow-mediated dilation (FMD) of the brachial artery is measured through high resolution vascular ultrasonography and is the most commonly used test for assessing EF. Briefly, the brachial artery diameter in the upper arm is measured from brightness (B) mode ultrasound images (Patel & Celermajer, 2006; Watts, Beye, Siafarikas, Davis et al., 2004). Ultrasound transducers must be greater than 7 MHz in order to obtain optimal resolution (Patel & Celermajer, 2006). Baseline measurements, which include diameter and blood flow velocity, are recorded for one minute. The FMD is induced by endothelial cells detecting changes in sheer stress during increasing turbulent flow following brief ischemia. Ischemia is caused by rapid inflation of a sphygmomanometer cuff wrapped around the forearm. The cuff is inflated to 250 – 300 mmHg for at least 4.5 minutes (Kasprzak et al., 2006; Patel & Celermajer, 2006; Watts, Beye, Siafarikas, O'Driscoll et al., 2004). Following cuff deflation, recordings are again measured for 1 minute. The percent change in brachial artery diameter from baseline to

45-60 seconds after deflation is recorded as FMD (Williams et al., 2005). After measuring FMD following brief ischemia, FMD is measured following sublingual administration of nitroglycerin (NTG). NTG causes vascular relaxation in an endothelium-independent manner and is often used as a control against the ischemic condition. At baseline, the ideal vessel diameter is 2.5 – 5 mm. A larger vessel may not be associated with significant increases in vasodilation, even when normal EF exists (Patel & Celermajer, 2006). It is often hard to obtain an accurate image in a vessel smaller than 2.5 mm. In adult subjects, vessel size is not often an issue, however, in children the artery of choice becomes the femoral artery (Patel & Celermajer, 2006). Due to differences in FMD with different size vessels, it is recommended that all values be reported as an absolute change in diameter (Kasprzak et al., 2006). Variations in FMD also exist between fasting and post-prandial conditions, therefore, it is important to test individuals in a fasting state.

FMD values are different among diverse populations. Individual's with known CHD, Type 2 diabetes, and healthy volunteers have FMD values that vary between -1.3 to 14%, 0.75 to 12%, and 0.2 to 21%, respectively (Berry, Skyrme-Jones, & Meredith, 2000; Kasprzak et al., 2006). These variations in baseline FMD values make it difficult in defining normal ranges. Researchers attribute these differences to arterial occlusion times, positioning of the occlusion cuff, and time point of vessel measurement following cuff deflation (Berry et al., 2000). Various studies have used an occlusion cuff on the upper arm rather than the forearm. When this occurs, brachial artery diameter measurements occur in the forearm rather than the upper arm. If an upper arm cuff is used to assess FMD then occlusion time must last for at least 5 minutes (Berry et al., 2000). Researchers have

suggested that brachial artery FMD was significantly greater when upper arm occlusion was used compared to forearm occlusion (Agewall et al., 2001; Berry et al., 2000; Peretz et al., 2007). Berry et al. also suggested that peak FMD occurred at different times depending on cuff placement (Berry et al., 2000). FMD peaked at 71 seconds with upper arm occlusion compared to 49 seconds with forearm occlusion. Participants in both upper and lower arm occlusion groups were separated based on their time to peak FMD. Although most subjects with forearm occlusion reached peak FMD within 60 seconds, there were still 6% of participants who did not see a peak FMD until after 60 seconds. Of those participants who had their upper arm occluded to assess FMD, 69% of the subjects experienced peak FMD after 60 seconds. These results have been shown in a more recent study and give rise to concern for variations found in studies that use the first 60 seconds to assess changes in FMD as well as comparing results using different arm occlusion sites (Peretz et al., 2007). While the brachial artery is often used to measure changes in FMD, the radial artery can also be measured following forearm occlusion. Agewall et al. compared FMD changes in the brachial and radial arteries following occlusion of both the upper arm and forearm (Agewall et al., 2001). FMD was greatest in the radial artery, regardless of where cuff occlusion occurred.

Venous Occlusion Strain-Gauge Plethysmography

Another non-invasive *in vivo* tool used to assess EF that is practical and relatively low-cost is venous occlusion strain-gauge plethysmography. In many laboratories, it is the preferred method in assessing EF because it does not require a radiological technologist as does the vascular ultrasonography. Strain-gauge plethysmography works similar to high

resolution vascular ultrasonography in measuring post-occlusive reactive hyperemia. Following an overnight fast, subjects are positioned in a supine position and acclimatize for at least thirty minutes (Thijssen, Bleeker, Smits, & Hopman, 2005). The arm is placed approximately 5 cm above the level of the heart and a sphygmomanometer cuff is placed around the upper arm. The cuff is attached to a rapid cuff inflator. A mercury-in-silastic strain gauge is placed on the largest portion of the forearm. A smaller cuff is placed around the wrist and inflated to super-systolic pressures prior to any measurement in attempt to minimize the contribution of hand blood flow, which has a different control function compared to forearm blood flow (FBF) (Leslie et al., 2004). This allows forearm volume to increase in conjunction with an increase in FBF. The increased forearm volume can be detected through increases in forearm circumference measured by changes in the mercury-in-silastic strain gauge (Leslie et al., 2004). A baseline measurement is recorded as the upper arm cuff is inflated above venous pressure but below arterial pressure. This allows venous outflow to be obstructed while arterial inflow remains the same. The upper arm cuff is inflated for ten seconds and deflated for five seconds while FBF is measured. Averages of five measurements are taken. Peak FBF is measured following post-occlusive hyperemia induced by inflating the upper cuff to super-systolic pressure for at least 5 minutes before FBF measurement is taken.

Technical considerations that should be considered for using venous occlusion strain-gauge plethysmography include appropriate venous occlusion pressure and duration of venous occlusion. It has been reported that several studies apply a standard venous occlusion of 50 mmHg while others employ a value dependent on subject's diastolic

pressure (Alomari et al., 2004). Researchers suggested that venous occlusion of 7, 14, 21, 28, and 35 mmHg below diastolic pressure had a stepwise decrease in venous capacitance, respectively (Alomari et al., 2004). The study also compared venous outflow with the same pressure values and illustrated a stepwise decrease as occlusion pressure decreased, suggesting a strong positive relationship between venous capacitance and venous outflow. Forearm inflow was not altered, providing a significant role for occlusion pressure as forearm circumference is related to venous outflow. Alomari et al. also showed that peak forearm blood inflow and venous outflow were both significantly correlated to cardiovascular fitness (Alomari et al., 2004). The positive relationship between peak oxygen consumption (VO_{2peak}) and both arterial inflow and venous outflow, clearly demonstrate the ability for arteries and veins to deliver nutrients and remove waste metabolites from blood during increasing levels of muscle contraction, potentially reducing muscular fatigue.

CLINICAL SIGNIFICANCE OF ENDOTHELIAL FUNCTION

Due to a strong association between ED and the initiating stages of atherosclerosis, as well as clinical symptoms such as unstable angina and myocardial infarction (MI), the assessment of EF is crucial in targeting individuals with impaired vascular reactivity (Vita & Keaney, 2002). Coronary ED has been shown to be an independent predictor of atherosclerotic disease progression and cardiovascular event rates in individuals with coronary artery disease (CAD), as well as those without CAD (Halcox et al., 2002; Schachinger, Britten, & Zeiher, 2000). Shachinger et al. evaluated the long term outcome (6.7±3.2 years) of individuals who were undergoing routine evaluation of chest pain or

percutaneous transluminal coronary angioplasty for single vessel disease (Schachinger et al., 2000). FMD measured by coronary angiography demonstrated a significant inverse relationship between the incidence of cardiovascular events and both flow-dependent and NTG-induced vasodilation. To determine if the incidence of cardiovascular events was inversely related to endothelial cell or smooth muscle cell function, the investigators normalized flow-dependent vasodilation to NTG-induced vasodilation. After normalization of NTG-induced vasodilation, flow-dependent vasodilation significantly predicted cardiovascular events, suggesting that impaired coronary vasodilation was directly related to endothelial cell function (Schachinger et al., 2000). Halcox et al. examined the change in coronary vascular resistance (CVR) during the arterial infusion of the endothelial-dependent vasodilator, acetylcholine (ACH), in patients with angiographic evidence of irregularity, as well as individuals with angiographically smooth coronary arteries (Halcox et al., 2002). The investigators demonstrated that individuals with the greatest change in CVR were more likely to have a cardiovascular event-free survival (46±3 months), independent of the presence of CAD.

Although the relationship between CAD and coronary ED is well supported, the invasive measure is not feasible in healthy individuals or even those with only suspected coronary ED. Since EF can be assessed years before any cardiovascular event, the need for a non-invasive measure of EF that can be used on multiple occasions during one's lifespan to determine the presence and effectiveness of treatment of atherosclerosis is appealing. FMD and FBF, as assessed by high resolution vascular ultrasonography and venous occlusion strain-gauge plethysmography, respectively, can provide non-invasive

assessment of EF in peripheral vessels. As previously mentioned, the relationship between ED in human coronary and peripheral arteries is well established and peripheral ED has also been shown to predict future cardiovascular events (Anderson et al., 1995; Kitta et al., 2009; Neunteufl et al., 1997; Shechter et al., 2007; Takase, Hamabe, Satomura, Akima, Uehata, Matsui, Ohsuzu, Ishihara, & Kurita, 2006; Tentolouris et al., 2004; Vita & Keaney, 2002; Yeboah, Crouse, Hsu, Burke, & Herrington, 2007).

Anderson et al. were the first to demonstrate a relationship between percent diameter changes in two different vascular beds of the same individual (Anderson et al., 1995). The investigators demonstrated a significant positive relationship with brachial and coronary vasodilation. The relationship was shown in patients with and without CAD, suggesting that ED is not restricted to vessels with evident atherosclerosis and may be an early "functional expression" of the atherosclerotic process (Anderson et al., 1995). Other studies supported FMD of the brachial artery as a useful non-invasive tool to measure EF and provided evidence that that ED may be an early "functional expression" of atherosclerosis (Kitta et al., 2009; Neunteufl et al., 1997; Takase, Hamabe, Satomura, Akima, Uehata, Matsui, Ohsuzu, Ishihara, & Kurita, 2006; Yeboah, Crouse, Hsu, Burke, & Herrington, 2007). Neunteufl et al. reported data from a multiple regression stepwise analysis suggesting that brachial artery FMD had a significant inverse correlation with the severity of CAD (Neunteufl et al., 1997). Subjects from the study with CAD had significantly less FMD than non-CAD subjects. NTG induced vasodilation was not significantly different between CAD and non-CAD subjects, suggesting that impairment in

vascular relaxation in CAD subjects was directly related to endothelial cell function as opposed to smooth muscle cell activity (Neunteufl et al., 1997).

More recently, Takase et al., suggested that the brachial artery's response to ACH was significantly correlated to the response of the coronary artery to ACH in individuals with low to moderate cardiovascular risk factors and only slight suspicion of CAD (Takase, Hamabe, Satomura, Akima, Uehata, Matsui, Ohsuzu, Ishihara, & Kurita, 2006). The investigators followed these same individuals for 53 ± 17 months and demonstrated that the peripheral vessel's dilatory capacity was useful in predicting the incident of long-term cardiovascular events. Employing the brachial artery's dilation capacity as a means to predict cardiovascular events has been well documented in other studies (Kitta et al., 2009; Shechter et al., 2007; Yeboah, Crouse, Hsu, Burke, & Herrington, 2007).

Shechter et al. assessed EF between healthy and stable CVD subjects (Shechter et al., 2007). Brachial artery FMD was significantly lower in CVD subjects compared to healthy controls and there was no significant difference in NTG-induced dilation between the two groups. All subjects were divided into groups based on cardiovascular risk factors such as gender, hypertension, hyperlipidemia, smoking history, diabetes mellitus and family history. FMD was significantly lower in males compared to females and non-significantly lower in diabetic compared to non-diabetic subjects (Shechter et al., 2007). This study reported no significant correlation between FMD and hypertension, hyperlipidemia, smoking and family history. When FMD was analyzed against an individual's number of CVD risk factors, there was a significant inverse relationship. Healthy individual's baseline brachial artery was significantly larger compared to the CVD

patients. In addition, the baseline brachial artery diameter and FMD had a significant inverse correlation. The investigators speculated that large diameter arteries are unable to reach maximal dilation, further illustrating the impaired vascular function of the CVD patients (Shechter et al., 2007). Of note, both healthy and CVD subjects had mean baseline brachial artery diameters greater than 5 mm, which is larger than the recommended size when assessing FMD. Shechter et al. followed up all subjects 15 ± 2 months for CVD endpoints (e.g. all-cause mortality, MI, hospitalization for heart failure or angina pectoris, cerebrovascular accident, CABG and percutaneous coronary interventions) (Shechter et al., 2007). CVD endpoints were significantly more frequent in subjects who had less than 6% brachial artery FMD change in response to reactive hyperemia.

Yeboah et al. were the first to evaluate brachial artery FMD as a predictor of cardiovascular events in older adults aged 72 to 98 years (Yeboah, Crouse, Hsu, Burke, & Herrington, 2007). Study participants were contacted biannually during a five year follow-up period to determine if any cardiovascular event had occurred. The investigators suggested that FMD was a significant predictor of cardiovascular events, even after adjusting for baseline CVD status and CVD risk factors including age and gender. Those individuals who had brachial artery FMD greater than the sex-specific medians were more likely to have event-free survival rates for cardiovascular events (Yeboah, Crouse, Hsu, Burke, & Herrington, 2007). This study was also the first to suggest that brachial artery diameter was inversely related to the incidence of cardiovascular events in older individuals. The relationship between FMD and baseline diameter size of the brachial artery was not shown in this study. However, these findings are similar to those reported

by Shechter et al. (2007), indicating that although individuals with a larger brachial artery baseline diameter size may not experience maximal FMD, the vascular reactivity is still significantly larger than individuals who experience a follow-up cardiovascular event. This further supports the view that impaired FMD is a measure of the early "functional expression" of the atherosclerotic process, independent of the brachial artery baseline diameter size.

To date, there are no known studies that have evaluated the prognostic impact of FBF assessed by strain gauge plethysmography in predicting the long-term outcome of individuals with and without CAD. However, strain gauge plethysmography has been used to demonstrate relationships among FBF and several cardiovascular disease risk factors. Ishibashi et al. examined FBF on individuals who were apparently healthy, with no known history of CVD and free from medications for hypertension, diabetes, and hyperlipidemia (Ishibashi et al., 2006). Several cardiovascular disease risk factors were assessed, which included age, hypertension, hypercholesterolemia, diabetic mellitus, smoking, obesity, and a family history of CVD. The investigators suggested that duration of hyperemia, when measuring FBF following the release of an upper arm cuff, was significantly shorter in individuals with CVD risk factors (Ishibashi et al., 2006). Long-term studies are warranted that evaluate the duration of hyperemia with clinically evident atherosclerotic disease later in life.

Alarmingly, it is speculated that ED is present in asymptomatic children as suggested by the existence of fatty streaks in postmortem studies (Napoli et al., 1999). As previously described, there is a strong relationship between ED and pro-inflammatory

conditions. Children with acute respiratory infections, a pro-inflammatory state, have decreased brachial artery FMD that has been shown to be reversible (M. Charakida et al., 2005). However, chronic pro-inflammatory conditions, such as obesity can be significant in the initiation of atherosclerosis. Due to ED being an early event in the progression of CVD, EF assessment could be of prognostic value since it reflects vascular biology.

If ED is associated with the pathogenesis of CVD, it is often proposed that reversal of ED will reduce the risk for CVD. Recently, Kitta et al. examined the effect of various medications used to treat CVD risk factors, along with suggested life-style changes, in evaluating improvements in brachial artery FMD and predicting future cardiovascular events (Kitta et al., 2009). Brachial artery FMD was assessed before and after a six month period that consisted of optimized therapy. At six months, approximately 58% of the individuals improved their FMD. This change in FMD was seen in the absence of changes in baseline brachial artery diameter, baseline brachial artery blood flow, increased brachial artery blood flow, and increased brachial artery diameter after the infusion of NTG (Kitta et al., 2009). Individuals with an improved FMD had a significantly higher survival rate without a cardiovascular event during a 36 month follow-up. These findings support the application of serial FMD measurements as a tool to assess therapeutic options involved in improving vascular reactivity. While subjects were advised to walk more than 30 minutes per day, an evaluation to measure adherence to physical activity (PA) was not included, making it difficult to articulate any benefits of PA in this particular study.

EXERCISE TRAINING AND ENDOTHELIAL FUNCTION

The beneficial effects of exercise on reducing risk for CVD are well known. Studies that have assessed the effects of exercise on ED have strengthened decisions to employ exercise training as an important therapy treatment for ED. As previously described, increasing shear stress on endothelial cells stimulates secretion of NO leading to smooth muscle cell relaxation and subsequent vascular dilation. This led investigators to initially hypothesis that any improvement in ED from exercise would be location specific due to an increased blood flow to contracting muscles. Kingwell et al. were the first to demonstrate in humans an enhanced resting NO production in an untrained limb following 4 weeks of cycle training (Kingwell, Sherrard, Jennings, & Dart, 1997). The researchers suggested that although no muscular adaptations occurred in the unexercised limb, there were systemic adaptations from exercise, including an increase in pulse pressure that enhanced the release of NO from non-trained vascular beds. Aside from the 4 week training study, an exercise session consisting of 30 minute on a cycle was performed to assess single-arm post-exercise FBF through venous occlusion plethysmography. Forearm blood flow was significantly increased after a single session of cycle exercise and remained elevated for approximately 60 minutes after cessation of exercise in a few of the subjects. This data suggests that regular exercise using a large muscle mass can induce adaptations in non-exercising limbs, which enhance EF. This important finding justifies the evaluation of FBF with common aerobic exercise modalities such as cycling and walking.

As exercise in large muscle groups has been shown to have a systemic effect on the body, localized training in smaller muscle groups (i.e. forearm) may not be associated with

systemic effects. Katz et al. examined the effect of exercise training on EF in adults with idiopathic dilated cardiomyopathy and congestive heart failure symptoms (Katz, Yuen, Bijou, & LeJemtel, 1997). The subjects performed non-dominant hand grip exercises 30 minutes per day for eight weeks. Peak reactive hyperemic FBF post-training was significantly increased in the trained arm, but not the untrained arm.

Exercise Training and EF in Subjects with CVD Risks

Many studies have shown an exercise training effect on EF with subjects who were at higher risk for CVD. Watts et al. evaluated the effect of exercise on EF during eight weeks of exercise training or eight weeks of non-training (Watts, Beye, Siafarikas, O'Driscoll et al., 2004). The study recruited obese adolescents who either refrained from all activity or performed 1 hour sessions of circuit training 3 times per week for eight weeks. Initially, brachial artery FMD was significantly different in obese versus normal control subjects. FMD significantly increased in obese adolescents after 8 weeks of exercise training, which resulted in statistically similar FMD values between obese adolescents that trained and normal subjects. Woo et al. examined the effect of diet alone versus diet with exercise on EF in overweight or obese adolescents (Woo et al., 2004). The diet intervention consisted of a hypocaloric menu that provided 900 – 1200 kcal a day. The exercise intervention consisted of circuit training exercise 2 times per week for 6 weeks. At the start of the study, overweight adolescents had an impaired brachial artery FMD compared to a non-obese control group. Although both groups had an improvement in FMD at the end of the study, the diet and exercise intervention resulted in a significant improvement in FMD compared to the diet alone. These changes in EF were not seen

following a sublingual dose of NTG, evidence that the changes in FMD were endothelium-dependent. Additionally, Hamdy et al. examined the effects of EF prior to and following a 6 month diet and exercise intervention in obese adults who had MetS (Hamdy et al., 2003). The intervention consisted of a diet accounting for 500 kcal per day of negative energy balance with supervised exercise 3 days per week along with 150 minutes per week of unsupervised activity. Baseline brachial artery diameter did not change during the intervention. FMD was significantly improved after the 6 month intervention. As shown in previously described intervention studies, sublingual administration of NTG did not result in significant differences in FMD at baseline compared to 6 months.

More recently, Schjerve et al. investigated the effect of various exercise training modes and intensities on EF in obese individuals (Schjerve et al., 2008). Subjects were randomized into three groups, which included strength training, moderate-intensity aerobic training, and high-intensity aerobic training. Participants completed 12 weeks of exercise training, performing supervised sessions twice per week and one home-based session each week. Aerobic exercise training was designed to control for caloric expenditure, as the high-intensity subjects performed 4x4-minute interval bouts at 85 – 95% maximal heart rate (HR_{max}) and the moderate-intensity subjects walked for 47 minutes at 60 – 70% of HR_{max} (Schjerve et al., 2008). The strength training group performed four sets of 5 repetitions at 90% 1 repetition max on a leg apparatus, in addition to three sets of thirty repetitions of abdominal and back exercises with a 30 second break between each set. Brachial artery FMD significantly increased after 12 weeks of training in all three exercise groups. However, post-training FMD of the high-intensity group was significantly greater

than the moderate-intensity and strength training group. The moderate-intensity and strength training group did not have a significantly different post-training FMD. Baseline brachial artery diameter and blood flow was not significantly different between groups before training and did not change after 12 weeks of training. Additionally, there was no significant difference in brachial artery FMD in response to nitroglycerin before or after 12 weeks of training in any of the groups (Schjerve et al., 2008). This study suggests that aerobic exercise training at high or moderate-intensity and strength training will improve EF in obese individuals.

Moreover, studies have evaluated other cardiovascular risk factors in determining the exercise training effect on EF in various populations, such as hypertensives, diabetics, and hypercholesterolemics (Higashi et al., 1999; Maiorana et al., 2001; Walsh, Yong et al., 2003). Higashi et al. examined the effect of exercise training on EF in hypertensive subjects (Higashi et al., 1999). The subjects performed 12 weeks of aerobic exercise, which consisted of brisk walking (52 ± 9% VO_2) for 30 minutes 5 -7 times per week. After 12-weeks, both systolic and diastolic blood pressures were significantly decreased. FBF was significantly increased in response to the infusion of acetylcholine at 12 weeks. This response was abolished with the infusion of N^G-monomethyl-$_L$-arginine (L-NMMA), a known inhibitor of NO. The study proposed that the increased shear stress from increased flow was responsible for changes in NO production and subsequent increased FBF.

Additionally, investigators have examined the effect of circuit exercise training on EF in subjects who are diabetic and those who are currently or have previously been hypercholesterolemic (Maiorana et al., 2001; Walsh, Yong et al., 2003). An 8-week

training schedule was developed to target the larger muscle groups of the lower body. Each supervised exercise session lasted one hour, with participants alternating every 45 seconds between seven resistance and eight aerobic exercise stations (Maiorana et al., 2001). Subjects were required to maintain 70 – 85% of HR_{max} while at the aerobic stations and lift 55 – 65% of their baseline maximal voluntary contraction at the resistance stations. Participants with a history of hypercholesterolemia had one at-home and two supervised weekly exercise sessions, while those with type 2 diabetes were instructed during three supervised weekly exercise sessions (Maiorana et al., 2001; Walsh, Yong et al., 2003). At home, the subjects were encouraged to perform continuous aerobic exercise at 70 – 85% of HR_{max} for 45 – 60 minutes. Investigators in both studies measured brachial artery FMD and FBF before training and then again at the end of the 8 week program. FMD in response to 5 minutes of forearm ischemia significantly increased after 8 weeks of circuit training in both subjects with type 2 diabetes and those who had been treated for hypercholesterolemia (Maiorana et al., 2001; Walsh, Yong et al., 2003). This change appeared to be endothelium dependent due to a similar non-significant FMD response to nitroglycerin. FMD did not significantly improve after 8 weeks of circuit training in untreated hypercholesterolemic subjects (Walsh, Yong et al., 2003). When comparing the ratio of flow to ACH in the infused arm to flow of the non-infused arm, FBF was significantly increased following 8 weeks of circuit training in subjects with type 2 diabetes, as well as those who had been treated for hypercholesterolemia (Maiorana et al., 2001; Walsh, Yong et al., 2003). The increased FBF response to ACH following circuit training was not observed during the infusion of sodium nitroprusside (SNP), an

endothelial-independent vasodilator. Likewise, infusion of L-NMMA in subjects who had been treated for hypercholesterolemia did not result in significant increases in FBF (Walsh, Yong et al., 2003). Similar to Higashi et al. (1999), both Maiorana et al. (2001) and Walsh et al. (2003) proposed that the increased endothelial wall shear stress was primarily responsible for the increase in FMD and FBF following circuit training in subjects with type 2 diabetes, as well as those who had been treated for hypercholesterolemia.

Interestingly, Green et al. investigated whether EF improvements observed with exercise resulted from short term repetitive exercise bouts or exercise associated improvements in CVD risk factor profiles (Green et al., 2003). Eight weeks of circuit training as described above resulted in an enhanced exercise capacity, emphasized by significant increases in resting heart rate, maximal exercise test duration, and absolute and relative VO_{2peak}. Other significant changes that resulted from 8 weeks of circuit training included an increased muscular strength and decreased peripheral adiposity. Eight weeks of circuit training did not significantly change plasma lipids, blood pressure, resting blood glucose, waist-to-hip ratio, or BMI (Green et al., 2003). Brachial artery FMD and FBF, in response to 5 minutes of forearm ischemia and ACH infusion, respectively, were significantly increased after 8 weeks of circuit training. Although exercise has been shown to have a systemic effect on EF, as described above, the study suggests that these changes in EF are not exclusively related to the effect that exercise has on CVD risk factors. This further demonstrates the impact exercise training has on the vascular system, with its frequent, periodic increase in shear stress.

Exercise Training and EF in CVD

Several studies have evaluated the effect of exercise training on EF in subjects with cardiovascular disease. Walsh et al. was the first to suggest that individuals with a history of coronary artery disease had improved EF in the brachial artery following eight weeks of circuit training as previously described (Walsh et al., 2003; Walsh, Yong et al., 2003). As demonstrated in other subject populations with impaired EF, brachial artery FMD significantly increased after eight weeks of exercise training. In keeping with previous studies described above, brachial artery response to NTG was not significantly different before versus after exercise.

The effect of aerobic exercise training only on EF in patients with CVD has shown similar results. Edwards et al. was the first to demonstrate significant improvements in brachial artery FMD in subjects with coronary artery disease following 12 weeks of aerobic exercise training (Edwards et al., 2004). Participants exercised 3-times per week on a treadmill or stationary cycle, progressing intensity and duration, from 40 – 85% of HR_{max} reserve and 15 – 50 minutes, respectively. The investigators also reported a significant increase in plasma nitrate and nitrite levels following 12-weeks of exercise training, suggesting a plausible mechanism for the improvement in EF (Edwards et al., 2004). Additionally, as few as four weeks of aerobic exercise training has been shown to improve EF in patients with CAD (Sixt et al., 2008). Subjects participated in supervised exercise sessions every day during the first week, followed by three weeks of a daily home-based exercise routine and supervised training sessions twice per week. Study findings suggest that relative VO_2 significantly improved without a concurrent loss in body weight after four weeks of exercise training. Subjects who participated in four weeks of

exercise training also significantly improved brachial artery FMD, while no difference in FMD was observed after infusion of NTG (Sixt et al., 2008).

Recently, Vona et al. investigated the effect of varying types of exercise training on EF in subjects who had recently experienced a myocardial infarction (Vona et al., 2009). Participants were randomly placed into one of four groups, which consisted of aerobic training, resistance training, combined training, or a control group. Each group, aside from the controls, underwent supervised training sessions four times a week for four weeks. Brachial artery diameter and FMD during reactive hyperemia was not significantly different between groups at baseline (Vona et al., 2009). After four weeks of training, brachial artery FMD significantly improved in all four groups, although significantly less in the control group. The investigators suggested that the increase in FMD observed in the control group was more likely a secondary response to the maximal exercise test conducted a day prior. Four weeks of exercise training, independent the mode of exercise appeared to improve EF in subjects who had recently experienced an uncomplicated myocardial infarction.

Exercise Training and EF in Healthy Individuals

Although most exercise training studies have evaluated subjects with an increased risk for CVD disease, a limited number of studies, as described below, have evaluated the effect of exercise training on EF in healthy subjects. Moreover, these studies are not in complete agreement that EF is enhanced following exercise training in healthy individuals. There are several studies that support an enhanced EF following exercise training in healthy individuals (Clarkson et al., 1999; Goto et al., 2003; Higashi et al., 1999;

Rakobowchuk et al., 2008). However, there are also studies that suggest that EF is not significantly enhanced following exercise training and may even be impaired depending upon the intensity employed during training (Bergholm et al., 1999; Goto et al., 2003; Rakobowchuk et al., 2005).

Initially, Higashi et al. assessed EF in healthy individuals following 12 weeks of aerobic exercise consisting of 30 minutes of brisk walking (52 ± 9% VO_{2max}) 5 -7 times per week (Higashi et al., 1999). Following 12 weeks of exercise, FBF was significantly increased in response to infused acetylcholine. This increased FBF response was abolished with the intra-arterial infusion of L-NMMA. Additionally, Clarkson et al. examined the effect of a 10-week basic military training camp on EF in 35 healthy males (Clarkson et al., 1999). Following exercise training, baseline vessel diameter of the brachial artery did not change compared to pre-training diameter size and brachial artery FMD during reactive hyperemia was significantly increased compared to pre-training values. The authors suggested that exercise at a reasonable intensity would be beneficial in inducing vascular change and improving EF. The military recruits performed a daily 3 mile run and undertook upper-body strength and endurance exercises.

However, in contrast to the previously described studies, one study did not support an enhanced EF following exercise training. Bergholm et al. evaluated the effect of a high-intensity 12-week aerobic exercise study on EF in moderately fit males (BMI 21.8 ± 0.4 kg/m^2, VO_{2max} 53 ± 2 ml/kg/min) (Bergholm et al., 1999). The subjects participated in four, one hour running sessions per week at an intensity that matched 70 – 80% of their VO_{2max}. FBF in response to ACH significantly decreased after 12 weeks of intense

exercise training compared to measurements taken before training. FBF in response to SNP was not significantly different in the subjects before compared to after the exercise training, suggesting the FBF response to intense exercise training was endothelium-dependent (Bergholm et al., 1999).

The inconsistent findings in exercise training effects on EF in healthy individuals led Goto et al. to investigate the effect of a 12 week exercise program using different exercise training intensities on EF (Goto et al., 2003). There were no baseline significant differences between the three groups who participated in either mild (25% VO_{2max}), moderate (50% VO_{2max}) or high (75% VO_{2max}) intensity exercise training. Following 12 weeks (30 minutes, 5-7 days each week) of aerobic exercise, only subjects who participated in moderate intensity exercise had an improvement in FBF following the infusion of acetylcholine. The infusion of L-NMMA abolished these effects, supporting the release of NO as the cause for vasodilation. The investigators suggested that the mild aerobic exercise was not a strong enough stimulus to attain a response, whereas high intensity aerobic exercise was associated with increasing biomarkers known to decrease the bioavailability of NO.

Furthermore, Rakobowchuk et al. evaluated the effect of resistance exercise on EF in 28 healthy young men (Rakobowchuk et al., 2005). The subjects completed a 12 week progressive protocol, exercising 5 times per week (60 sessions). Brachial artery diameter and peak FMD during reactive hyperemia significantly increased after 6 weeks of training and remained elevated at 12 weeks. However, peak and average shear rate in the brachial artery assessed through a linear array pulse Doppler ultrasound probe were not

significantly different at any point. When brachial artery FMD was normalized for shear stimulus, there was no significant FMD change at any time during the training period. Although EF assessed by FMD did not change during 12 weeks of resistance training, the findings suggest that resistance exercise could represent a stable adaptation by enhancing resistance vessel function.

Currently, studies appear to support an improvement in EF in healthy individuals following moderate-intensity aerobic exercise training. These positive changes in EF with moderate-intensity training are not well supported in high-intensity aerobic exercise training or strength exercise training models. Recently, Rakobowchuk et al. compared the effects of moderate-intensity aerobic training to high-intensity, low-volume, sprint interval training on EF (Rakobowchuk et al., 2008). The authors suggested that both groups had a comparable significant improvement in FMD. However, the measurement of FMD was different than in the studies previously described. Rather than measure FMD in the brachial artery, the authors measured FMD in the popliteal artery. The FMD response of the popliteal artery did not measure the systemic effects of the exercise training, rather the direct effects of a trained limb making it difficult to compare the results of this study to those previously reported.

In 2008, Heylen et al. described the impact of exercise training frequency on EF in male Wister rats (Heylen et al., 2008). The study suggested that the thoracic aorta's sensitivity to ACH was significantly greater in rats that exercised at a moderate intensity five days per week, compared to one day per week of moderate PA. The authors provided evidence that there is a frequency-dependent dose of moderate PA that will elicit

endothelial-dependent improvements in vascular reactivity. Studies investigating the effect of exercise frequency training on EF are warranted in human populations, especially those individuals who are at increased risk for CVD, where exercise training appears to have an established effect on EF.

ACUTE BOUTS OF EXERCISE AND ENDOTHELIAL FUNCTION

While most studies examine EF following exercise training, EF following acute exercise has not been extensively researched. Only a few studies have evaluated the effect of a single bout of exercise on EF in healthy individuals. Initially, Baynard et al. recruited healthy adult men who were either endurance trained or resistance trained (Baynard, Miller, & Fernhall, 2003). FBF was measured prior to and immediately after (5 minutes) a maximal exercise test measuring oxygen consumption. Resting FBF was higher in the resistance trained group. The endurance trained group had a higher resting reactive hyperemic FBF. Following maximal exercise, reactive hyperemic FBF declined at slower levels compared to resting reactive hyperemic FBF in the endurance trained group. The resistance trained group had significantly higher reactive hyperemic FBF following exercise compared to resting values. A subsequent study examined FBF in subjects for 2 hours following maximal exercise (Bousquet-Santos, Soares, & Nobrega, 2005). FBF was measured prior to and 10, 60, and 120 minutes after maximal exercise in 9 healthy adults. Compared to resting values, baseline FBF was significantly increased at 10 minutes post exercise, but not 60 and 120 minutes post exercise. Compared to resting values, reactive hyperemic FBF was significantly increased at 10 and 60 minutes post exercise, but returned to near baseline numbers at 120 minutes post exercise. It is important to point out

that this study did not use peak FBF values rather the area under the curve following three minutes of FBF measurements. These results are very similar to the FBF rate of decline, which may be controlled by a different mechanism than peak FBF. Finally, Rognmo et al. recently investigated the effect of a single high-intensity exercise bout on brachial artery FMD in highly-trained endurance athletes (VO_{2max} 75.9 ± 0.8 mL/kg/min) versus sedentary controls (VO_{2max} 47.7 ± 1.7 mL/kg/min) (Rognmo et al., 2008). The study consisted of subjects performing an exercise protocol on the treadmill which included a 15 minute warm-up (60 – 70% HR_{max}) and 5 intervals of high-intensity (90% HR_{max}) running for 3 minutes. During each interval, subjects performed an active recovery (60 – 70% HR_{max}) for two minutes. Each subject had brachial artery FMD measured prior to, and 1, 24, and 48 hours post exercise. The authors suggested that baseline FMD was not significantly different between highly-trained endurance athletes and sedentary, healthy individuals (Rognmo et al., 2008). FMD measured 1 hour after a single high-intensity exercise bout was significantly decreased compared to baseline values in highly-trained endurance athletes and only slightly decreased in the sedentary controls. FMD did not significantly change in the sedentary control group following exercise and returned to baseline values in the highly-trained endurance athletes at 24 and 48 hours after exercise. Brachial artery diameter was significantly larger in the highly-trained endurance athletes compared to the sedentary controls. However, brachial artery diameter significantly increased 1 hour post-exercise compared to baseline diameter measurements in both groups (Rognmo et al., 2008). Although there was a significant reduction in FMD in the highly-endurance trained group, the vessel diameter was most likely maximally dilated at 1 hour post-exercise,

decreasing shear stress and any subsequent vasodilatory effect. The large diameter size observed in highly-trained endurance athletes is unlike that of individuals with coronary artery disease. Rather, the decreased FMD after exercise in the highly-trained endurance group is attributed to an improved "functional" vessel.

Harris et al. was the first to investigate the effects of a single exercise bout on EF in overweight individuals (Harris, Padilla, Hanlon, Rink, & Wallace, 2008). Subjects were categorized as either trained or untrained depending on physical activity profiles and placed randomly in one of three groups, where they walked on a treadmill for 45 minutes at 25, 50, or 75% of maximal exercise capacity. Brachial artery FMD was assessed prior to and 1 hour post exercise. Baseline FMD was not significantly different between groups. Walking 45 minutes at varying exercise intensities did not significantly alter FMD in trained or untrained overweight individuals (Harris et al., 2008). However, when FMD was normalized to reflect the absolute response to exercise, trained overweight individuals significantly improved FMD 1 hour after exercise and untrained overweight individuals significantly decreased FMD 1 hour after exercise.

Likewise, Umpierre et al. was the first to investigate the effect of sub-maximal exercise on EF in subjects with chronic heart failure (Umpierre, Stein, Vieira, & Ribeiro, 2009). Subjects performed a single exercise bout for 25 minutes at 10% below a predetermined, second ventilatory threshold. FBF was measured prior to, immediately after, 10, 30, 60 minutes, and 24 hours post-exercise. FBF was not significantly different at baseline between individuals with chronic heart failure and healthy controls. FBF significantly increased in chronic heart failure subjects in response to exercise immediately

after and 10 minutes post-exercise before returning back to baseline values at 30 minutes post-exercise (Umpierre et al., 2009). FBF significantly increased in healthy controls in response to exercise immediately after, 10, and 30 minutes post-exercise before returning back to baseline values 60 minutes post-exercise. Interestingly, there were no significant FBF differences between the two groups when FBF was increased above baseline.

The studies above present important findings in further understanding the effects of acute exercise on EF. However, an extensive review of literature has produced no study in which the extended time-course for EF following a single bout of maximal exercise is shown, especially in obese individuals. As seen later in this review, there are studies that evaluate an extended time-course of inflammatory biomarkers following exercise that may have an effect on the EF. This information would be of primary importance, especially when measuring EF in response to exercise training where maximal exercise tests are typically performed before and after the training intervention in an attempt to evaluate changes in maximal exercise capacity. There does not appear to be an established optimal time before EF is assessed following a training program's last exercise bout. Previously, measures have varied between 24 and 72 hours after the final exercise bout.

EXERCISE TRAINING AND ENDOTHELIAL DYSFUNCTION BIOMARKERS

Several studies described below include the measurement of ED biomarkers that are provoked by exercise training. In a study previously described, Hamdy et al. evaluated the effects of exercise training on adhesion molecules ICAM-1 and VCAM-1 in subjects with MetS (Hamdy et al., 2003). Following exercise training, ICAM-1 was significantly reduced while there was no change in VCAM-1. ICAM-1 is associated with the

development of atherosclerosis through enhancement of foam cell formation and any improvement in blood concentration could potentially be linked to a risk reduction in the development of CVD. Additionally, Niebauer et al. examined the relationship between eight weeks of exercise training and inflammatory cytokines in subjects with chronic heart failure (Niebauer, Clark, Webb-Peploe, & Coats, 2005). During the eight weeks, subjects cycled on a home ergometer for 20 minutes at 70 – 80% of HR_{max}. The investigators suggested that there was no effect of exercise training on TNF-α, IL-6, and ICAM-1 in both subjects with chronic heart failure and age-matched healthy controls (Niebauer et al., 2005). Furthermore, Stewart et al. examined the influence of exercise training on inflammatory markers IL-6, TNF-α, and CRP in young and old subjects who were physically active and inactive, but otherwise healthy (Stewart et al., 2007). The 12 week training intervention consisted of circuit training performed 3 days per week. When comparing all groups, there was no significant difference in IL-6 and CRP baseline concentrations. TNF-α concentration was significantly higher in younger subjects at baseline, independent of being physically active or inactive. Following exercise training, the young and old physically inactive had a significant decline in CRP levels. There was no exercise training effects on TNF-α or IL-6 in any group. Previous findings from Fisher et al. agreed that no relationship existed between TNF-α levels and PA (Fischer, Berntsen, Perstrup, Eskildsen, & Pedersen, 2006). However, self-reported qualitative assessments of PA suggested a significant negative linear association with PA and pro-inflammatory cytokines, CRP and IL-6 (Fischer et al., 2006).

ACUTE EXERCISE AND ENDOTHELIAL DYSFUNCTION BIOMARKERS

Few studies have evaluated the effect of acute exercise on biomarkers associated with atherosclerosis. Kinugawa et al. examined the effect of maximal exercise on plasma TNF-α and IL-6 in subjects with CVD (Kinugawa et al., 2003). At baseline, subjects with CVD had significantly higher levels of plasma TNF-α and IL-6 as compared to healthy controls. Baseline plasma TNF-α and IL-6 had a significant positive correlation with norepinephrine levels and a significant negative correlation with VO_{2peak}. Maximal exercise significantly increased plasma TNF-α and IL-6 in subjects with CVD and healthy controls. Additionally, von Kanel et al. compared the result of acute exercise on atherosclerotic risk markers and suggested that mean arterial blood pressure and VO_{2peak} were independently associated with plasma ICAM-1 and IL-6, respectively (von Kanel, Hong, Pung, & Mills, 2007). After a 20 minute sub-maximal exercise test on the treadmill, plasma IL-6 and ICAM-1 were both significantly higher than baseline. However, after 25 minutes post-exercise, plasma ICAM-1 declined to baseline values while IL-6 was significantly higher than post exercise values. Finally, in a study previously described, Harris et al. examined the effect of a single bout of exercise at various intensities on plasma TNF-α and IL-6 levels one hour post-exercise in overweight trained and untrained men (Harris et al., 2008). The authors suggested that baseline plasma TNF-α was not significantly different between trained versus untrained overweight men and that exercise did not significantly alter plasma TNF-α in either group independent of the exercise intensity. Plasma IL-6 was not significantly different at baseline between trained versus untrained overweight men. However, both groups had a comparable significant increase in plasma IL-6 after participating in moderate and high intensity exercise (Harris et al., 2008).

To date, there are no known studies that evaluate the extended time course of inflammatory markers after a single bout of exercise. In 2007, a study by Louis et al. provided evidence that suggests the extended evaluation of inflammatory markers after exercise may be essential in the understanding of time controlled release of specific cytokines (Louis, Raue, Yang, Jemiolo, & Trappe, 2007). The extended evaluation of inflammatory markers may also provide a much better understanding in the evaluation of vascular reactivity after exercise. Louis et al. investigated the TNF-α mRNA and IL-6 mRNA expression changes following a single aerobic exercise bout, which consisted of running for 30 minutes on the treadmill at 75% of each subject's VO_{2max} (Louis et al., 2007). TNF-α mRNA was significantly elevated immediately after exercise and again 12 and 24 hours after exercise. IL-6 mRNA was significantly elevated immediately after exercise and again 2, 4, 8, 12, and 24 hours after exercise.

CONCLUSION

The critical effect of endothelial wall activation and its associated role in initiating atherosclerosis warrants further research of the mediating receptors and circulating agents that allow for the control of vascular homeostasis. Assessing EF and the biomarkers associated with its control is vital in understanding interventions that may help not only slow the progression of CVD but negate specific CVD risk factors. The underlying mechanisms that control EF changes following exercise training have received a large amount of attention and continue to be evaluated. PA has been shown as an important mediator for improving EF through the generation of shear stress. Current research supports the importance of NO on vasodilation and previous studies agree with the role

shear stress plays in stimulating NO release from endothelial cells, contributing to the increased vascular blood flow during exercise. Technical considerations in the assessment of exercise induced alterations of EF are beneficial in contributing to the proposed mechanisms of adaptation.

Direct non-invasive tools used to assess EF include high resolution vascular ultrasonography and venous occlusion strain-gauge plethysmography. These tools allow researchers to mimic the shear stress which is often associated with exercise. Inflatable cuffs wrapped around the upper arm and wrist allow for control of blood flow in the forearm. Turbulent shear stress can be generated following a brief period of ischemia due to cuff inflation. The reactive hyperemia has been closely associated with NO availability and the change from baseline in FBF and FMD are directly related to EF.

During the assessment of EF, an intra-arterial catheter can be used to infuse different agents such as acetylcholine and L-NMMA. Sublingual doses of nitroglycerin are also commonly used during the assessment of EF. These agents have helped researchers characterize the role of endothelial cells in mediating vascular relaxation and have strengthened the role NO has on EF. Unfortunately, many laboratories do not have the means, for various reasons, to access these clinical drugs. This emphasizes the importance in understanding various mechanisms that control for NO availability. Biomarkers, such as TNFα, IL-6, ET, and CRP, can be easily measured and are suggested to be directly involved in the regulation of NO availability.

Myokines, known as cytokines released from skeletal muscle, are generally secreted in response to PA. Although several cytokines will soon be acknowledged as

myokines, only one cytokine has been recognized as a myokine. IL-6 is known to have numerous secretion sites, including adipose and skeletal tissue. Acute exercise studies support the role of cytokines released from areas other than adipose tissue and as described in this review, it is proposed that myokines may have different functional roles than adipokines. Myokines may help support the well understood mechanics of glucose homeostasis during exercise and increased blood flow to contracting muscles in need of more oxygen.

Future research directions include an extended time course of acute exercise-induced adaptation on EF. The time course of exercise induced adaptations on EF has been evaluated in only a few studies giving rise to the need for a similar study in healthy and obese subjects. Exercise training study results are inconclusive as to whether the exercise interventions improve EF or whether improvements in EF are simply a reflection of the alterations from the last exercise bout performed. A study designed to address this issue would be beneficial in establishing an optimal time point to assess EF following an exercise training program. Findings may also allow researchers to further understand the impact an acute exercise bout can have on regulating the vasodilatory capacity of the arterial wall.

Manuscript 1

THE EXTENDED TIME COURSE OF FOREARM BLOOD FLOW
FOLLOWING A SINGLE MAXIMAL BOUT OF EXERCISE

THE EXTENDED TIME COURSE OF FOREARM BLOOD FLOW FOLLOWING A SINGLE MAXIMAL BOUT OF EXERCISE

Abstract

Chronic exercise is thought to improve endothelium-dependent vasodilation; however, studies evaluating endothelial dysfunction (ED) following an exercise training program lack a standardized time frame in which to measure vascular function (VF). Although most studies require subjects to abstain from exercise for 24 hours prior to any VF measures, no study to date has assessed VF longer than 24 hours after the cessation of exercise. **Purpose:** To evaluate VF, as determined by the assessment of forearm blood flow (FBF) and vascular reactivity (VR) before and up to 48 hours after a single bout of maximal exercise in healthy male volunteers. **Methods:** Twelve male subjects volunteered to participate. FBF was assessed before and during reactive hyperemia (RH). FBF measures were obtained prior to (PRE-E), immediately after (POST-E), and at 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after exercise. Total excess flow, calculated as the difference between baseline FBF and FBF during RH, was used as an indicator of VR. Blood samples were also obtained at each time point to evaluate the response of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which are potential modifiers of VF. **Results:** Baseline FBF and FBF during RH were significantly ($P < 0.05$) increased POST-E before returning to baseline values by POST-1. VR was significantly ($P < 0.05$) increased POST-E and was not significantly ($P < 0.05$) reduced until POST-48. Concentrations of IL-6 and TNF-α were unchanged in response to exercise. **Conclusions:** These results suggest that measurements used to verify

improvements in VF following exercise training should be employed after a minimum of 48 hours following physical activity.

Introduction

In healthy individuals, the quiescent state of the vascular system is maintained by vasoactive compounds produced by endothelial cells. These compounds are responsible for cellular adhesion, thromboresistance, smooth muscle cell proliferation, vessel wall inflammation, and most importantly, modulating vascular tone (5). Reduced endothelium-dependent vasodilation, also known as endothelial dysfunction (ED), is associated with the early sub-clinical stages of atherosclerosis and often the result of a decreased nitric oxide (NO) bioavailabity (12, 18). Several inflammatory bio-markers, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are directly related to NO bioavailability and subsequently, vascular function (VF) (14, 15, 22). TNF-α has been shown to inhibit NO release from endothelial cells and degrade nitric oxide synthase mRNA, which can lead to an impairment of endothelium-dependent vasodilation (15, 22). The role of IL-6 has recently received renewed attention as its origin of secretion is now thought to determine its function. Whereas IL-6 released from adipose tissue is associated with an increase in the pro-inflammatory marker C-reactive protein (15), IL-6 secreted from contracting skeletal muscle is associated with the stimulation of anti-inflammatory cytokines and inhibition of TNF-α (14). Furthermore, exercise training studies do not support a change in resting TNF-α or IL-6 concentrations (13, 19).

TNF-α and IL-6 concentrations have been shown to significantly increase immediately following acute exercise in healthy individuals, as well as those with

inflammatory disease (10). However, there are no studies that evaluate the extended time course of inflammatory markers after a single bout of exercise. Louis and colleagues recently demonstrated that TNF-α mRNA in muscle was significantly elevated immediately after acute exercise, as well as at 12 and 24 hours post-exercise (11). Likewise, IL-6 mRNA in muscle was significantly elevated immediately after acute exercise, and at 2, 4, 8, 12, and 24 hours post-exercise. An extended evaluation of inflammatory markers after acute exercise may contribute to a further understanding of the mechanisms influencing changes in VF following exercise.

Chronic aerobic exercise training is thought to improve ED with much of the improvement in the vasodilatory capacity being attributed to the effects of an increase in exercise-induced shear stress production of NO (7, 8, 21). However, studies evaluating ED following an exercise training program lack a standardized time frame in which to measure VF following the final training bout. There are no studies that have included a specified amount of time after training to measure VF, with most reporting an abstinence of exercise for 24 hours (6-8, 17, 21, 23).

Few studies have evaluated the effect of acute exercise on VF. Bousquet-Santos and colleagues evaluated VF by measuring forearm blood flow (FBF) and vascular reactivity (VR) in healthy sedentary volunteers for two hours following a maximal exercise bout (4). VR, calculated as the excess blood flow following a 5 minute bout of ischemia, was significantly elevated immediately after exercise and remained elevated for an hour, before returning to baseline within two hours of exercise. Likewise, Umpierre and colleagues measured FBF in stable chronic heart failure (CHF) patients and healthy

controls up to 24 hours after a sub-maximal exercise bout (20). Although no significant differences in FBF were seen between the groups across time, the FBF in CHF patients and healthy volunteers were back to baseline within 30 and 60 minutes, respectively.

Neither of the aforementioned studies that evaluated FBF after exercise assessed inflammatory markers, which as previously stated, may affect prolonged post-exercise FBF measures. Therefore, the purpose of this study was to evaluate an extended time course of FBF and vascular reactivity (VR) changes following a maximal aerobic bout of exercise for up to 48 hours. Additionally, we aimed to evaluate the changes in blood markers associated with vascular endothelium activation following maximal exercise to further understand possible contributions to FBF response.

Methods

Subjects

Thirteen apparently healthy male subjects between the ages of 20 and 25 years volunteered to participate in this study. Subjects were excluded if they were smokers, had been diagnosed with diabetes or high blood pressure, had any form of known cardiovascular disease, or were taking medications that would potentially affect vascular function. Subjects were also excluded if they were participating in high levels of physical activity as determined by the International Physical Activity Questionnaire at the time of the study. Subjects were instructed to refrain from exercise for 3 days prior to the start of the study and during the course of the study. Subjects were also instructed to refrain from alcohol and caffeine for 24 hours prior to testing and during the 3 days of testing. Written informed consent was obtained from each subject before participation in the study. All

procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Study Design

Subjects were instructed to report to the Human Performance Laboratory at 6:30 a.m. following an overnight fast. Subjects completed a medical history questionnaire and physical activity readiness questionnaire (PAR-Q) prior to the start of testing. Additionally, a physical activity diary was employed during the course of the study to evaluate physical activity outside of the study protocol. After a 30-min rest period, a 7 ml blood sample was obtained from an anticubital vein. Body composition was assessed with dual-energy x-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI). Resting heart rate (HR), blood pressure (BP), and FBF measures were taken prior (PRE-E) to the maximal exercise test. Blood samples and subsequent BP and FBF measures were taken immediately after exercise (POST-E) and 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after the cessation of exercise. All FBF measures were taken on the right arm, opposite of all blood sampling. All subjects were asked to remain in the laboratory until after the 2 hour post-exercise measurements. On days 2 and 3, subjects reported to the laboratory following an overnight fast 30 minutes prior to times corresponding to 24 and 48 hours after the cessation of exercise, respectively. A blood sample, BP, and FBF were taken following a 30-minute rest period.

Maximal Exercise Protocol

Each subject was fitted with a heart rate monitor and respiratory gas analysis equipment (VMAX Spectra, Sensormedics Corp., Yorba Linda, CA) before completing a

graded exercise test to exhaustion on a treadmill. Following a 3-minute warm-up period, the treadmill speed was increased to elicit 80% of each individual's age-predicted maximal heart rate within 4 minutes. Thereafter, grade was increased by 2% every 2 minutes until the subject could no longer maintain the treadmill pace. The protocol was designed to last 8 – 12 minutes. Heart rate was obtained every minute and rating of perceived exertion (RPE) was obtained once every exercise stage. Following an immediate blood pressure measurement, a 1-minute post-exercise capillary blood lactate sample (Lactate Scout, Sports Resource Group, Inc.) was obtained and used as a partial criterion to ensure that the subject gave a maximal effort. Breath-by-breath oxygen consumption was averaged every 10 seconds and the highest averaged value was identified as the maximal oxygen consumption (VO_{2max}).

Forearm Blood Flow Measurement

Forearm blood flow (FBF) was assessed using mercury-in-rubber strain-gauge plethysmography (MSGP; Model AI6, D.E. Hokanson, Inc., Bellevue, WA). Blood pressure cuffs were positioned around each subject's upper right arm and right wrist, and a mercury-in-rubber strain gauge was placed around the forearm approximately 10 cm distal to the olecranon process (1). During each trial, the wrist cuff was inflated to a pressure of 240 mmHg prior to each measurement to occlude hand circulation. Baseline FBF was determined by rapidly inflating the upper cuff to 40 mmHg for 10 seconds to occlude venous flow during a 20 second cycle. Nine measurements were recorded to determine the average rate of volume change during venous occlusion (ml/100 ml of forearm tissue volume/min). Subsequently, the upper arm cuff was inflated to 240 mmHg to induce

forearm ischemia for a period of 5 minutes. After 5 minutes of occlusion, the cuff was released and FBF, as described above, was determined during a 3-minute period of reactive hyperemia (RH). Total FBF, both at baseline and during RH were assessed as an area under the curve (AUC), calculated as a flow-time index. Vascular reactivity (VR), indicated by total excess blood flow above baseline, was calculated as total FBF during RH minus total FBF at baseline.

Biochemical Analyses

Blood samples for analysis of TNF-α and IL-6 were collected into serum separator tubes and allowed to clot for 30 minutes before being centrifuged for 15 minutes at ~1000 x g. Serum was immediately aliquoted into microtubes and stored at -80°C until analyzed. Concentrations of TNF-α and IL-6 were determined through enzyme high-sensitivity immunoassay according to manufacturer's specifications (R&D Systems, Minneapolis, MN). All samples were run in duplicate and the mean concentration of each sample was used in statistical analysis.

Statistical Analyses

A one-way ANOVA (SPSS, Chicago, IL: V17.0) was used to determine the impact of a maximal exercise test on FBF, VR, and serum cytokine (IL-6 and TNF-α) concentration changes across six different time periods (PRE-E, POST-E, POST-1, POST-2, POST-24, and POST-48). When indicated by a significant *F*-ratio, a Bonferroni post-hoc analysis was performed to identify differences. Additionally, Pearson product-moment correlations were utilized to evaluate relationships among cardiorespiratory fitness values, vascular function, and cytokine (IL-6 and TNF-α) responses following the maximal

effort. Descriptive statistics were used to illustrate subject demographics and exercise-induced physiological variables to the maximal bout of exercise. All data are expressed as mean ± SEM unless otherwise noted. Statistical significance was set at $P < 0.05$.

Results

Subject Characteristics and physiological responses to acute maximal exercise

Of the thirteen subjects that volunteered for this study, one individual requested to withdraw from the study due to an aversion to the blood sampling procedures. Therefore, 12 subjects were used in the final analyses. Subject characteristics are presented in Table 1. Table 2 provides the physiological responses to the maximal exercise test. The total duration of the maximal exercise test did not include the warm-up. One volunteer had difficulty breathing through the respiratory gas sensor and had the flow sensor removed during the maximal exercise test, however the subject continued with the protocol until expressing volitional fatigue. Therefore, 11 subjects were used for all VO_{2max} data analyses.

FBF in response to acute maximal exercise

The AUC for FBF at baseline and during RH are shown in Figures 1 and 2, respectively. A one-way ANOVA revealed a significant *F*-ratio for FBF, both at baseline and during RH, across time ($P = 0.0001$ and $P = 0.001$, respectively). A Bonferroni post-hoc analysis revealed that FBF, at baseline and during RH, was significantly ($P < 0.0001$) elevated POST-E compared to PRE-E, before returning to baseline values within POST-1 ($P < 0.01$, POST-E vs. POST-1). FBF, at baseline and during RH, remained at values similar to PRE-E at POST-2, POST-24, and POST-48. VR, expressed as total excess

blood flow, is shown in Figure 3. A one-way ANOVA revealed a significant ($P = 0.014$) F-ratio for VR across time. Furthermore, a Bonferroni post-hoc analysis revealed that VR was significantly elevated ($P = 0.017$) POST-E compared to PRE-E. VR remained elevated, although not significantly different from PRE-E, at POST-1, POST-2, and POST-24 before returning to values at POST-48 significantly ($P = 0.044$) lower than POST-E.

Moreover, VR PRE-E was not significantly correlated to VO_{2max} per lean muscle mass ($r = 0.348$, $P = 0.295$). However, VR PRE-E was significantly correlated to BMI ($r = .749$, $P = 0.005$), fat mass ($r = 0.812$, $P = 0.001$), and percent fat ($r = 0.651$, $P = 0.022$).

IL-6 and TNF-α response to acute maximal exercise

Figure 4 illustrates the IL-6 and TNF-α response to acute maximal exercise. A one-way ANOVA revealed that IL-6 and TNF-α were not significantly different across time ($P = 0.173$ and $P = 0.653$, respectively). Additionally, IL-6 and TNF-α were not significantly correlated to VR across time ($r = 0.050$, $P = 0.678$ and $r = 0.172$, $P = 0.158$, respectively). The mean coefficient of variation for all IL-6 and TNF-α samples run was 17.98 ± 2.00% and 10.94 ± 1.03, respectively.

Discussion

This is the first study that has evaluated FBF and VR at time points up to 48 hours after a single maximal bout of aerobic exercise. The main finding of this study was that VR following a single maximal bout of aerobic exercise was not significantly reduced from the immediate post-exercise measure until 48 hours after the cessation of exercise. FBF and FBF during RH significantly increased immediately after exercise, before returning to pre-exercise levels within 1 hour of exercise. Additionally, IL-6 and TNF-α

did not significantly change from baseline values for the duration of this study. However, IL-6 did increase up to 2 hours following exercise, before decreasing below pre-exercise concentrations.

Similar to other studies measuring FBF and VR following an acute aerobic exercise bout, our study did show an increase in FBF and VR immediately following exercise (2-4, 9). Kingwell and colleagues demonstrated an increase in FBF immediately following 30 minutes of cycling at 65% of VO_{2max} in healthy individuals (9). However, it is unclear how long the increase in FBF lasted after exercise, as the investigators only presented results from three subjects who continued to have an increased FBF at 60 minutes post exercise. FBF and VR have been measured after a maximal exercise test in only two other studies. Bousquet-Santos and colleagues demonstrated a significant increase in FBF during RH and VR immediately following a maximal exercise test in healthy sedentary individuals (4). The increase in FBF during RH and VR were still significantly elevated at 1 hour post exercise before returning to baseline levels within 2 hours post exercise. It is important to note that our healthy males had a greater cardiorespiratory fitness level, demonstrated by a greater relative VO_{2max} (50.9 ± 1.1 vs. 44.8 ± 2.1, $mlO_2/kg/min$), than those previously reported. Rognmo and colleagues speculated that lower fitness levels were often associated with smaller arteries that may have a greater vascular dilation response due to an increased hyperemic shear stress (16). The previously reported FBF during RH and VR that was significantly increased 1 hour after maximal exercise may have been attributed to a subject population with smaller arteries and a subsequent greater hyperemic shear stress. Baynard and colleagues evaluated the difference in FBF during

RH in resistance trained versus endurance trained men following a maximal exercise test (2, 3). FBF during RH was significantly elevated immediately after exercise in resistance and endurance trained individuals.

IL-6 and TNF-α were not significantly different at any time point up to 48 hours following a maximal bout of aerobic exercise. This finding is in contrast to previous reports that have demonstrated a significant increase in IL-6 and TNF-α following a maximal bout of exercise (10). Additionally, Louis and colleagues recently suggested that the concentration of IL-6 and TNF-α mRNA cytokine release in response to exercise varied significantly up to 48 hours post-exercise (11). An acute sub-maximal bout of aerobic exercise significantly increased IL-6 mRNA immediately and 4, 8, 12 and 24 hours post-exercise. TNF-α mRNA demonstrated a temporal response to exercise as well with significant increases immediately and 2, 12, and 24 hours following an acute sub-maximal bout of aerobic exercise. Louis and colleagues did not determine the functional protein expression translated from the increased mRNA markers following acute aerobic exercise. Any change in IL-6 and TNF-α during an extended time-course following exercise could affect endothelium-dependent vasodilation. However, the IL-6 and TNF-α responses to acute exercise in the current study suggest that FBF and VR at rest and following a single maximal aerobic bout of exercise are independent of the response of these blood markers in healthy males.

These results must be viewed within the context of this study's limitations. We did not measure the size of the arteries in the forearm under investigation, therefore can not conclude that variation in shear stress was responsible for differences seen in our study

compared to results previously reported. It is also important to note that we did not take a sample of blood between our immediate post-exercise measure and 1 hour after exercise and are unable to conclude that a shortened inflammatory response did not occur within our measurement period. Additionally, if the functional protein expression of IL-6 and TNF-α varied following exercise in a similar response to the mRNA expression previously indicated, then measurement time becomes crucial in determining a change in biomarker activity. Lastly, although the sample size for this study is small, it is in accordance with other studies investigating this area of research (2-4, 16, 20).

In conclusion, a single maximal bout of aerobic exercise resulted in an immediate increased VR that did not significantly reduce from the immediate post-exercise value until 48 hours after exercise. Additionally, FBF at baseline and during RH significantly increased immediately after exercise before falling to pre-exercise values within 1 hour. These changes in FBF and VR following exercise demonstrate that although healthy males have an increased vascular conductance immediately after exercise, they also have a significantly enhanced reactive vasodilation immediately following exercise. Although VR at 1, 2, 24, and 48 hours after exercise were not significantly different from pre-exercise values, these results suggest that any increase observed in VR up to 24 hours after exercise may be directly due to the previous exercise bout. We did not measure VR between 24 and 48 hours and can not speculate any changes that may have occurred between those two measurement periods. Exercise training studies that may use VR as an assessment of vascular improvements following exercise need to be cautious in taking pre and post measurements at different times of the day due to any diurnal variations that could

exist. Therefore, although further research is necessary to understand VF following a single maximal bout of aerobic exercise, this study recommends that any measurement used to assertively elucidate improvements in VF following exercise training should be performed at least 48 hours after the last exercise session. IL-6 and TNF-α did not change in response to a single maximal bout of exercise and do not support the changes seen in FBF following the exercise session. Future studies are warranted that investigate the acute extended time course effects of various exercise intensities and VR measurement times in healthy individuals.

Table 1 Subject characteristics, N = 12

Variable	Mean ± SEM
Age (years)	21.6 ± 0.4
Weight (kg)	67.8 ± 2.8
BMI (kg/m^2)	21.9 ± 0.3
Percent Fat (%)	19.8 ± 1.4
Fat Mass (kg)	13.0 ± 1.2
Lean Mass (kg)	51.9 ± 1.9
Resting HR (bpm)	56.0 ± 2.2
Resting SBP (mm Hg)	119.3 ± 4.2
Resting DBP (mm Hg)	66.6 ± 2.1
Baseline TNF-α (pg/mL)	1.2 ± 0.1
Baseline IL-6 (pg/mL)	1.3 ± 0.5

BMI, Body Mass Index; HR, Heart Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TNF-α, Tumor Necrosis Factor – alpha; IL, Interleukin.

Table 2 Physiological responses to acute maximal exercise, N = 12

Variable	Mean ± SEM
Maximal Lactate (mmol/L)	10.6 ± 0.7
Maximal RPE	19.5 ± 0.1
Maximal HR (bpm)	195.9 ± 2.3
Testing Time (s)	709.9 ± 33.6
Relative VO_{2max} (mLO_2/BM/min)	50.9 ± 1.1
Relative VO_{2max} (mLO_2/LM/min)	66.1 ± 1.2
Absolute VO_{2max} (LO_2/min)	3.4 ± 0.1

RPE, Rating of Perceived Exertion; HR, Heart Rate; VO_{2max}, Maximal Oxygen Consumption; BM, Body Mass (kg); LM, Lean Mass (kg).

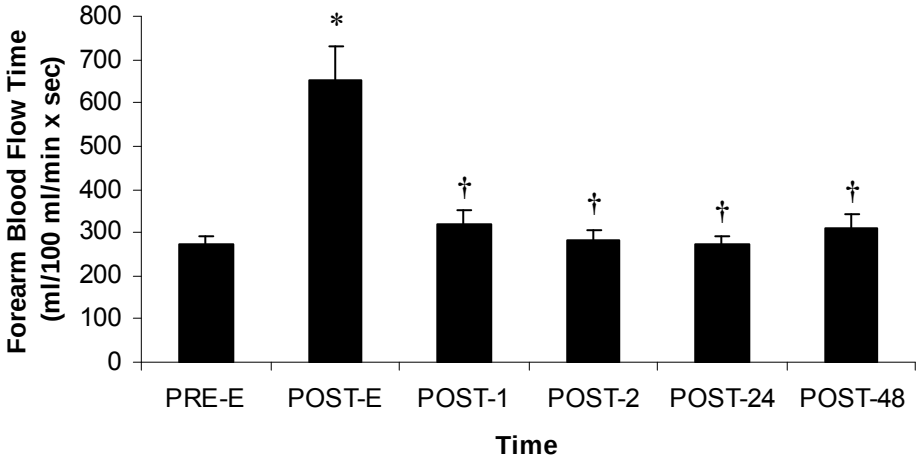

Figure 1. Forearm blood flow during baseline calculated as the area under the curve (mean ± SEM). *$p<0.05$ vs. PRE-E, †$p<0.05$ vs. POST-E.

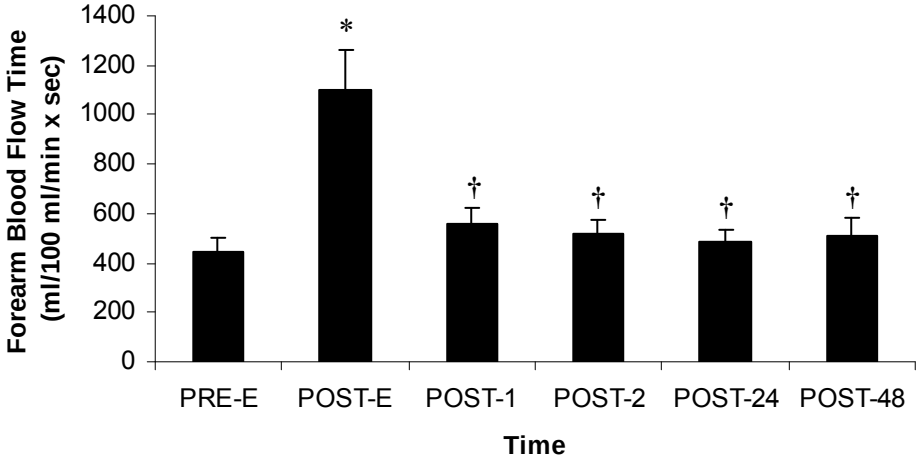

Figure 2. Forearm blood flow during reactive hyperemia calculated as the area under the curve (mean ± SEM). *p<0.05 vs. PRE-E, †p<0.05 vs. POST-E.

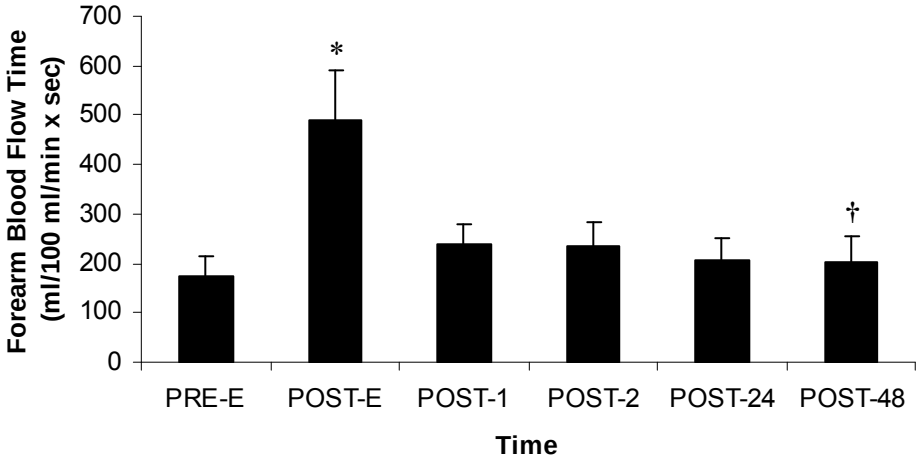

Figure 3. Vascular reactivity, calculated as total RH FBF minus total baseline FBF (mean ± SEM). *$p<0.05$ vs. PRE-E, †$p<0.05$ vs. POST-E.

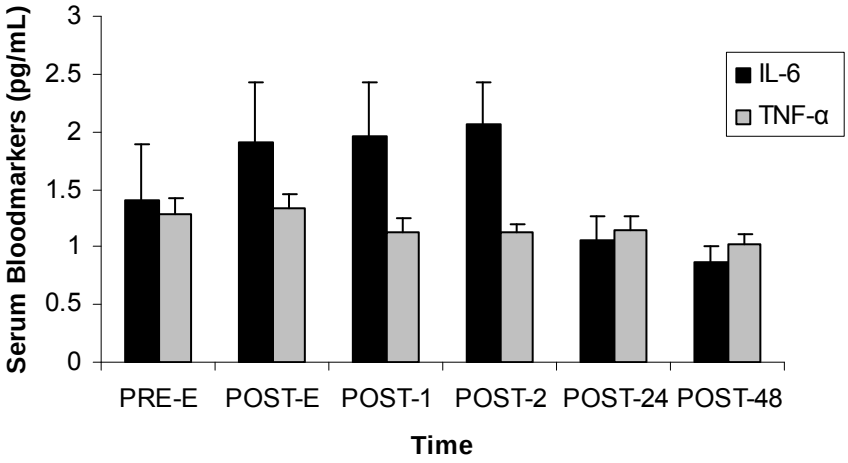

Figure 4. The effect of acute maximal exercise on serum concentrations of IL-6 and TNF-α (mean ± SEM).

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Manuscript 2

FOREARM BLOOD FLOW RESPONSE TO AN ACUTE MAXIMAL EXERCISE BOUT IN OBESE AND NORMAL WEIGHT MALES

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Abstract

One of the earliest sub-clinical stages associated with atherosclerosis is endothelial dysfunction (ED), which has been shown to predict future cardiovascular events. Chronic exercise is thought to improve endothelium-dependent vasodilation; however, few studies have evaluated the effects of acute exercise on vascular function (VF). In addition, no studies have compared VF responses in obese and non-obese individuals following acute exercise. **Purpose:** To evaluate VF, as determined by the assessment of forearm blood flow (FBF) and vascular reactivity (VR) before and up to 48 hours after a single bout of maximal exercise in obese and non-obese males. **Methods:** Twelve obese (37.0 ± 1.1 kg/m^2) and twelve non-obese (21.9 ± 0.3 kg/m^2) males volunteered to participate. FBF was assessed before and during reactive hyperemia (RH). FBF measures were obtained prior to (PRE-E), immediately after (POST-E), and at 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after exercise. Total excess flow, calculated as the difference between baseline FBF and FBF during RH, was used as an indicator of VR. Blood samples were also obtained to evaluate the response of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), which are potential modifiers of VF. **Results:** Baseline FBF and FBF during RH were significantly ($P < 0.05$) increased in both groups POST-E before returning to baseline values by POST-1. VR was enhanced in both groups POST-E, although the magnitude of change was significantly greater in non-obese males. Concentrations of IL-6 and TNF-α were unchanged in response to exercise in both groups.

Conclusions: An acute bout of maximal exercise significantly increased forearm endothelium-dependent vasodilation in non-obese and obese males. Additionally, an increased reactive vasodilation was observed only in non-obese males following exercise.

Introduction

The most recent prevalence report of overweight and obesity in the United States estimates that the incidence of obesity has continued to rise, as 32.2% of all individuals over the age of 20 years are classified as obese (21). This is particularly alarming given that obesity is widely considered an independent risk factor for cardiovascular disease (CVD) (23). Atherosclerosis, a group of diseases distinguished by arterial wall thickening, accounts for approximately three-fourths of all CVD deaths (27). Additionally, atherosclerosis is a progressive condition where pathological changes will occur many years earlier in arteries prior to the onset of adult clinical symptoms. One of the earliest sub-clinical stages in the atherosclerotic process is the impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (ED) (29). ED can be detected prior to the development of atherosclerotic plaques in both coronary and peripheral vessels and has been associated with the pathogenesis of numerous CVD risk factors (7). The assessment of vascular function can therefore be useful in detecting the onset of atherosclerosis, quantifying cardiovascular risk, and determining the effectiveness of intervention programs (14, 38).

The endothelium is a dynamic paracrine organ made up of a single layer of cells and found between the lumen and the smooth muscle cells of blood vessels. This layer of cells respond to physical and chemical signals by secreting factors that regulate vascular

tone, cellular adhesion, cell migration and proliferation, and vascular inflammation (6, 7). In healthy individuals, the quiescent state of the vascular system is maintained by vasoactive compounds released by endothelial cells, most notably nitric oxide (NO). Vascular shear stress that accompanies increased blood flow during exercise has been shown to stimulate the release of NO (20). Chronic aerobic exercise training is known to decrease CVD risks and numerous studies have shown that vascular function improves with recurring bouts of aerobic exercise in both healthy individuals and those at higher risk of CVD (5, 10, 12, 13, 19, 28, 34, 36, 39). Moreover, the improvement in vascular function following aerobic exercise training appears to be independent of any simultaneous improvement in CVD risk factors (11).

Research supports the potential role of systemic inflammation in the pathophysiology of ED (4, 8, 17, 18, 22, 24, 35). Obesity is characterized as a state of chronic low-grade inflammation and several circulating inflammatory markers, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are directly related to adiposity (4, 8, 17). TNF-α has been shown to inhibit NO release from endothelial cells and degrade nitric oxide synthase mRNA, which can lead to an impairment of endothelium-dependent vasodilation (24, 35). The role of IL-6 has recently received renewed attention as its origin of secretion is now thought to determine its function. Whereas IL-6 released from adipose tissue is associated with an increase in the pro-inflammatory marker of C reactive protein (24), IL-6 secreted from contracting skeletal muscle is associated with the stimulation of anti-inflammatory cytokines and inhibition of TNF-α (22). Although no studies have evaluated the extended time course of IL-6 and TNF-α following exercise, Louis and

colleagues demonstrated increased IL-6 and TNF-α mRNA changes in skeletal muscle up to 24 hours following an acute bout of exercise (18).

Few studies have evaluated the effect of acute exercise on vascular function. Bousquet-Santos and colleagues measured forearm blood flow (FBF) and vascular reactivity (VR) in healthy sedentary volunteers for two hours following a maximal exercise bout (3). VR was significantly elevated immediately after exercise and remained elevated for an hour before returning to baseline within two hours of exercise. Likewise, Umpierre and colleagues measured FBF in stable chronic heart failure (CHF) patients and healthy controls up to 24 hours after a sub-maximal exercise bout (31). No significant differences in FBF were observed between the groups across time and FBF had returned to baseline in both groups within one hour.

The extended response of FBF and VR to maximal exercise in obese individuals has not been evaluated. Therefore, the purpose of this study was to evaluate the FBF and VR changes in obese and non-obese males following a maximal aerobic exercise bout. Additionally, we evaluated the changes in blood markers associated with vascular endothelium activation following maximal exercise in both groups.

Methods

Subjects

Twenty five male subjects between the ages of 19 and 29 years volunteered to participate in this study. Subjects were categorized by their BMI into either a non-obese (n = 13, BMI $\leq$ 25 kg/m^2) or obese (n = 12, BMI $\geq$ 30 kg/m^2) group. Subjects were excluded from the study if they were smokers, had been diagnosed with diabetes or high blood

pressure, had any form of known cardiovascular disease, or were taking medications that would potentially affect vascular function. Subjects were also excluded if they were participating in high levels of physical activity as determined by the International Physical Activity Questionnaire at the time of the study. Subjects were instructed to refrain from exercise for 3 days prior and alcohol and caffeine for 24 hour prior to the start of the study and during the course of the study. Written informed consent was obtained from each subject before participation in the study. All procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Study Design

Subjects were instructed to report to the Human Performance Laboratory at 6:30 a.m. following an overnight fast. Subjects completed a medical history questionnaire and physical activity readiness questionnaire (PAR-Q) prior to the start of testing. Additionally, a physical activity diary was employed to evaluate physical activity outside of the study protocol. After a 30-min rest period, a 7 ml blood sample was obtained from an anticubital vein. Body composition was assessed with dual-energy x-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI). Resting heart rate (HR), blood pressure (BP), and FBF measures were taken prior (PRE-E) to the maximal exercise test. Blood samples and subsequent BP and FBF measures were taken immediately after exercise (POST-E) and 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after the cessation of exercise. All FBF measures were taken on the right arm, opposite of all blood sampling. All subjects were asked to remain in the laboratory until after the 2 hour post-exercise measurements. On days 2 and 3, subjects reported to

the laboratory, following an overnight fast, 30 minutes prior to times corresponding to 24 and 48 hours after the cessation of exercise, respectively. A blood sample, BP, and FBF were taken following a 30-minute rest period.

Maximal Exercise Protocol

Each subject was fitted with a heart rate monitor and respiratory gas analysis equipment (VMAX Spectra, Sensormedics Corp., Yorba Linda, CA) before completing a graded exercise test to exhaustion on a treadmill. Following a 3-minute warm-up period, the treadmill speed was increased to elicit 80% of each individual's age-predicted maximal heart rate within 4 minutes. Thereafter, grade was increased by 2% every 2 minutes until the subject could no longer maintain the treadmill pace. The protocol was designed to last 8 – 12 minutes. Heart rate was obtained every minute and rating of perceived exertion (RPE) was obtained once every exercise stage. Following an immediate blood pressure measurement, a 1-minute post-exercise capillary blood lactate sample (Lactate Scout, Sports Resource Group, Inc) was obtained and used as a partial criterion to ensure that the subject gave a maximal effort. Breath-by-breath oxygen consumption was averaged every 10 seconds and the highest averaged value was identified as the maximal oxygen consumption (VO_{2max}).

Forearm Blood Flow Measurement

Forearm blood flow (FBF) was assessed using mercury in-rubber strain gauge plethysmography (MSGP; Model AI6, D.E. Hokanson, Inc., Bellevue, WA). Blood pressure cuffs were positioned around each subject's upper right arm and right wrist, and a mercury-in-rubber strain gauge was placed around the forearm approximately 10 cm distal

to the olecranon process (1). During each trial, the wrist cuff was inflated to a pressure of 240 mmHg prior to each measurement to occlude hand circulation. Baseline FBF was determined by rapidly inflating the upper cuff to 40 mmHg for 10 seconds to occlude venous flow during a 20 second cycle. Nine measurements were recorded to determine the average rate of volume change during venous occlusion (ml/100 ml of forearm tissue volume/min). Subsequently, the upper arm cuff was inflated to 240 mmHg to induce forearm ischemia for a period of 5 minutes. After 5 minutes of occlusion, the cuff was released and FBF, as described above, was determined during a 3-minute period of reactive hyperemia (RH). Area under the curve (AUC), calculated as a flow-time index, provided values for total FBF both at baseline and during RH. Vascular reactivity (VR), indicated by total excess blood flow above baseline, was calculated as total FBF during RH minus total FBF at baseline.

Biochemical Analyses

Blood samples for analysis of TNF-α and IL-6 were collected into serum separator tubes and allowed to clot for 30 minutes before being centrifuged for 15 minutes at 1000 x g. Serum was immediately aliquoted into microtubes and stored at -80°C until analyzed. Concentrations of tumor necrosis factor-α and interleukin-6 were determined through enzyme high-sensitivity immunoassay according to manufacturer's specifications (R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate and the mean concentration of each sample was used during the statistical analysis.

Statistical Analysis

A mixed between-within repeated measures (RM) ANOVA (SPSS, Chicago, IL: V16.0) was used to determine the impact of a maximal exercise bout on systolic blood pressure (SBP), FBF, VR, and serum cytokine (IL-6 and TNF-α) concentration changes between the two groups (non-obese and obese males) across six different time periods (PRE-E, POST-E, POST-1, POST-2, POST-24, and POST-48). If a main effect was observed within groups, a one-way ANOVA was used to determine differences across time, with a Bonferroni post-hoc analysis being performed to identify differences. Additionally, Pearson product-moment correlations were utilized to evaluate relationships among cardiorespiratory fitness values, vascular function, and cytokine (IL-6 and TNF-α) concentration responses following the maximal effort. Subject demographics were compared using descriptive statistics and independent *t*-tests. All data are expressed as mean ± SEM unless otherwise noted. Statistical significance was set at $P < 0.05$.

Results

Subject Characteristics

Of the twenty-five subjects that volunteered for this study, one individual in the non-obese group requested to withdraw from the study due to an aversion to the blood sampling procedures. Therefore, 12 subjects in both the non-obese and obese group were used in the final analyses. Subject characteristics for both non-obese and obese males are presented in Table 1. Body mass, BMI, body fat percentage, fat mass, fat-free mass, resting HR and SBP were significantly ($P < 0.05$) different between the groups. Additionally, PRE-E TNF-α concentration was significantly ($P = 0.037$) elevated in obese males compared to non-obese males.

Physiological variables in response to acute maximal exercise

Table 2 provides the physiological variables in response to the maximal exercise test. The total duration of the maximal exercise test (not including warm-up) was significantly ($P < 0.05$) higher in the non-obese males compared to the obese males. One non-obese male had difficulty breathing through the respiratory gas sensor. The subject had the flow sensor removed during the maximal exercise test, however the subject continued with the protocol until expressing volitional fatigue. Therefore, the non-obese group had 11 subjects for all VO_{2max} data analyses. VO_{2max} expressed per kilogram of body mass and lean mass was significantly ($P < 0.05$) greater in non-obese males. Absolute VO_{2max} was significantly ($P < 0.05$) greater in obese males. Maximal lactate, RPE, and HR were similar in both groups. Furthermore, RM ANOVA revealed that SBP had significant main effects for group ($P < 0.001$) and time ($P < 0.001$). A group x time interaction was not significant for SBP. A one-way ANOVA revealed a significant ($P < 0.001$) *F*-ratio for SBP across time for both groups. Furthermore, a Bonferroni post-hoc analysis revealed that SBP was significantly elevated ($P < 0.05$) POST-E compared to PRE-E, before returning to values similar to PRE-E at POST-1, POST-2, POST-24, and POST-48.

FBF in response to acute maximal exercise

The AUC for PRE-E FBF, at baseline and during RH, for both groups are shown in Figure 1. As demonstrated in Figure 1, obese males had a significant elevation in FBF (Obese, 406.0 ± 32.8 ml/100 ml/min·sec vs. Non-obese, 271.4 ± 19.3 ml/100 ml/min·sec; $P = 0.002$) and FBF during RH (Obese, 714.8 ± 54.3 ml/100 ml/min·sec vs. Non-obese,

447.0 ± 51.0 ml/100 ml/min·sec; $P = 0.002$) at PRE-E measures. RM ANOVA revealed that FBF, both at baseline and during RH, had significant main effects for group (Baseline FBF, $P = 0.014$; RH FBF, $P = 0.013$) and time (Baseline FBF, $P = 0.001$; RH FBF, $P = 0.001$). A group x time interaction was not significant for FBF at baseline or during RH. A one-way ANOVA revealed a significant ($P < 0.001$) *F*-ratio for FBF, at baseline and during RH, for both groups across time. Furthermore, a Bonferroni post-hoc analysis revealed that FBF, at baseline and during RH, for both groups was significantly elevated ($P < 0.05$) POST-E compared to PRE-E, before returning to values similar to PRE-E at POST-1, POST-2, POST-24, and POST-48.

VR, expressed as total excess blood flow above baseline, is shown in Figure 2. RM ANOVA revealed that VR had significant main effects for group ($P = 0.033$) and time ($P = 0.026$). A group x time interaction was not significant for VR. A one-way ANOVA revealed a significant ($P = 0.014$) *F*-ratio for VR across time for only non-obese males. Furthermore, a Bonferroni post-hoc analysis revealed that VR was significantly elevated ($P = 0.017$) POST-E compared to PRE-E in non-obese males. VR remained elevated in non-obese males, although not significantly different from PRE-E, at POST-1, POST-2, and POST-24 before returning to values at POST-48 significantly ($P = 0.044$) lower than POST-E. In obese males, a one-way ANOVA revealed that VR was not significantly ($P = 0.497$) different across time. As demonstrated figure 2, an independent sample *t*-test revealed a significantly higher VR PRE-E in obese males compared to non-obese males (308.8 ± 29.7 ml/100 ml/min·sec vs. 175.5 ± 38.7 ml/100 ml/min·sec, $P = 0.012$).

The VR measures across time were significantly correlated to observed SBP across time (r = 0.359, $P < 0.001$). Furthermore, VR PRE-E was not significantly correlated to VO_{2max} per lean muscle mass (r = -0.207, $P = 0.343$). However, VR PRE-E was significantly correlated to BMI (r = .475, $P = 0.019$), fat mass (r = 0.446, $P = 0.029$), lean mass (r = 0.527, $P = 0.008$), and percent fat (r = 0.508, $P = 0.011$). Figure 3 illustrates the relation between VR and percent fat.

IL-6 and TNF-α response to acute maximal exercise

One non-obese male did not have a blood sample collected at 1 hour post exercise and one obese male had an undetectable IL-6 concentration at 24 hours post exercise. Therefore, the non-obese group had 11 subjects for IL-6 and TNF-α analysis at 1 hour post exercise and the obese group had 11 subjects for IL-6 analyses at 24 hours post exercise. Although IL-6 was not significantly different between groups at PRE-E, TNF-α was significantly ($P = 0.037$) elevated in obese compared to non-obese males (Table 1). RM ANOVA revealed that IL-6 had a significant main effect for group ($P = 0.032$) and time ($P = 0.008$). A group x time interaction was not significant with IL-6. A one-way ANOVA revealed a significant ($P = .014$) *F*-ratio for IL-6 across time for only obese males. A Bonferroni post-hoc analysis did not reveal any differences in IL-6 across time in obese males. However, a paired-sample *T*-test between the non-obese and obese male's peak IL-6 (POST-2) and lowest IL-6 (POST-48) revealed a significant reduction ($P < 0.05$) of 57.63 and 66.82%, respectively.

Additionally, RM ANOVA revealed that TNF-α had a significant main effect for group ($P = 0.017$) and time ($P = 0.027$). A group x time interaction was not significant

with TNF-α. A one-way ANOVA with TNF-α samples from all males combined revealed a non-significant ($P = 0.786$) F-ratio across time. Additionally, when TNF-α was compared within non-obese and obese males, a one-way ANOVA revealed a non-significant F-ratio for non-obese ($P = 0.653$) and obese males ($P = 0.978$) across time.

Furthermore, VR, BMI, percent fat, and fat mass were not significantly correlated to IL-6 or TNF-α. The mean coefficient of variation for all IL-6 and TNF-α samples run was 16.0 ± 1.3% and 9.9 ± 0.6%, respectively.

Discussion

This is the first study that evaluated FBF and VR in obese males after a single maximal bout of aerobic exercise. The main finding in this study was that a single maximal bout of aerobic exercise resulted in an immediate post-exercise significant increase in VR in non-obese individuals only. Although obese individuals did not demonstrate a significant increase in VR following a single maximal bout of exercise, the VR response following exercise was not significantly different between non-obese and obese males. FBF response to exercise was not significantly different at baseline and during RH between non-obese and obese males. FBF and FBF during RH significantly increased in both groups immediately after exercise, before returning to PRE-E levels within 1 hour of exercise.

Furthermore, the exercise response of IL-6 and TNF-α was not significantly different in non-obese and obese males. The magnitude of change in IL-6 at POST-48 from POST-2 was greater in obese males. No response was observed in TNF-α for up to 48 hours following a single bout of maximal exercise in non-obese and obese males.

Similar to previous studies, following an acute aerobic exercise bout, our study revealed an increase in FBF and VR immediately following exercise (2, 3, 15, 32). Kingwell and colleagues demonstrated an increase in FBF immediately following 30 minutes of cycling at 65% of VO_{2max} in healthy individuals (15). However, it is unclear how long the increase in FBF lasted after exercise, as the investigators only showed results from three subjects who continued to have an increased FBF at 60 minutes post exercise. FBF and VR have been measured after a maximal exercise test in only two other studies. Bousquet-Santos and colleagues demonstrated a significant increase in FBF during RH and VR immediately following a maximal exercise test in healthy sedentary individuals (3). The increase in FBF during RH and VR were still significantly elevated at 1 hour post exercise before returning to baseline levels within 2 hours post exercise. Baynard and colleagues evaluated the difference in FBF during RH in resistance trained versus endurance trained men following a maximal exercise test (2). Similar to our study, there were significant differences in VO_{2max} and testing time when comparing the two groups. FBF during RH was significantly elevated after exercise, although no differences were shown between the two groups both prior to and following exercise. However, Baynard and colleagues did not report the FBF after exercise, which would have allowed for differentiation of improved VR or vascular conductance after exercise (2).

ED has been associated with obesity, as well as the progression of CHF (30, 37). In a recent study, Umpierre and colleagues evaluated the difference in FBF and FBF during RH following a sub-maximal cycle test in CHF patients versus healthy controls (31). There were no significant differences in FBF or FBF during RH between the CHF

patients and healthy controls prior to exercise (31). FBF following exercise had a similar temporal response between the two groups. FBF was significantly elevated after the exercise session and returned to pre-exercise values within 1 hour after exercise. FBF during RH was significantly elevated in both groups at 30 minutes post-exercise and significantly different between CHF patients and healthy controls. Although our FBF during RH response was not significantly different between non-obese and obese males immediately following exercise, we did not measure FBF at 30 minutes post-exercise in non-obese and obese males. These findings between CHF patients and healthy controls, in which a difference in endothelium-dependent vasodilation is expected across groups, were similar to our findings. In our study, although the magnitude of change in VR was higher in non-obese males, our obese males presented normal endothelium-dependent vasodilation following a single maximal aerobic bout of exercise. Additionally, this study is the first to demonstrate an enhanced vascular conductance in obese males immediately following a maximal bout of exercise. An increased vascular conductance immediately after exercise had previously been shown in non-obese healthy individuals (3).

Although IL-6 was not significantly different at baseline between our non-obese and obese males, TNF-α was significantly elevated in obese males at pre-exercise levels. While both markers have been shown to be significantly correlated with adiposity levels, Van Guilder and colleagues suggested that this correlation is only present among obese individuals who also have the metabolic syndrome (MetS) (24, 26, 33). Although a mechanism responsible for the difference in obese individuals with and without MetS is unknown, obese individuals without MetS had similar baseline concentrations of IL-6 and

TNF-α to that of normal weight individuals (33). In the current study, we did not access the presence of the MetS in our obese males. However, the similarity in IL-6 and TNF-α previously reported between normal weight individuals and obese individuals without the MetS may help explain the current study's similar temporal response to IL-6 and TNF-α following exercise in non-obese and obese males. Additionally, it has previously been reported that IL-6 and TNF-α significantly increase following a maximal bout of exercise (16). In spite of these earlier findings, IL-6 and TNF-α did not significantly increase following exercise in our study. However, it is important to note that in both groups, there was a significant reduction between the highest IL-6 concentration (POST-2) and lowest IL-6 concentration (POST-48). Although we do not know the origin of secretion for the IL-6 measured in this study, these results potentially demonstrate an anti-inflammatory effect of muscle derived IL-6 following maximal exercise. It is important to note that we did not take a sample of blood between POST and 1 hour after exercise and are unable to conclude that a shortened response did not occur within our measurement periods. Furthermore, the IL-6 and TNF-α response to acute exercise led us to believe that FBF and VR at rest and following a single maximal aerobic bout of exercise are independent of these blood markers in non-obese and obese males.

These results must be viewed within the context of this study's limitations. Studies have demonstrated with an infusion of a NO inhibitor, such as N^{G}-monomethyl-$_{L}$-arginine (L-NMMA), that FBF is dependent on NO bioavailability in both sedentary and trained individuals (13, 15). Shear stress, generated by flow during exercise and RH, is one of the most important mechanical regulators of endothelial NO synthase (9). Although we did

not measure shear stress or NO metabolites, we can not discount that a difference in shear stress may have contributed to the distinct VR magnitude changes observed between non-obese and obese males after exercise. Conversely, we also did not measure the size of the arteries in the forearm under investigation. Rognmo and colleagues speculated that lower fitness levels were often associated with smaller arteries that may have a greater vascular dilation response due to an increased hyperemic shear stress (25). Our non-obese males had a significantly lower cardiorespiratory fitness level, demonstrated by a lower VO_{2max} per FFM. The significantly enhanced FBF and FBF during RH in obese males may have been attributed to a smaller artery size and subsequent enhanced hyperemic shear stress. This is further illustrated by the significant main effect for SBP between groups, in which SBP was elevated in obese individuals across all time points. Lastly, there were no FBF measurements between the end of exercise and 1 hour following exercise. As described above, changes in FBF following exercise may have occurred within the first hour after exercise (31).

In conclusion, a single maximal aerobic bout of exercise results in increased FBF, at baseline and during RH, in non-obese and obese males. Additionally, non-obese males had a greater magnitude change than obese males in VR immediately following exercise. These changes in FBF and VR following exercise demonstrate that although non-obese and obese males have an increased vascular conductance immediately after exercise, only non-obese males have a significantly enhanced reactive vasodilation following exercise. TNF-α did not change in response to a single maximal bout of exercise and does not further explain the changes seen in FBF following the exercise session. Likewise,

although the response of IL-6 to maximal exercise was not significantly different between non-obese and obese males, both groups demonstrated a significant reduction in IL-6 at peak values, 2 hours post-exercise, compared to 48 hours post-exercise. This change could help explain an anti-inflammatory effect of muscle-derived IL-6. However, the IL-6 response to exercise did not explain the observed response to FBF following the exercise session. Future studies are warranted that investigate the acute effects of various exercise intensities and VR measurement times in non-obese and obese individuals.

Table 1 Subject characteristics

Variable	Non-Obese	Obese
Age (years)	21.6 ± 0.4	23.0 ± 1.0
Weight (kg)	67.8 ± 2.8*	121.3 ± 5.0
BMI (kg/m^2)	21.9 ± 0.3*	37.0 ± 1.1
Percent Fat (%)	19.8 ± 1.4*	41.3 ± 1.6
Fat Mass (kg)	13.0 ± 1.2*	48.5 ± 3.5
Lean Mass (kg)	51.9 ± 1.9*	67.9 ± 2.5
Resting HR (bmp)	56.0 ± 2.2*	64.0 ± 2.9
Resting SBP (mm Hg)	119.3 ± 4.2*	134.6 ± 3.2
Resting DBP (mm Hg)	66.6 ± 2.1	73.0 ± 2.7
Baseline IL-6 (pg/mL)	1.4 ± 0.5	1.7 ± 0.5
Baseline TNF-α (pg/mL)	1.3 ± 0.1*	1.7 ± 0.2

* $P < 0.05$, Non-obese vs. Obese. Values are mean ± SEM.

BMI, Body Mass Index; HR, Heart Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; IL, Interleukin; TNF-α, Tumor Necrosis Factor alpha.

Table 2 Physiological variables in response to acute maximal exercise

Variable	Non-Obese	Obese
Maximal Lactate (mmol/L)	10.6 ± 0.7	9.9 ± 0.7
Maximal RPE	19.5 ± 0.1	19.1 ± 0.2
Maximal HR (bpm)	195.9 ± 2.3	197.0 ± 1.7
Test Duration (s)	709.9 ± 33.6*	545.0 ± 20.7
Relative VO_{2max} (mLO_2/kg of BW/min)	50.9 ± 1.1*	34.8 ± 1.2
Relative VO_{2max} (mLO_2/kg of LM/min)	66.1 ± 1.2*	61.8 ± 1.6
Absolute VO_{2max} (LO_2/min)	3.4 ± 0.1*	4.1 ± 0.1

* $P < 0.05$, Non-obese vs. Obese. Values are mean ± SEM.

RPE, Rating of Perceived Exertion; HR, Heart Rate; VO_{2max}, Maximal Oxygen Consumption; BW, Body Weight; LM, Lean Mass.

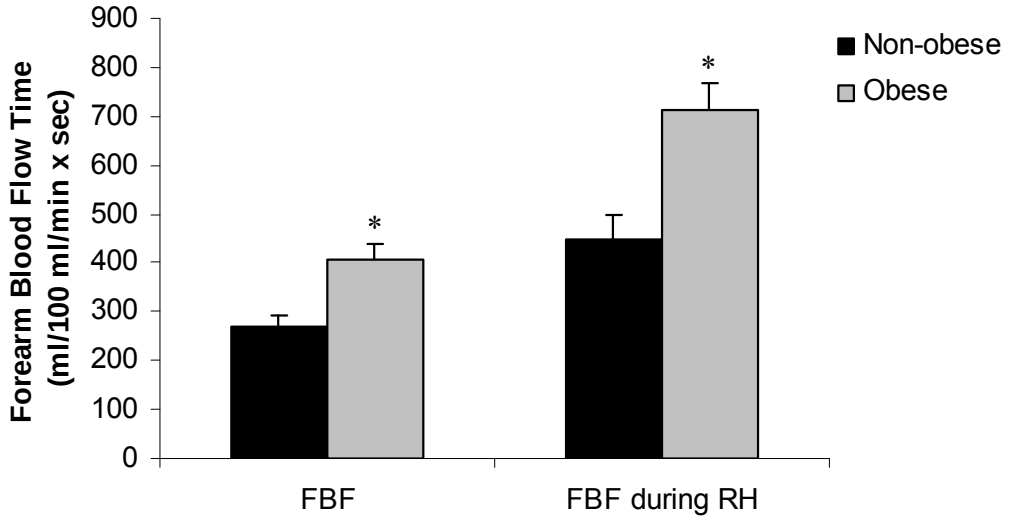

Figure 1. PRE-E Forearm blood flow calculated as the area under the curve (mean ± SEM). *$p<0.05$ Non-obese vs. Obese

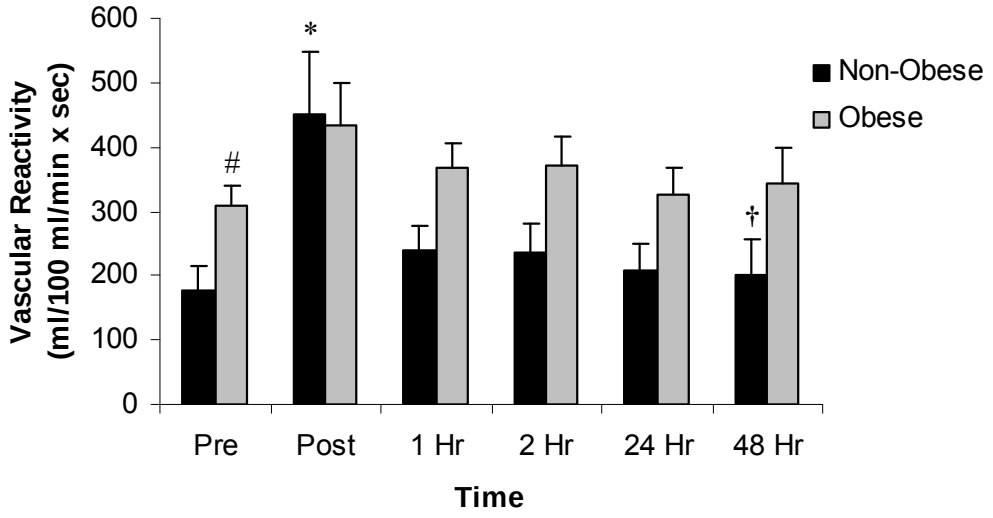

Figure 2. Vascular reactivity, calculated as total RH FBF minus total baseline FBF (mean ± SEM). #p<0.05, PRE-E, Obese vs. Non-obese; *p<0.05, Non-obese, POST-E vs. PRE-E; †p<0.05, Non-obese, POST-48 vs. POST-E.

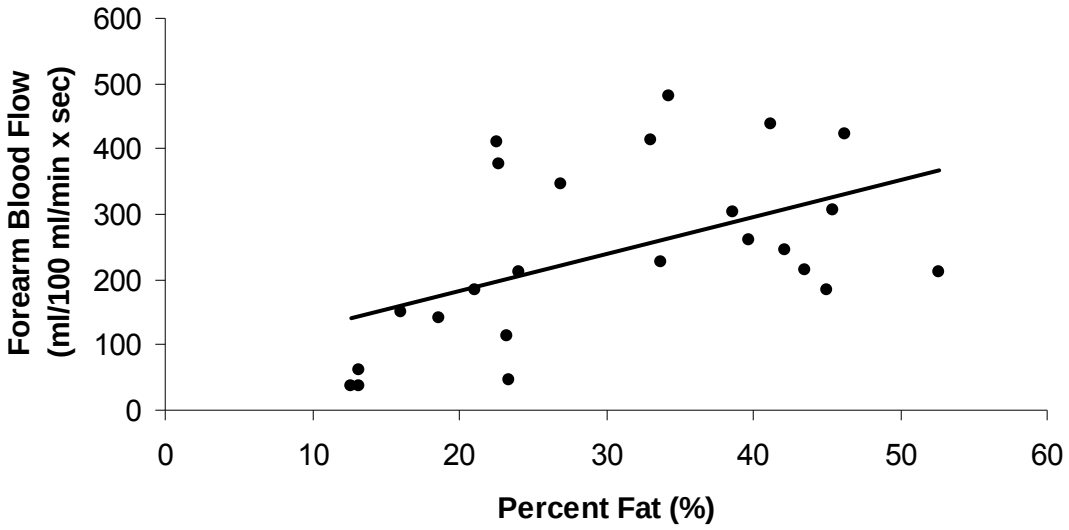

Figure 3. Relation between VR PRE-E and Percent Fat (r = 0.508, *P* = 0.011).

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APPENDIX A

Expanded Methods

Subjects

Forty male subjects between the ages of 18 and 30 years with body mass index (BMI) values below 25 kg/m^2 (N = 20) and greater than 30 kg/m^2 (N = 20) were recruited for the study through flyers posted on campus and through classroom visits. All classroom visits were approved by the instructor and/or department chair. Classroom visits lasted less than 5 minutes and consisted of a research staff member describing the project and answering any questions posed by potential subjects. Potential subjects were not encouraged to make their interest in participating known during the classroom visit, but were given a contact number for the Health and Human Performance Laboratory so that contact could be made at a later time. Potential subjects were made aware that their participation was completely voluntary and that the course instructor would not be made aware of who did or did not participate in the project.

Twenty five male subjects between the ages of 19 and 29 years volunteered to participate in this study. Subjects were categorized by their BMI into either a non-obese (n = 13, BMI $\leq$ 25 kg/m^2) or obese (n = 12, BMI $\geq$ 30 kg/m^2) group. Subjects were excluded from the study if they were smokers, had been diagnosed with diabetes or high blood pressure, had any form of known cardiovascular disease, or were taking medications that

would potentially affect vascular function. Subjects were also excluded if they were participating in high levels of physical activity as determined by the International Physical Activity Questionnaire (IPAQ) at the time of the study. The IPAQ is a short (4 questions), 7-day physical activity recall for use with young and middle-aged adults. The types of activity assessed in the IPAQ short include walking, moderate-intensity and vigorous-intensity activities. The amount of activity for each type assessed was given a separate score that required the summation of the duration (minutes) and frequency (days). Walking, moderate-intensity, and vigorous-intensity were designated MET values of 3.3, 4.0, and 8.0 respectively. High physical activity consisted of individuals meeting one of 2 criteria: 1.) Vigorous-intensity activity on at least 3 days and accumulating at least 1,500 MET-minutes/week or 2.) 7 or more days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3,000 MET-minutes/week. Subjects were instructed to refrain from exercise for 3 days prior and alcohol and caffeine for 24 hour prior to the start of the study and during the course of the study. Written informed consent was obtained from each subject before participation in the study. All procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Study Design

Subjects were instructed to report to the Human Performance Laboratory at 6:30 a.m. following an overnight fast. Subjects completed a medical history questionnaire and physical activity readiness questionnaire (PAR-Q) prior to the start of testing. Additionally, a physical activity diary was employed to evaluate physical activity outside of the study protocol. After a 30-min rest period, a 7 ml blood sample was obtained from

an anticubital vein. Body composition was assessed with dual-energy x-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI). Resting heart rate (HR), blood pressure (BP), and FBF measures were taken prior (PRE-E) to the maximal exercise test. Blood samples and subsequent BP and FBF measures were taken immediately after exercise (POST-E) and 1 (POST-1), 2 (POST-2), 24 (POST-24), and 48 (POST-48) hours after the cessation of exercise. All FBF measures were taken on the right arm, opposite of all blood sampling. All subjects were asked to remain in the laboratory until after the 2 hour post-exercise measurements. On days 2 and 3, subjects reported to the laboratory, following an overnight fast, 30 minutes prior to times corresponding to 24 and 48 hours after the cessation of exercise, respectively. A blood sample, BP, and FBF were taken following a 30-minute rest period.

Maximal Exercise Protocol

Each subject was fitted with a heart rate monitor and respiratory gas analysis equipment (VMAX Spectra, Sensormedics Corp., Yorba Linda, CA) before completing a graded exercise test to exhaustion on a treadmill. Following a 3-minute warm-up period (3 mph at 0% grade), the treadmill speed was increased to elicit 80% of each individual's age-predicted maximal heart rate within 4 minutes. Thereafter, grade was increased by 2% every 2 minutes until the subject could no longer maintain the treadmill pace. The protocol was designed to last 8 – 12 minutes. Heart rate was obtained every minute and rating of perceived exertion (RPE) was obtained once every exercise stage. Following an immediate blood pressure measurement, a 1-minute post-exercise capillary blood lactate sample (Lactate Scout, Sports Resource Group, Inc) was obtained and used as a partial

criterion to ensure that the subject gave a maximal effort. Breath-by-breath oxygen consumption was averaged every 10 seconds and the highest averaged value was identified as the maximal oxygen consumption (VO_{2max}).

Forearm Blood Flow Measurement

Forearm blood flow (FBF) was assessed using mercury in-rubber strain gauge plethysmography (MSGP; Model AI6, D.E. Hokanson, Inc., Bellevue, WA). Blood pressure cuffs were positioned around each subject's upper right arm and right wrist, and a mercury-in-rubber strain gauge was placed around the forearm approximately 10 cm distal to the olecranon process (1). During each trial, the wrist cuff was inflated to a pressure of 240 mmHg prior to each measurement to occlude hand circulation. Baseline FBF was determined by rapidly inflating the upper cuff to 40 mmHg for 10 seconds to occlude venous flow during a 20 second cycle. Nine measurements were recorded to determine the average rate of volume change during venous occlusion (ml/100 ml of forearm tissue volume/min). Subsequently, the upper arm cuff was inflated to 240 mmHg to induce forearm ischemia for a period of 5 minutes. After 5 minutes of occlusion, the cuff was released and FBF, as described above, was determined during a 3-minute period of reactive hyperemia (RH). Each measurement period was edited one at a time. Area under the curve (AUC), calculated as a flow-time index, provided values for total FBF both at baseline and during RH. Flow-time index was calculated as the summation of two consecutive time periods, divided by 2 and multiplied by the measurement time (20 seconds). Vascular reactivity (VR), indicated by total excess blood flow above baseline, was calculated as total FBF during RH minus total FBF at baseline.

Biochemical Analyses

Blood samples for analysis of TNF-α and IL-6 were collected into serum separator tubes and allowed to clot for 30 minutes before being centrifuged for 15 minutes at 1000 x g. Serum was immediately aliquoted into microtubes and stored at -80°C until analyzed. Concentrations of tumor necrosis factor-α and interleukin-6 were determined through enzyme high-sensitivity immunoassay according to manufacturer's specifications (R&D Systems, Minneapolis, MN).

Masks and gloves were worn during the preparation of all assay kits as a precautionary measure. Distilled water was used to prepare a wash buffer. Specifications of plate washer were set as follows:

Plate: R&D Systems

Bottom Shape: Flat

Centering: 0.8 mm

Asp. Hor. Pos.: 2.0 mm

Asp. Vert. Pos.: 12.0 mm

Bot. Vert. Pos.: 11.0 mm

B.W. Vert. Pos.: 9.0 mm

Horizontal Speed: 4

Vertical Speed: 4

Asp. Downw. Speed: 0

Disp. Upw. Speed: 2

Bot. Downw. Speed: 6

Bot. Upward Speed: 9

Shaking Amplitude: 0

Shaking Speed: 0

Kit: R&D Systems

Method 1: Wash

Mode: Plate

Crosw. Asp.: No

Asp. Time: 1.0 s

Volume: 400 µl

Overflow: 3.2 mm

Liquid: Wash W1

Flow: 00

Nr. Of Cycles: 6

Soaking: 30 s

Met. Intervals: 0 m 0 s

Method 2: Asp.

Mode: Plate

Crosw. Asp.: No

Asp. Time: 1.5 s

Nr. Of Cycles: 1

Soaking: 0 m 0 s

Serum cytokine concentration was determined using a microplate reader (Model 680 XR, Bio-Rad Laboratories, Inc., Hercules, CA). The reading parameters for the endplate protocol were set 490 nm (measurement filter) and 650 nm (reference filter). All samples were analyzed in duplicate and the mean concentration of each sample was used during the statistical analysis.

Statistical Analysis

A mixed between-within repeated measures (RM) ANOVA (SPSS, Chicago, IL: V16.0) was used to determine the impact of a maximal exercise bout on systolic blood pressure (SBP), FBF, VR, and serum cytokine (IL-6 and TNF-α) concentration changes between the two groups (non-obese and obese males) across six different time periods (PRE-E, POST-E, POST-1, POST-2, POST-24, and POST-48). If a main effect was observed within groups, a one-way ANOVA was used to determine differences across time, with a Bonferroni post-hoc analysis being performed to identify differences. Additionally, Pearson product-moment correlations were utilized to evaluate relationships among cardiorespiratory fitness values, vascular function, and cytokine (IL-6 and TNF-α) concentration responses following the maximal effort. Subject demographics were compared using descriptive statistics and independent *t*-tests. All data are expressed as mean ± SEM unless otherwise noted. Statistical significance was set at $P < 0.05$.

VITA

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Academic Education

Ph.D. Virginia Commonwealth University, 2009
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HPEX 375	Exercise Physiology
HPEX 350	Sport Nutrition
HPEX 200	Strength, Endurance and Flexibility Training
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HPR 302	Techniques to Evaluating Fitness
HPR 105	Concepts in Physical Fitness
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Honors and Awards

American Heart Association's Award: 2002 Student Scholar in Cardiovascular Disease and Stroke
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Certifications

ACSM Health Fitness Specialist, 2008 - present
ACSM Health Fitness Instructor, 2001 – 2008
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Publications

Franco R.L., Evans R.K. Vascular function changes following an acute bout of

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Professional Presentations

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Rogers M., **Franco R.L.**, Whitehead M.T., Evans R.K., Soukup J., Webster M.J., Scheett T.P. Effect of the police corps law enforcement training program on aerobic and anaerobic power. *Southeast Region American College of Sports Medicine*, Birmingham, AL, February, 2009.

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Franco R.L., J.T. Soukup, J.C. Taylor, R.K. Evans. The Effect of Moderate Congestive Heart Failure on Myosin Heavy Chain Distribution in the Rat Diaphragm. *Southeast Region American College of Sports Medicine*, Atlanta, GA, January, 2003.

Grants

Principle Investigator, "Effects of a progressive running program on body weight status, cardiorespiratory fitness, and cardiovascular risk factors of obese adolescents". Saucony Run for Good Foundation, December, 2009. **($10,000.00; Not Funded)**

Investigator, "Time course of vascular function changes following an acute maximal exercise bout in obese and normal weight males". VCU School of Education Research Initiation Award, July 1, 2007. **($8,998.00; Funded)**

Investigator, "Alterations in physical fitness, physical activity behavior and attitudes, quality of life and cardiovascular disease risk factors following gastric bypass surgery". VCU School of Education Research Initiation Award, July 1, 2006. **($5,000.00; Funded)**

Investigator**,** "Effects of Immulina supplementation on Exercise-induced muscle soreness and indicators of inflammation and tissue damage". Nordic Immotech, APS, December, 2006. **($12,697.00; Not Funded)**

Co-Investigator, "The effect of myocardial volume overload-induced congestive heart failure on myocardial MCT1 content". American Heart Association Student Award, 2002. **($2,000.00; Funded)**