ACUTE BRACHIAL ARTERY RESPONSES TO EXERCISE IN HEALTHY MALES

ACUTE BRACHIAL ARTERY RESPONSES TO ENDURANCE AND HIGH-INTENSITY INTERVAL EXERCISE IN YOUNG HEALTHY MALES

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TITLE: Acute Brachial Artery Responses to Endurance and High-Intensity Interval Exercise in Young Healthy Males

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ABSTRACT

Purpose: Habitual aerobic exercise improves vascular function; however, the acute vascular response to exercise is poorly understood. The present investigation compared the time course of acute changes in vascular function following two different types of aerobic exercise. **Methods:** Ten untrained males (23 ± 2 yrs) completed one bout of sustained moderate-intensity cycling (END) (30 mins at 55% peak power) or high-intensity interval (HIT) cycling (10 one-minute intervals at 80% peak power) on different days. Endothelium-dependent dilation was assessed by brachial artery flow-mediated dilation (baFMD) at baseline, immediately post-exercise, 1 hour post-exercise and 24 hours post-exercise. Endothelium-independent dilation was assessed via nitroglycerin (NTG) at all time points, except 1 hour post-exercise. **Results:** baFMD values were not significantly different between END and HIT at any time point. Immediately post-exercise baFMD values were unchanged from baseline. 1 Hour post-exercise, relative ($p \leq 0.001$) and absolute ($p \leq 0.05$) baFMD values were attenuated compared to all other time points for both HIT (%FMD baseline: 5.9 ± 2.3%; 1 hour post-exercise: 2.5 ± 1.5%) and END (%FMD baseline: 6.8 ± 2.4%; 1 hour post-exercise: 2.6 ± 1.9%). Relative ($p \leq 0.05$) and absolute ($p \leq 0.05$) NTG responses were attenuated immediately post-exercise compared to baseline for both HIT (%NTG baseline: 18.8 ± 4.4%; immediately post-exercise: 12.3 ± 3.1%) and END (%NTG baseline: 18.3 ± 3.1%; immediately post-exercise: 10.9 ± 4.9%). **Conclusions:** Immediately post-exercise, endothelium-dependent dilation is maintained; but reduced 1 hour following exercise cessation. Similar acute vascular responses are found following HIT and END.

Key words: endothelium; interval exercise; flow-mediated dilation; nitroglycerin

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TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

Tables from Chapter 2: Acute brachial artery responses to endurance and high-intensity interval exercise in young healthy males

LIST OF FIGURES

Figures from Chapter 2: Acute brachial artery responses to endurance and high-intensity interval exercise in young healthy males

LIST OF ABBREVIATIONS AND SYMBOLS

%FMD	Relative flow-mediated dilation
%NTG	Relative NTG
AbsFMD	Absolute flow-mediated dilation
AbsNTG	Absolute NTG
Ach	Acetylcholine
baFMD	Brachial artery flow-mediated dilation
CAD	Coronary artery disease
cGMP	Cyclic guanosine monophosphate
CHF	Congestive heart failure
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
END	Moderate-intensity endurance exercise
eNOS	Endothelial nitric oxide synthase
FMD	Flow-mediated dilation
HIT	High- intensity interval exercise
HR	Heart rate
ICC	Intraclass correlation coefficient
MBV	Mean blood velocity
MI	Myocardial infarction
NO	Nitric oxide
NormFMD	Normalized flow-mediated dilation
NTG	Nitroglycerin
PAD	Peripheral arterial disease
PPO	Peak power output
PW	Pulsed wave
ROS	Reactive oxygen species
RPM	Revolutions per minute
SBP	Systolic blood pressure
SR	Shear rate
SR_{AUC}	Area under the shear rate vs. time curve
VO_2 peak	Peak oxygen consumption

CHAPTER 1

Literature Review

1.1 Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for approximately 30% of all deaths. The most lethal cardiovascular diseases are coronary artery disease (CAD) and cerebrovascular disease, which are responsible for heart attacks and strokes respectively [1]. Both heart attack and stroke are typically the manifestation of an underlying pathology known as atherosclerosis, in which the arterial walls become rich with plaque, ultimately resulting in narrowing of the lumen and reduced arterial compliance. In turn, blood flow is hindered and, in the event one of these plaques ruptures, heart attack or stroke can occur [1].

While the burden of CVD has begun to stabilize or decrease slightly in high-income countries, this has been offset by a marked increase in incidence in low- and middle-income countries, thereby contributing to rising CVD rates globally. This trend will likely continue with the ever-growing reach of globalization, as developing countries continue to industrialize and urbanize, with parallel increases in CVD risk factors [2]. CVD risk factors include hypertension, diabetes, hypercholesterolemia, obesity, tobacco use, physical inactivity and excessive intake of alcohol, salt, fat and calories [1]. While many of these risk factors are modifiable, increasing physical activity levels represents a unique opportunity for mitigating CVD risk because of the effect increasing activity has on improving multiple CVD risk factors.

1.2 Exercise training and risk reduction

Physical inactivity is a predictor of all-cause and CVD mortality [3, 4] and has been attributed to 37% of all CAD-related deaths; second only to hyperlipidemia [5]. Increasing activity level has been shown to improve blood pressure [6-8], glucose regulation [9, 10], blood lipids [11-13], obesity [14, 15] and inflammatory biomarkers [16, 17], with an overall CVD risk reduction of 30-50% [16]. Exercise also plays a role in smoking cessation, reducing cigarette cravings and tobacco withdrawal symptoms [18, 19]. Moreover, clinical populations, including individuals with documented CVD and those who have suffered a CVD-related event, stand to benefit from habitual exercise. Meta-analyses of cardiac rehabilitation programs have found mortality rates 20-32% lower in individuals enrolled in exercise-based programs compared to non-exercise based rehabilitation [20-23]. Patients with chronic heart failure have demonstrated reduced mortality, fewer hospitalizations, fewer cardiac events and improved health related quality of life through participation in regular exercise programs [24-26]. The benefits of habitual exercise are experienced by healthy individuals, those with multiple CVD risk factors and a variety of clinical populations [27, 28] and moving forward, should be a mainstay of CVD prevention, management and rehabilitation.

1.3 The exercise stimulus

During a bout of dynamic, submaximal aerobic exercise, such as walking, running or cycling, the metabolic demands of active skeletal and cardiac muscle increase. Heart rate and stroke volume also increase, augmenting cardiac output 5-8 fold over resting values and mildly increasing systolic blood pressure. Sympathetic output is enhanced, causing vasoconstriction of the renal and splanchnic regions, while a variety of local

factors allow vascular beds in active skeletal muscle to overcome sympathetic stimulation and dilate [29]. The vasodilation in the vascular beds of cardiac and active skeletal muscle results in a reduction in local resistance, facilitating greater blood flow to these regions. All these factors contribute to a response termed exercise hyperemia, in which blood is diverted from the viscera and directed towards cardiac and active skeletal muscle [29]. One of the regulators of the vascular response to exercise, and the focus of this paper, is a layer of cells lining the vasculature called the endothelium.

1.4 The endothelium

The endothelium is a thin layer of cells lining the interior of the entire circulatory system and is responsible for maintaining vascular homeostasis. Endothelial cells produce a variety of factors that collectively regulate blood flow and blood pressure [30]. Nitric oxide (NO) is the most potent endothelial-derived vasodilator, synthesized from L-arginine by eNOS - the endothelial isoform of NO synthase – in response to shear stress against the vascular wall or a variety of signaling molecules [31]. Once released, NO diffuses through the abluminal cell surface into smooth muscle cells where it activates a signaling cascade, resulting in the formation of cGMP. cGMP prevents calcium from entering the smooth muscle cell, reducing intracellular calcium concentration, promoting smooth muscle cell relaxation and causing the vessel to dilate [31-33]. This form of vasodilation is termed endothelium-dependent vasodilation and is involved in the maintenance of resting vascular tone and the changing hemodynamic states during exercise [34-36].

Additionally, NO has many anti-atherogenic properties including inhibition of platelet aggregation [35, 37], prevention of low-density lipoprotein oxidation [38, 39] and prevention of smooth muscle cell proliferation and migration [37, 39, 40]. Dysfunction of the endothelium, characterized by insufficient NO release and or elevated NO scavenging, is correlated with several risk factors for coronary artery disease, including hypercholesterolemia, smoking, hypertension, diabetes, estrogen deficiency, advanced age and obesity [35, 41-43]. Moreover, endothelial dysfunction has been identified as one of the primary steps in atherosclerosis development, evident before plague accumulation detectable by angiography or ultrasound assessment [39]. Habitual physical activity has been demonstrated to improve endothelial function in populations with CAD, type II diabetes, obesity, hypercholesterolemia, hypertension and congestive heart failure [36, 44], as well as preventing age-related decline in function [45]. Improved endothelial function in response to exercise training is believed to represent one of the mechanisms involved in the partially unexplained risk-reduction for CVD associated with exercise [44, 46].

1.5 Assessing vascular function

1.5.1 Flow-mediated dilation (FMD) assessment

First described by Celermajer *et al.* in 1992, flow-mediated dilation (FMD) is a non-invasive assessment used to gauge endothelial function in peripheral conduit arteries [47]. FMD describes the arterial dilation that occurs in response to increased blood flow through an artery, generated through exercise, localized heating, vasodilator infusion or physical occlusion and reperfusion.

FMD assessments are commonly performed in the brachial, radial, femoral or popliteal arteries and typically begin by inflating a pneumatic cuff to suprasystolic pressure, occluding blood flow to the distal region of a given limb [48]. During cuff inflation, the arterioles downstream of the inflation site dilate, reducing vascular resistance. When the cuff is deflated, reactive hyperemia ensues as blood rushes through the artery supplying the ischemic vascular bed. The substantial increase in flow through the artery increases shear stress along the endothelium, activating a variety of signaling pathways culminating in NO release and vasodilation [49]. While the specific pathway through which shear stress elicits NO release is incompletely understood, there is considerable evidence for the involvement of endothelial calcium-activated potassium channels. When these channels open in response to the mechanical forces associated with shear stress, the cell becomes hyperpolarized, promoting calcium entry, which stimulates the production and release of NO [50-52]. While this mechanism has been suggested as the primary contributor to FMD [53], it should be acknowledged that there are multiple mechanosensitive arterial structures and other vasodilators contributing to arterial dilation in response to increased flow [32, 54, 55].

1.5.1.1 NO-mediated FMD

Depending on the protocol used, FMD assessments can elicit vasodilation that is almost exclusively dependent on endothelial-NO. If these specifications are followed, an FMD assessment under resting conditions can be used as an assay of NO-bioavailability [49]. Multiple studies have demonstrated that brachial artery dilation following a distal cuff placement and five-minute occlusion is primarily NO mediated, whereas dilation in response to longer bouts of occlusion and/or proximal cuff placement involves additional

mechanisms [55, 56]. Placement of the occluding cuff is of critical importance for evoking an exclusively NO mediated response. Multiple investigations have revealed that placing the cuff proximal to the site of FMD measurement yields greater dilation than distal placement and was previously believed to represent maximum endothelium-dependent dilation [57-60]. Further investigation revealed that the larger dilation elicited by proximal cuff placement appears to be only partially NO mediated and therefore a poor measure of endothelium-derived NO [57].

Furthermore, arterial infusion of the vasodilator acetylcholine has been used to perform FMD assessments in the past; however, the gradual increase in flow evoked by acetylcholine appears to involve other vasodilatory mechanisms, in addition to endothelial-NO [55]. FMD should be performed using rapid increases in flow, such as achieved by inflation and deflation of a pneumatic cuff. In summary, investigations to date suggest that NO-mediated FMD requires a five-minute occlusion period with an instantaneous increase in flow and cuff placement distal to the site of measurement.

1.5.1.2 Brachial artery FMD

Because endothelial dysfunction is a systemic phenomenon, brachial artery FMD (baFMD) has been identified as a suitable surrogate measure for global endothelial function; notably including function in the coronary arteries [37, 61]. This relationship is evident in healthy individuals with angiographically normal coronary arteries and patients with varying degrees of atherosclerosis [62, 63]. Furthermore, there is considerable evidence indicating that baFMD is prognostic of future cardiovascular events across multiple populations including healthy subjects, as well as patients with CAD and peripheral arterial disease (PAD) [64-66]. In the most robust study to date, Yeboah et al.

found baFMD to be an independent predictor of future cardiovascular events in over 2500 older adults [67]; however this finding is not conclusive [68, 69].

Under resting conditions and following previously described procedures [32, 53], baFMD assessments have proven to be both reliable and reproducible, offering the least amount of variability among techniques currently used to non-invasively assess endothelial function *in vivo* [70-75]. Additionally, performing serial baFMD assessments does not affect measures in healthy young males, as similar baFMD values have been reported in this demographic with as many as five baFMD assessments over two hours [76-78]. One investigation found that healthy young subjects demonstrated similar baFMD values with as little as five minutes between successive measures [79]. Repeated baFMD assessments may affect endothelial function in clinical populations however, as Zhu *et al.* found an effect of repeated measures in obese subjects, but not in healthy lean controls. In the obese subjects, simply performing two baFMD assessments transiently improved endothelial function two hours later [78]. The study authors concluded that individuals with baseline dysfunction my experience an improvement in baFMD values following repeated bouts of reactive hyperemia; an effect not seen in their healthy lean controls. More investigation into the effect of repeated baFMD assessments on clinical populations is needed, but all studies to date have reported no effect for repeated measures in lean young men. Coretti et al., in their *Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery* suggest a minimum of ten minutes between successive FMD measures [53].

There is considerable evidence that baFMD responses exhibit diurnal variation in healthy young populations, with the most common finding that baFMD values are

reduced in the morning [80-84]. The precise pattern of this variation remains unclear, with reports of both comparable [80] and significantly different baFMD values at 800 and 1200 hours in healthy young men [81]. While the definitive minimum amount of time within which measures can be taken in order to avoid this variability remains unclear, Harris *et al*. found similar baFMD values in young healthy males at 800 and 1000 hours [76]. While it appears that baFMD assessments can be taken over 2-4 hours in the morning without diurnal variation, measures should be taken at the same time of day for within subject repeat comparisons.

Interestingly, the diurnal variation of baFMD appears to have an impact on the vascular response to exercise. Jones *et al*. compared the baFMD response to a bout of exercise in the morning and afternoon. In the morning, baFMD values were similar before and 30 minutes following a bout of aerobic interval exercise consisting of three 10-minute bouts of cycling at 70% VO_2 peak, with each bout separated by 10 minutes of rest [84]. In the afternoon, diurnal variation was evident, with pre-exercise baFMD values significantly higher than in morning. Following the afternoon exercise bout, baFMD was attenuated, with values similar to those found in the morning [84]. While the conclusion of 'impaired baFMD values' following the exercise bout in the afternoon may be correct, it is important to realize that the impairment simply returned baFMD values to pre- and post-exercise morning values.

Using baFMD to assess the acute endothelial responses to exercise presents multiple challenges considering the changing hemodynamics experienced during physical activity. Harris *et al*. demonstrated that baFMD assessments performed following a bout of aerobic exercise were reproducible for measurements immediately post-exercise and

every hour for three hours thereafter [85]. A review by Padilla *et al*. specifically examined the use of baFMD in assessing acute vascular responses following a bout of exercise, concluding that baFMD was methodologically appropriate for examining acute responses to exercise [86].

1.5.2 Nitroglycerin assessment

While baFMD is a measure of arterial dilation in response to increased shear stress and subsequent NO release, vasodilation can also occur in response to other endogenous stimuli or exogenously administered substances. Nitroglycerin (NTG) is a potent NO donor, that is converted to NO within the vascular smooth muscle, causing muscular relaxation and arterial dilation independent of endothelial involvement [87]. Arterial dilation in response to NTG administration is termed endothelium-independent dilation. NTG is commonly administered to assess vascular smooth muscle function in conjunction with baFMD assessments, allowing observed reductions in baFMD values to be interpreted as a reduction in endothelial NO bioavailability, not an underlying impairment of the vascular smooth muscle [88, 89]. When administered via sublingual spray, NTG has been shown to cause brachial arterial dilation within two minutes [90], with peak diameters reached at approximately five minutes in both CAD patients and healthy young adults [88, 91]. In response to sublingual NTG administration, brachial artery dilation has demonstrated a dose-response relationship [92]. In healthy young men, larger doses of sublingual NTG elicited larger dilation. This investigation administered NTG multiple times during each testing session and used a washout period of two hours (no explanation or reference was provided for use of a two hour washout period) [92]. Currently, the duration of NTG-induced arterial dilation as well as the combined effects

of NTG and exercise are poorly documented and make determining a suitable washout period difficult. Previous investigations have assessed vascular smooth muscle function pre-exercise and approximately 1 hour post-exercise, via sublingual NTG administration [93-95], while others have used a subset of study participants to test vascular responsiveness to NTG [96, 97].

1.6 Collection of vascular measures

For both baFMD and NTG assessments, brachial artery images and blood velocities are obtained using ultrasound. High-resolution longitudinal brightness mode (B-mode) images of the brachial artery proximal to the brachial bifurcation are obtained at baseline, after which the occluding cuff is instantaneously inflated to a pressure at least 50 mmHg greater than systolic blood pressure, in order to occlude arterial inflow to the forearm. For non-hypertensive subjects, a standardized pressure of 200 mmHg is commonly used. The cuff remains inflated for five minutes and upon deflation, three minutes of brachial artery images and corresponding blood velocities are collected using B-mode and pulse wave Doppler respectively.

NTG assessments require the acquisition of a baseline image, after which 0.4 mg of NTG is administered sublingually. B-mode images are typically obtained every minute for five minutes thereafter, until it is determined that maximal dilation has been achieved.

1.7 Analysis of vascular measures

1.7.1 baFMD analysis

Determining the magnitude of dilation in response to baFMD assessment requires a comparison of lumen diameters pre- and post-occlusion. End-diastolic frames are extracted from all images according to the R-spike of the simultaneously collected ECG and from these images lumen diameters are determined. End-diastolic images are used in an attempt to account for varying vascular compliance between individuals and to improve the accuracy of comparisons at different time points. FMD values are presented as absolute FMD (AbsFMD) and relative FMD (%FMD) and are calculated as follows:

Equation 1 $$AbsFMD = Peak\ Diameter(mm) - PreOcclusion\ Diameter\ (mm)$$

Equation 2 $$\%FMD = \frac{AbsFMD \times 100\%}{PreOcclusion\ Diameter}$$

Several studies have reported an inverse relationship between vessel calibre and magnitude of FMD [47, 48, 63, 98]. Greater dilation in smaller arteries is believed to result from augmented shear stress in smaller arteries and has led to the proposition that FMD values should be normalized to the shear stimulus eliciting maximal dilation. Calculating shear stress requires a measure of blood viscosity; however, shear rate (SR) provides an adequate surrogate measure and is used throughout the literature [48]. Shear rate incorporates a measure of mean blood velocity (MBV) and is calculated as follows:

Equation 3 $$ShearRate = \frac{MBV \times 8}{Diameter}$$

To calculate NormalizedFMD, the SR corresponding to each end-diastolic diameter is calculated. SR values from the point of cuff deflation to the point maximum

diameter is achieved are graphed vs. time and the area under this curve (SR_{AUC}) is considered the eliciting shear rate. Normalized FMD is calculated as follows:

Equation 4 $$NormalizedFMD = \frac{\%FMD}{SR_{AUC}}$$

Following exercise, baFMD values should be interpreted with caution, as a result of altered hemodynamic states and sympathetic output, which can affect arterial diameter, shear stress and vascular responsiveness [86]. Post-exercise, baFMD values may not be as accurate a barometer of NO bioavailability as they are at rest, as a result of these multiple regulatory mechanisms affecting arterial diameter [94, 99]. Multiple investigations have demonstrated inconsistencies between groups following normalization [100-102]. It is suggested that normalized FMD values should only be considered when there is a clear relationship between SR_{AUC} and %FMD [32, 100]. Recently, Llewellyn *et al.* found highly variable shear rate values for baFMD assessments performed post-exercise, resulting in a very weak relationship between SR_{AUC} and %FMD. They concluded that variables in addition to SR_{AUC} were contributing to the baFMD response at this time point and therefore baFMD assessments performed post-exercise should not be normalized to SR_{AUC} [99].

1.7.2 NTG assessment analysis

End-diastolic frames are extracted from NTG images for a comparison of pre- and post-NTG diameters. NTG values are presented as absolute NTG (AbsNTG) and relative NTG (%NTG) and are calculated as follows:

Equation 5 $$AbsNTG = Peak\ Diameter(mm) - PreNTG\ Diameter(mm)$$

Equation 6 $$\%NTG = \frac{AbsNTG \times 100\%}{PreNTG\ Diameter}$$

1.8 Endothelial adaptations to exercise

1.8.1 Chronic vascular adaptations

As discussed, physical activity dramatically reduces risk for CVD; however, more than 40% of this risk reduction cannot be accounted for by modification of traditional risk factors [16, 103, 104]. There is mounting evidence that vascular adaptations may account for a substantial portion of exercise-related CVD risk reduction [34, 36, 44]. The augmented blood flow experienced during exercise causes an increase in shear stress along the vessel wall and is believed to be an essential stimulus for both acute and chronic vascular adaptations to exercise [36, 44, 97, 105]. During lower-limb exercise, shear stress increases in the vascular beds of both active and inactive muscles, an effect not seen during upper-limb exercise, which elicits shear stress elevations in the active muscle beds only [36, 44]. As a result, shear stress induced vascular adaptations are seen systemically with lower body training; whereas forearm training evokes changes localized exclusively to the forearm vasculature [36, 44]. The endothelium is responsible for detecting changes in shear stimulus and NO release and as such, is implicated as one of the primary regulators of vascular adaptations to exercise [31, 44].

Vascular adaptation to exercise training is believed to go through two phases, both mediated, at least in part, by repeated bouts of elevated shear stress. The initial phase involves upregulation of eNOS, resulting in greater NO bioavailability [36, 106]. Increased NO bioavailability associated with short-term training is evident during exercise [107], as well as at rest [108]. If training persists, the second phase of vascular adaptation occurs in which vessel diameter increases, accompanied by a return of eNOS expression and NO activity to basal levels [36, 106]. Both short-term functional and

long-term structural adaptations are believed to be NO-mediated and manifest in an attempt to normalize shear stress against the vessel wall [36, 46].

1.8.2 Acute vascular response

While it is clear that elevated shear stress is essential for chronic vascular adaptations to exercise, altered shear stress also evokes acute alterations in vascular function. More specifically, it appears that acutely enhanced antegrade and mean shear stress improves endothelial function [46, 97, 109]. Multiple investigations have demonstrated that during low-intensity cycling exercise, the brachial artery experiences an increase in blood flow associated with an increase in mean shear rate [97, 109-111]. This increased flow is associated with arterial dilation [110] and an increase in baFMD values [46, 97, 111]. Tinken *et al*. demonstrated that simply reducing the amount of antegrade shear in the brachial artery during cycling abolished improvements in baFMD following exercise cessation [97].

Enhanced antegrade and mean shear rate during exercise are hypothesized to increase NO release and acutely improve baFMD values post-exercise [46, 111]. While increased NO has been reported during and immediately post-exercise [94, 107, 109, 112, 113], the correlation between increase NO and enhanced baFMD values in the post-exercise setting is not well established and increased NO has also corresponded to reduced baFMD in some instances [94]. While multiple mechanisms are involved in vascular function post-exercise [86], the importance of NO has been demonstrated by infusing an NO antagonist, which eliminated acute exercise-induced elevations in baFMD values [112, 114, 115].

In contrast to antegrade and mean shear stress, oscillatory and retrograde shear stress impairs endothelial function [46, 111, 116]. One of the proposed mechanisms through which this impairment is thought to occur is an increase in oxidative stress by reactive oxygen species (ROS) that has been documented post-exercise [117-121]. Increased ROS production by endothelial cells in response to enhanced retrograde and oscillatory shear stress has been found in vitro and in animal models [122-124]. ROS has been shown to quench NO [125] and impair eNOS [122] preventing further NO synthesis and causing endothelial dysfunction [126]. In accordance with these findings, impaired baFMD values in response to enhanced oscillatory and retrograde shear stress has been demonstrated in humans both at rest [116] and following exercise [111].

Further evidence for the role of ROS in post-exercise vascular function comes from studies using ascorbic acid (ROS scavenger) supplementation with exercise. Johnson *et al*. found that augmenting retrograde shear stress using partial forearm occlusion eliminated post-exercise improvements in baFMD values in healthy young males. When ascorbic acid was administered prior to exercise with enhanced retrograde shear stress, post-exercise improvements in baFMD remained, leading the authors to conclude that ascorbic acid reduced oxidative stress, preserving baFMD values [111]. Further investigations using antioxidant therapy with exercise have reported similar findings in patients with intermittent claudication [127, 128]. While it is known that multiple mechanisms are involved in the relationship between endothelial function and retrograde/oscillatory shear stress, these studies point to the involvement of ROS specifically affecting baFMD values.

During cycling, both antegrade and retrograde shear stress through the brachial artery are elevated [46, 110, 111]; however, at low-intensity, the rise in antegrade flow appears to offset the potentially deleterious impact of increased retrograde flow and preserve endothelial function [46]. The interplay between shear stress, NO availability, ROS formation and baFMD values in response to exercise is complex and appears to be altered by training status, antecedent endothelial dysfunction, temporality and exercise intensity and duration.

1.9 baFMD post-exercise

1.9.1 Training status

The training status of individuals is believed to have a significant effect on baFMD values post-exercise, in part because active individuals demonstrate a greater resistance to acute exercise-induced oxidative stress [117]. In support of this theory, Harris *et al*. found impaired baFMD values post-exercise for sedentary overweight men, while active overweight men experienced improved baFMD values post-exercise [129]. Similarly, Hwang *et al*. found reduced baFMD post-exercise in subjects who reported little to no exercise and unchanged baFMD values in subjects who exercised three or more times weekly [130]. Data from multiple studies indicate that trained men are less likely to experience reductions in baFMD values [119] compared to untrained men [131, 132]; however, this finding is not universal [94]. The finding that acute baFMD response to exercise is related to activity level is not surprising, considering the well-documented improved baseline endothelial function found in individuals who exercise regularly [36].

1.9.2 Antecedent dysfunction

Habitual whole body exercise training has consistently shown to improve vascular function in several populations with risk factors for endothelial dysfunction, including patients with hypertension, hypercholesterolemia, diabetes, obesity, CAD and congestive heart failure (CHF) [36]. Additionally, regular exercise has proven to ameliorate the age-associated decline in endothelial function [45]. While vascular adaptations to exercise training are well established in populations with antecedent endothelial dysfunction, improvements in healthy populations are less obvious [36], but have been reported [133, 134].

Multiple studies have highlighted the benefits of regular exercise for individuals with risk factors for endothelial dysfunction; however, fewer investigations have examined how the endothelium acutely responds to exercise in these populations. Thirty to sixty minutes following a bout of exercise, improvements in baFMD values have been reported in postmenopausal women [93], CAD patients [95] and obese subjects [78]. Interestingly, improvements in healthy young subjects 30-60 minutes post-exercise have seldom been reported, with multiple studies finding unchanged or impaired baFMD values at this time [84, 93, 94, 99, 119, 130, 132, 135, 136]. Young healthy subjects are more likely to experience increased baFMD immediately post-exercise [97, 111, 119], while data on clinical populations at this time point is scarce. Thus, it appears that underlying vascular health and time-elapsed post-exercise interact to have a significant impact on dilatory capacity post-exercise.

1.9.3 Temporal relationship

1.9.3.1 Immediately post-exercise

While there have been numerous investigations into the effects of sustained exercise training on endothelial function, fewer studies have examined the acute endothelial response to a bout of aerobic exercise. Literature on measures of baFMD performed within 30 minutes of exercise cessation is sparse, perhaps because of the potentially confounding effects of changing hemodynamic states, altered conduit vessel caliber and enhanced sympathetic output experienced during exercise [86]. The investigations that have been conducted to date offer varied results including unchanged baFMD in a group of elderly males [127], renal transplant recipients [137] and healthy young men [119, 132]; improved baFMD in healthy young [97, 111, 119], young obese [78] and middle-aged sedentary individuals [137]; and finally impaired baFMD in a group of young trained [131] and untrained [132] men, immediately following dynamic lower-limb exercise. Johnson *et al*. found both improved and unchanged baFMD values post-exercise in trained young men, depending on the combination of exercise intensity and duration used (discussed later) [119].

Additionally, acute handgrip exercise revealed improvements in baFMD immediately post-exercise in healthy subjects [97, 105], while individuals with treated hypertension experienced reduced baFMD values [138]. It is difficult to draw conclusions from this limited and inconsistent sample, other than to note that the baFMD response immediately post-exercise is variable and may be affected by risk factors for endothelial dysfunction, physical fitness and exercise intensity. In summary, 0-30 minutes post-exercise, the most common finding is of improved baFMD in young

individuals [78, 97, 105, 111, 119] with minimal data on clinical populations indicating they are unlikely to experience increased baFMD values at this time point [127, 137, 138].

1.9.3.2 30-60 Minutes post-exercise

The majority of investigations examining the acute baFMD response to exercise perform assessments 30-60 minutes following dynamic lower limb exercise. At this time, improvements in baFMD have been reported in postmenopausal women [93, 139], CAD patients [95], active overweight men [129] and obese men [78]; while no change was reported in premenopausal women [93], sedentary young men [94], healthy young males [84, 132, 135], non-elite runners [136] and trained young men [119]; and finally impaired baFMD was found in healthy young subjects [84, 99], overweight sedentary individuals [129] and highly endurance trained men [94]. As previously discussed, it appears that 30-60 minutes post-exercise, populations with risk factors for endothelial dysfunction (post menopause, CAD, obesity) [78, 93, 95, 139] commonly report elevated baFMD values, while healthy subjects experience unchanged or reduced baFMD values [84, 93, 94, 99, 119, 130, 132, 135, 136].

A study performed by Hwang *et al*. assessed baFMD post-exercise after 'heart rate returned to baseline' in a group of healthy active and sedentary males and females. Considering that the study population was not trained, we will assume that the post-exercise baFMD assessments were made more than 30 minutes following exercise cessation, in accordance with previous findings that found elevated HRs in similar populations 30+ minutes post-exercise [78, 93, 99, 140]. As previously discussed, they found an overall reduction in baFMD values; however, when stratified for activity level,

active individuals experienced unchanged values, while inactive individuals saw reduced baFMD values post-exercise [130].

1.9.3.3 1+ Hours post-exercise

In examining how endothelial function is altered post-exercise, it is important to understand the duration of these functional changes. Johnson *et al*. found antegrade and mean shear rates two hours post-exercise were different from baseline [141]; however, the affect of this altered hemodynamic state on baFMD values remains to be seen (baFMD assessments were not performed). Understanding how long reported reductions in dilatory capacity last could have clinical implications, as it has been suggested that repeated bouts of prolonged acute endothelial dysfunction might be atherogenic [127].

Several investigations have reported a return to baseline baFMD values 1 hour post-exercise in healthy populations [84, 93, 94, 119, 132, 135, 136]. In trained men, Johnson *et al*. reported baseline baFMD values for a variety of exercise intensities and durations at one and two hours post-exercise [119]. In contrast, Zhu *et al*. reported improved baFMD at one and two hours post-exercise in a group of obese men. The improvements seen in baFMD values at two hours were greater than the improvements experienced 1 hour post-exercise; however, the authors note that the increased baFMD values in obese subjects may be partially the result of repeated baFMD assessments, which they found improved baFMD values in obese subjects even in the absence of exercise [78].

The acute vascular effects of an exercise bout may be even more pronounced with the introduction of a stimulus shown to acutely impair endothelial function. Acute hyperglycemia [142] and hyperlipidemia [143] are both known to cause endothelial

dysfunction; however, it appears that a bout of exercise may offer protection. In healthy young men, when an exercise bout was completed immediately following glucose ingestion, the reduction in baFMD values seen with glucose ingestion alone was avoided for three hours post-exercise [140]. Similar findings were reported in another group of healthy young men, who completed an exercise bout two hours following consumption of a high-fat meal and experienced baFMD values greater than baseline two hours following exercise cessation [144].

While changes in baFMD values lasting a few hours post-exercise are not uncommon, there is some evidence that vascular function may be altered for several hours following exercise cessation. Tyldum *et al*. found improved baFMD values in slightly overweight middle-aged men 16-18 hours after exercise completion. Conversely, Rognmo et al. reported basal baFMD values 24 hours post-exercise, in both sedentary and trained young men; who experienced no change and reduced baFMD values 1 hour post-exercise respectively [94].

The protective effects of exercise on the vasculature are also reported when exercise is completed prior to glucose or fat consumption. In a group of middle-aged subjects, exercise completed 17 hours prior to glucose ingestion afforded protection for 2.5 hours afterwards [145]. A similar group of subjects consumed a high-fat meal 16-18 hours following an exercise bout and not only avoided the reduction in baFMD values seen in the control group, but saw baFMD values significantly greater than baseline for four hours following meal consumption and at 22 hours post-exercise [96].

Evidence of improvement two hours post-exercise in obese men [78] and 16-22 hours post-exercise in overweight middle-aged men [96] further implicates the possibility

that individuals with a priori endothelial dysfunction experience greater acute benefits than their healthy counterparts. While healthy populations report baseline vascular function 1 hour or more post-exercise [84, 93, 94, 119, 132, 135, 136], if fat [144] or glucose [140] ingestion is used to induce acute endothelial dysfunction, the acute effects of exercise on baFMD values become apparent two to three hours post-exercise. These findings support the hypothesis that healthy young individuals have close to optimal function at baseline, which is why acute vascular improvements following exercise are not reported in the fasting state, but are experienced with acute dysfunction induced by glucose or fat ingestion.

1.9.4 Exercise duration and intensity

Of the aforementioned studies investigating the acute baFMD responses to exercise, few have considered the effects of duration or intensity. The majority of investigations examining the vascular response to exercise employed interventions of 30-45 minutes sustained moderate-intensity lower limb exercise [78, 93, 97, 99, 119, 129, 136, 137, 144], typically between 50-60% of VO_2 peak. While some studies used more intense or longer duration exercise, direct comparisons are rare, but tend to focus on the period immediately following exercise cessation.

Studies that have examined vascular responses to different intensities and durations have found differences. In healthy, untrained men, Birk *et al*. found that, immediately post-exercise, %FMD was unchanged in response to 30 minutes of cycling at 50% of maximum heart rate. When exercise intensity was increased to 70 or 85%, %FMD was significantly attenuated [132]. While pre-occlusion diameter was also elevated in an intensity-dependent fashion, controlling for pre-occlusion diameter and SR

indicated that exercise intensity was an independent predictor of dilatory capacity post-exercise [132]. Johnson *et al*. demonstrated that the interaction between exercise intensity and duration affects baFMD values immediately post-exercise in trained young men as well. Improved baFMD values were found immediately post-exercise for isocaloric bouts of high-intensity short duration and moderate intensity moderate duration exercise conditions. However, if the *duration* of moderate intensity exercise was increased, improvements in baFMD values were eliminated [119]. As well, if exercise *intensity* was increased for an identical duration, the improvements in baFMD values previously seen were once again eliminated [119]. The finding of improved baFMD values following moderate-intensity exercise in trained men [119] and unchanged values in untrained men [132], is likely the result of the increased resistance to oxidative stress in trained individuals [117]. Likewise, Bailey *et al*. reported reduced baFMD immediately following high-intensity exercise in untrained young men [131]. Investigations that have reported improvements immediately post-exercise typically used low-intensity exercise in young healthy populations [97, 111] or moderate-intensity exercise and trained subjects [119].

In patients with intermittent claudication, Silvestro *et al*. reported no change in baFMD values following sub-maximal exercise, but marked impairment following maximal exercise; however, it should be noted that in this study population, sub-maximal exercise consisted of approximately three minutes of treadmill walking, while maximal exercise consisted of approximately six minutes [127]. Overall, immediately post-exercise, endothelial function appears to be closely related to relative exercise intensity.

While the effect of intensity is apparent immediately post-exercise, the duration of this effect appears to be short-lived as both Johnson and Birk reported a return to baseline baFMD values by 1 hour post-exercise for all exercise doses in trained and untrained men respectively [119, 132]. In further support of these findings, Harris *et al.* found similar baFMD responses 1 hour following 45 minutes of walking at 25%, 50% and 75% VO_2 peak in both active and inactive overweight young men [129].

The relationship between exercise intensity and baFMD values appears to be governed in part by shear stress, as it has been reported that different intensities [141, 146] and durations [147] of exercise result in different shear stress profiles through the vasculature. Specifically, immediately post-exercise, higher intensity exercise results in greater antegrade and mean shear rates than less intense exercise, while retrograde and oscillatory shear rates are comparable between intensities [141, 148].

Despite these similar retrograde and oscillatory shear patterns (stimuli for ROS production), ROS generation is not equivalent and appears to vary with exercise intensity. Goto *et al*. reported increased levels of oxidative stress following 30 minutes of cycling at 75% VO_2 peak, while 25% and 50% did not alter oxidative stress [121]. Using an alternative marker, Johnson *et al*. reported elevated oxidative stress following different durations of exercise at 80% VO_2 peak, while bouts of 30 or 60 minutes at 50% VO_2 peak yielded no changes [119]. Additional findings of enhanced oxidative stress in acute high-intensity exercise settings [149], as well as over weeks long high-intensity training studies [150, 151] indicate that oxidative stress is intensity-dependent.

Correlating oxidative stress and baFMD values post-exercise has proven challenging, as a result of the multiple mechanisms involved in vascular patency

following an exercise bout [86]. However, as previously discussed, two studies have demonstrated that ROS plays a critical role in vascular function, by using ascorbic acid supplementation to prevent reduced baFMD values post-exercise [111, 127].

Acknowledging that intensity is an important factor governing the acute endothelial response to exercise, it becomes important to understand how to adjust exercise doses to obtain optimal benefits. While sustained moderate-intensity exercise (END) has long-shown to improve health in many ways, beyond vascular adaptations, a growing assemblage of evidence suggests that comparable benefits can be achieved with shorter duration, higher-intensity exercise [152]. High-intensity interval training (HIT) consists of short intervals of strenuous activity, interspersed with periods of low-intensity exercise or complete rest. This method of increasing intensity is different from the previously discussed studies, which increased intensity simply by increasing workload, but did not offer intermittent rest periods.

1.10 HIT versus END

1.10.1 Response to training

Investigations comparing HIT and END have focused primarily on cardiorespiratory and metabolic outcomes following several weeks of training. Multiple studies have found that, in as little as 6 weeks of training, HIT can evoke similar cardiovascular and metabolic adaptations as END, despite up to one third the time commitment and one tenth the total training volume required for a HIT protocol [133, 153, 154].

In terms of vascular function, in 20 healthy, untrained young men, over 6 weeks of training, HIT evoked similar changes to END for both distensibility and FMD of the popliteal artery: surrogate markers of peripheral vascular structure and function, respectively [133]. Interestingly, in clinical populations, the vascular adaptations to HIT appear to be equivalent or perhaps superior to END. Currie *et al*. found similar improvements in baFMD values in a group of CAD patients who undertook 12 weeks of either END or HIT [155]. In a similar study, heart failure patients who completed 12 weeks of a HIT protocol demonstrated greater improvements in baFMD values, in addition to greater improvements in VO_2 peak, left ventricular remodeling and ejection fraction [156]. Furthermore, when patients with metabolic syndrome were randomized to 16 weeks of either HIT or END, those who completed the HIT protocol demonstrated greater improvements in baFMD values, VO_2 max and overall risk factor reduction [157].

1.10.2 Acute response

While chronic adaptations to HIT appear to be comparable or superior to END, differences in the acute responses to these types of exercise remain largely unexamined. Minimal data however, appear promising for HIT. In CAD patients, Currie *et al.* found similar improvements in baFMD values 1 hour post-exercise in response to both END and HIT, despite less total time and work for the HIT stimulus [95]. Tyldum *et al*. compared the responses to HIT and END exercise protocols in middle-aged men and found that while both exercise bouts improved baFMD 16-18 hours following exercise cessation, HIT resulted in a greater improvement [96]. Furthermore, following consumption of a high-fat meal - a stimulus known to evoke endothelial dysfunction [158] - END reduced the level of lipemia-induced dysfunction; however dysfunction was

still present. In contrast, HIT not only prevented dysfunction from lipid consumption, but augmented baFMD values above baseline levels for up to four hours following consumption and 22 hours after exercise cessation [96]. This limited sample appears promising for HIT, but further research comparing the acute vascular responses to HIT and END in different populations is needed.

1.10.3 Advantages of HIT

Determining the effectiveness of HIT versus END may prove useful because HIT overcomes one of the most commonly cited barriers to regular physical activity: 'lack of time' [159-162]. Younger individuals are particularly likely to cite 'lack of time' as the reason for low levels of exercise [159]. Additionally, HIT was cited as more enjoyable and preferred over END in a group of healthy young males [163] and CAD patients [164], illustrating that HIT may also be effective in combating the 'lack of enjoyment' barrier to exercise [159]. Higher levels of adherence [165] and more consistent completion of exercise sessions [166] have been reported for HIT exercise regimes in the cardiac rehabilitation setting. Thus, HIT may effectively overcome two barriers to regular physical activity, increasing adherence to exercise programs, especially among younger populations for whom lack of time is perhaps the most significant barrier.

1.11 Personalizing exercise prescription

1.11.1 Healthy populations

As discussed previously, individuals with a priori endothelial dysfunction are more likely than their healthy counterparts to experience vascular adaptations to habitual exercise training [36]. Likewise, acute improvements in baFMD 30-60 minutes

following a bout of exercise are reported in clinical populations with prior endothelial dysfunction [78, 93, 95], but are rarely seen in healthy individuals [84, 93, 94, 99, 119, 135, 136]. It has been suggested that more intense training is required for healthy populations to experience chronic improvements in vascular function [36]. This theory is disputed however; as some have contended that intense exercise training results in elevated oxidative stress and impairment of endothelial function [150, 151]. If this is the case, perhaps the intermittent periods of rest provided during HIT could temporarily reduce ROS generation and oxidative stress, attenuating potential impairments seen with sustained high-intensity exercise. Examining the acute and chronic responses to HIT in healthy individuals warrants further investigation, in order to determine if higher exercise intensities are in fact more likely to stimulate vascular adaptations in healthy populations.

1.11.2 Clinical populations

For clinical populations or individuals with low exercise-capacity, determining the relationship between exercise dose and acute vascular function may mean the difference between health-promoting exercise and acutely increasing risk of a cardiovascular event. Several investigations have revealed that during and immediately following an acute bout of exercise, there occurs a period where individuals are at increased risk for cardiac events [167-169]. While difficult to quantify, it is estimated that 22% of myocardial infarctions (MI) [170] and up to 17% of sudden cardiac deaths [171] are exertion-related. Individuals who are inactive, have multiple cardiac risk factors or have atherosclerosis are at the greatest risk for exertion-related events [172-174]; however, it has been demonstrated that even the most active individuals experience increased risk during and immediately following exercise [171, 173]. It has also been

reported that exertion-related MIs result in more complications than those occurring at rest, further increasing the burden of these events [172]. It is encouraging to note that risk of exertion-related events can be reduced with regular exercise [171, 175]. The phenomenon of exertion-related cardiac events is especially troublesome for sedentary individuals with multiple risk factors for CVD, as they are in greatest need of health-promoting exercise, and yet, incur the most risk during and immediately after activity. The mechanism behind exertion-related risk is poorly understood, underscoring the need for further investigation into the acute cardiovascular response to exercise.

Regular HIT exercise has demonstrated added benefit in patients with CAD [155], CHF [156] and metabolic syndrome [157]. Additionally, it has been suggested that HIT forms of exercise may allow patients with compromised cardiac function to work at higher exercise intensities, albeit for a shorter duration, providing periods of rest that reduce total cardiac stress [25]. However, it is possible that high-intensity exercise is not suitable for certain clinical populations, as some investigations have reported acute reductions in baFMD values following intense exercise. In patients with intermittent claudication, it has been reported that a single bout of maximal treadmill walking lasting only six minutes resulted in a substantial reduction in baFMD, while submaximal treadmill walking elicited no change [127]. When compared to healthy controls, CAD patients had comparable baFMD values during moderate intensity exercise, while values at baseline and peak exercise were significantly lower [176]. These results differed from those of Currie *et al*., who found CAD patients experienced acute improvements in baFMD values following HIT [155], and may be explained by several differences including the periods of rest offered by HIT, time point of baFMD assessment or the use

of peak exercise used in the previous study. These conflicting data point to the importance of individualizing exercise prescriptions, particularly in clinical populations, in order to mitigate risk and maximize benefit.

1.12 Summary

Interpreting the results of all these studies collectively presents several challenges due to various populations used, different exercise modalities, intensities and durations and the range of time points at which measures were taken. Moreover, it should be noted that not all of the investigations discussed utilized an FMD protocol shown to elicit an exclusively NO-dependent dilation and therefore should be considered with caution [78, 93, 94]. With that in mind, and acknowledging the potential difficulties assessing baFMD post-exercise, it appears that healthy populations undergo a brief period of enhanced baFMD values immediately following low to moderate-intensity exercise. If the exercise is of long duration or high-intensity, reduced baFMD values are likely. Thirty to sixty minutes post-exercise, healthy subjects demonstrate reduced or baseline baFMD values and baseline values 24 hours following exercise cessation.

Clinical populations, likely with underlying endothelial dysfunction, appear to experience a transient reduction in baFMD values immediately post-exercise, followed by elevated baFMD values 30-60 minutes post-exercise. Brachial artery FMD assessments greater than 1 hour post-exercise have seldom been reported in clinical populations.

1.13 Rationale for study

The acute endothelial response to an exercise bout is poorly understood and the literature to date remains inconsistent. With regard to time course, reports of unchanged, increased and decreased baFMD values exist immediately post-exercise. Additionally, altered vascular function has been reported up to 18 hours post-exercise [96] while others report a return to baseline 1 hour post-exercise [119, 132]. Further research is needed into the time course of acute vascular responses to exercise. As well, identifying the acute vascular response to exercise may help in further understanding chronic vascular adaptations, and how such adaptations may contribute to overall CVD risk reduction experienced with exercise.

While the benefits of habitual exercise on vascular function are well established, the most favourable mode, intensity and duration of exercise remain unclear. The majority of research to date has focused on traditional endurance exercise; however, a growing body of literature suggests that high-intensity interval training may be equally as or perhaps more effective at stimulating health-promoting adaptations to exercise. When considering that HIT accommodates two commonly cited barriers to exercise – lack of time and enjoyment – it is clear there is potential for increased adherence to a HIT regime, compared to an exercise program focused on END. Furthermore, high-intensity exercise may elicit greater improvements in clinical populations and may be necessary for young healthy individuals devoid of risk factors to incur vascular improvements associated with exercise. To date, a study comparing the acute vascular effects of HIT and END in healthy populations has not been completed.

1.14 Purpose and hypothesis

The purpose of the present study was to 1) examine the time course of acute changes in baFMD values following a bout of exercise and 2) compare how these responses differ between traditional endurance exercise and high-intensity interval exercise. We hypothesize that immediately following exercise cessation baFMD values will be unchanged for END and reduced for HIT, with a return to baseline for both conditions 1 hour following that will remain at the 24-hour time point.

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CHAPTER 2

ACUTE BRACHIAL ARTERY RESPONSES TO ENDURANCE AND HIGH-INTENSITY INTERVAL EXERCISE IN YOUNG HEALTHY MALES

2.1 INTRODUCTION

The burden of cardiovascular disease (CVD) continues to rise globally [1]; highlighted by increasing rates of modifiable risk factors in the developing world [2]. Modifiable risk factors for CVD include smoking, abnormal lipids, hypertension, diabetes, abdominal obesity, physical inactivity and excessive intake of alcohol, salt, fat and calories [1, 3]. Exercise training offers a unique opportunity to modify risk for CVD because it improves almost every risk factor including blood lipids [4-6], blood pressure [7-9], glucose regulation [10, 11], obesity [12, 13] and physical inactivity. Additionally, there is evidence that exercise reduces cigarette cravings and tobacco withdrawal symptoms, aiding in the cessation process [14, 15]. Exercise not only reduces the risk for CVD, but also improves the health of individuals with documented CVD, demonstrated by reduced mortality, fewer cardiac events and improved quality of life [16-19]. Overall, peak-exercise capacity is the best predictor of all cause mortality [20], indicating that habitual exercise should play a role in CVD prevention, management and rehabilitation.

It is estimated that physical inactivity increases risk of heart disease and stroke by 50% [21], while regular physical activity can reduce risk for CVD by 30-50% [22, 23]. Interestingly, a portion of the reduction in CVD risk associated with exercise remains unexplained through modification of traditional risk factors [23]. Recently, it has been postulated that vascular adaptations to exercise training contribute to this unexplained risk-reduction associated with exercise [24]. Vascular adaptations to exercise are

mediated by the endothelium, in response to increased shear stress, and include a short-term increase in NO bioavailability and long-term structural changes [25, 26]. During dynamic lower limb exercise, vascular shear stress increases systemically, resulting in systemic improvements in endothelial function. Conversely, upper limb exercise elicits increased shear stress in the vasculature supplying active musculature only, confining improvements in endothelial function to the vascular beds supplying the upper limbs [24].

Habitual dynamic lower limb exercise has almost unanimously shown to improve endothelial function in patients with hypertension, hypercholesterolemia, diabetes, CAD, congestive heart failure, obesity [25], and those with age-associated decline in endothelial function [27]. While it is clear clinical populations with risk factors for endothelial dysfunction experience training-induced vascular adaptations to exercise training, improvements in healthy populations are not as common, although have been reported [25].

While the vascular responses to exercise training are becoming clear, how the vasculature acutely responds to a single exercise bout is poorly understood. Studies performed to date have used a wide variety of study populations and exercise protocols and assessed endothelial function at various time points post-exercise. As a result, acute vascular function has been reported to improve [28-36], diminish [36-43] and remain unaltered from baseline [31-33, 39, 40, 42, 44-46] in response to exercise. These studies indicate that vascular function post-exercise exhibits a temporal response that varies according to sex, presence of risk factors for endothelial dysfunction, training status and the combination of exercise intensity and duration. Further investigation is required into

the time-course of changes in vascular function post-exercise and how these changes might be affected by different exercise doses.

Despite the well-known benefits of habitual exercise, rates of physical activity remain low, with 'lack of time' frequently cited as one of the primary barriers to regular exercise participation [47-49]. There is growing evidence indicating that metabolic and cardiovascular adaptations associated with traditional endurance training (END) can be achieved through high-intensity interval training (HIT) [50-53], with the added benefit of requiring less time and being more enjoyable [54, 55]. Additionally, there is some evidence in clinical populations that high-intensity interval exercise may induce greater improvements in cardiovascular function, compared to sustained moderate-intensity exercise [56].

The evidence for HIT is promising; however, few investigations have compared the acute vascular responses of HIT and END [34, 57], and none have done so in healthy young men. Comparisons between HIT and END are required in order to improve understanding of the dose-response relationship between exercise and improved vascular function. Additionally, understanding the acute effects of a single bout of HIT may contribute to elucidating the mechanism behind the greater improvement in cardiovascular function previously reported with HIT training in patients with heart failure [56]. The purpose of the present investigation was to examine the time course of acute changes in baFMD following exercise in healthy young men, and determine if these responses differ between HIT and END exercise.

2.2 METHODS

2.2.1 *Study design*

A within-subject, repeated measures, crossover design was used to examine the time course of brachial artery endothelial responses acutely following two different exercise bouts. Participants reported to the lab on five separate occasions. Pre-visit instructions for the first visit included refraining from vigorous physical activity 48 hours prior, alcohol and caffeine avoidance 12 hours prior and eating a small, typical meal approximately two hours prior to arrival in the laboratory. The first visit consisted of familiarization with the laboratory environment and a VO_2 peak cycle ergometer test. Pre-visit instructions for the remaining visits were identical to the first visit, with the exception of the small meal, which was replaced with a minimum eight hour fast. The subsequent visits consisted of a baseline brachial artery flow mediated dilation (baFMD) assessment, an acute cycle exercise bout and baFMD assessments immediately post-exercise, 1 hour post-exercise and 24 hours post-exercise (Figure 1). Brachial artery nitroglycerine-mediated dilation (NTG) assessments were performed 10 minutes following all baFMD measures, save the 1 hour post-exercise time point. The two different exercise bouts were separated by an average of 9 ± 5 days. All vascular assessments were performed in a quiet, temperature controlled room (22.6 ± 0.5° C) with participants in a supine position. Within subjects, testing sessions were conducted at the same time of day beginning between 800 and 1000 hours. All subjects reported successful completion of pre-visit instructions.

Base line		26-36min Exercise		0hr	1hr		24hr
FMD NTG		HIT or END		FMD NTG	FMD	→	FMD NTG

Figure 1: Schematic of Testing Sessions

2.2.2 *Subjects*

Ten males age 20-30 (23.3 ± 2.1 years) were recruited from McMaster University campus and surrounding area for participation in the present study. Exclusion criteria included any risk factors for endothelial dysfunction including hypercholesterolemia, hypertension, smoking, diabetes, obesity and cardiovascular disease. Participants had to be free of any contraindications to exercise and possess average aerobic fitness levels (41 – 55 ml kg^{-1} min^{-1}) [58] based on a progressive peak oxygen uptake test (VO_2 peak). The experimental protocol was approved by the Hamilton Health Sciences Research Ethics Board in accordance with the Helsinki Declaration on the use of human subjects and all participants provided written informed consent prior to study participation.

2.2.3 *Peak oxygen uptake test*

Participants reported to the laboratory between 800 and 1200 for completion of a VO_2 peak test on a cycle ergometer (Excalibur Sport V2.0; Lode BV, Groningen, The Netherlands). Respiratory measures (pulmonary oxygen consumption [VO_2] and expired carbon dioxide [VCO_2]) were obtained using a unidirectional flow mouthpiece and a Medisoft metabolic cart with ExpAir software featuring an on-line gas collection system (model E10010001000, Medisoft, Georgia, United States) to provide continuous expired gas data.

The incremental exercise test consisted of a two-minute warm-up at 50 watts and thereafter increased 1 watt every 2 seconds until one or more of the following cessation criteria were met: 1) volitional fatigue, 2) sustained pedal cadence < 40 rpm 3) chest pain, 4) sustained ventricular tachycardia. Respiratory measures were recorded on a minute-by-minute basis and heart rate was monitored via single lead electrocardiograph throughout the test. VO_2 peak was determined to be the highest 30-second average oxygen consumption (Table 1). The peak power output (PPO) was the maximum wattage attained before cessation criterion was met. PPO was subsequently used to determine the exercise intensities for the exercise protocols described below.

2.2.4 *Exercise protocols*

There were two exercise protocols used in this study and each included a three-minute warm-up and cool-down at 30% PPO. The endurance protocol (END) consisted of 30 minutes of seated cycling at 55% PPO, while the high-intensity interval protocol (HIT) consisted of 10 one-minute seated cycling intervals at 80% PPO, alternating with one-minute intervals at 30% PPO. Exercise protocols were modeled after a similar study by Currie *et al*. that compared the acute effects of exercise intensities on baFMD in coronary artery disease patients [34]. All exercise was completed on an electrically braked cycle ergometer (Excalibur Sport V2.0; Lode BV, Groningen, The Netherlands). Participants were instructed to keep pedal cadence between 60 and 80 rpm and the order of END and HIT was randomized and counterbalanced between subjects.

2.2.5 *Outcome measures*

Heart rate and blood pressure monitoring

Resting values for each participant are reported as the average of two 30-second periods from both sessions, and were obtained in the supine position following ten minutes of rest (Table 1). Heart rate was continuously monitored throughout vascular assessments using single-lead electrocardiography (ECG) (ML 123, ADInstruments Inc., Colorado Springs, CO, USA). Blood pressure was continuously monitored noninvasively via a small pneumatic cuff around the finger (Nexfin monitor, Model 1, BMEYE B.V., Amsterdam, The Netherlands). Signals for heart rate and blood pressure were acquired simultaneously using a commercially available data acquisition system (Powerlab model ML795, ADInstruments, Colorado Springs, USA) and software program (LabChart 7; ADInstruments, Colorado Springs, USA). Post-exercise values are reported as a 30-second average immediately following exercise cessation.

Vascular measures

All vascular assessments were completed with the participant in the supine position. For acquisition of brachial artery ultrasound images, the right arm was supinated and abducted 80°- 90° from the torso. Ultrasound images of the right brachial artery were taken on the medial side of the upper arm, 5-10 cm proximal to the antecubital fossa. Arterial images were collected in the longitudinal plane at a frame rate of 7.7 frames per second (Vivid Q; GE Medical Systems, Horten, Norway). A duplex image comprised of a two-dimensional grayscale ultrasound image and blood velocity measures were obtained via pulsed wave (PW) Doppler at a frequency of 4MHz with a 12MHz linear array probe for brachial diameter and blood velocity assessment.

Endothelium-dependent function: flow-mediated dilation

A pneumatic cuff was placed around the right forearm, 5 cm distal to the antecubital fossa, while the ultrasound probe was positioned as described above. The probe was adjusted until visualization of brachial artery lumen-intima boundaries was deemed optimal, with the caveat that insonation angle of the PW Doppler not exceed 70°: a requirement for accurate measurement of blood velocity [59]. The PW Doppler sample volume was maximized to insonate the complete lumen diameter. The mixed audio signal from the PW Doppler was continuously fast-Fourier transformed using an external spectral analysis system (model Neurovision 500M TCD; Multigon Industries, Yonkers, NY, USA), to provide an intensity-weighted mean blood velocity (MBV). Intensity-weighted MBV has been determined to most accurately represent the speed of blood cells within the artery [59].

At each data collection time point a 30-second baseline image was collected, followed by inflation of a pneumatic cuff to 200 mmHg using a rapid cuff inflator (model E20 and AG101; Hokanson, Bellevue, WA, USA). Following five minutes of occlusion, the cuff was deflated and three minutes of data was recorded with simultaneous arterial diameter and MBV signals. FMD procedures were followed in accordance with those designed to elicit dilation provoked predominantly by endothelial-NO [60, 61].

Endothelium-independent function: nitroglycerin administration

Ten minutes following cuff deflation, a nitroglycerin (NTG) assessment was performed to determine endothelium-independent function. Pre-NTG images of the same segment of the brachial artery visualized during the baFMD assessments were acquired for ten cardiac cycles after which, a 0.4 mg dose of NTG was administered sublingually.

Images were then acquired for ten cardiac cycles every minute for ten minutes. NTG was administered again following the baFMD assessment immediately post-exercise but was not administered 1 hour post-exercise in order to avoid possible side effects elicited by administration of multiple NTG doses.

2.2.6 *Data analysis*

Brachial artery blood velocity

MBV was analyzed offline using LabChart 7 Pro for Windows (Powerlab ML 795; ADInstruments, Colorado Springs, CO, USA). Pre-occlusion velocities were calculated as the 30-second average MBV during pre-occlusion image acquisition and MBV post-occlusion was averaged using moving 5-heart cycle bins.

Brachial artery diameter

Using DICOM editing software (Sante DICOM Editor 3.1.13, Santesoft, Athens, Greece), end-diastolic frames were extracted from all ultrasound image files and merged into a single DICOM file. End-diastolic frames are consistently used in analysis of arterial diameter to limit the effects of varying arterial compliance between subjects and to limit artifacts due to pulse-related diameter changes [59].

Arterial diameters were calculated within a region of the artery displaying the clearest resolution using semi-automated edge detection software (AMS Image and Data Analysis 2.1; Gothenburg, Sweden). Edges were detected by identifying differences in brightness at the wall-lumen interface at approximately 100 points along both arterial walls [62]. If, upon visual inspection, the arterial border was inaccurately identified, manual adjustments were made.

Pre-occlusion diameters were calculated as the average end-diastolic diameter within the 30-second pre-occlusion image acquisition period. Following cuff deflation, five-diameter rolling averages were used and the largest five-diameter bin was deemed the maximum diameter reached following cuff deflation [59]. Diameters pre- and post-NTG administration were calculated as the average over ten heart cycles. Absolute and relative FMD and NTG were calculated as follows:

Equation 1 $$AbsFMD = Peak\ Diameter\ (mm) - PreOcclusion\ Diameter\ (mm)$$

$$AbsNTG = Peak\ Diameter\ (mm) - PreNTG\ Diameter\ (mm)$$

Equation 2 $$\%FMD = \frac{AbsFMD \times 100\%}{PreOcclusion\ Diameter}$$

$$\%NTG = \frac{AbsNTG \times 100\%}{PreNTG\ Diameter}$$

It has been recommended that FMD values be normalized to the shear stress achieved during cuff release [60, 63]. The shear stress calculation incorporates a measure of blood viscosity; however, shear rate (SR) has proven to be a reliable surrogate measure and is used throughout the literature [64]. The calculation for shear rate is as follows:

Equation 3 $$Shear\ Rate = \frac{MBV \times 8}{Diameter}$$

Calculating the area under the Shear Rate vs. Time to Maximum Diameter curve (SR_{AUC}) provides the eliciting shear rate value. Normalized FMD is calculated as follows:

Equation 4 $$NormalizedFMD = \frac{\%FMD}{SR(AUC)}$$

2.2.7 *Statistical analysis*

Statistical analyses were conducted using SPSS (SPSS, version 11.5; Chicago, IL, USA). Intra-class correlations (ICC) and coefficients of variation (CV) were performed to assess the day-to-day reliability of baseline vascular measures. Vascular measures and

hemodynamic variables were compared between exercise conditions: HIT vs. END, as well as time point: baseline, immediately post-exercise, 1 hour post-exercise, 24 hours post-exercise using a two-way repeated measures factorial analysis of variance (ANOVA). Mauchly's test of sphericity was used to assess homogeneity of variance among measures. Bonferroni correction was used for pairwise comparisons. Descriptive statistics are presented as mean ± standard deviation and $p < 0.05$ is considered statistically significant.

2.3 RESULTS

Ten males aged 21-26 (23.3 ± 2.1 years) participated in the current study. Participants were non-hypertensive (MAP: 83.7 ± 6.5 mmHg) and non-obese (BMI 25 ± 3 kg/m^2). Subjects demonstrated average aerobic fitness levels (46 ± 5 ml $O_2 \bullet kg^{-1} \bullet min^{-1}$) and all were able to complete both END and HIT exercise bouts. No medications were taken by any of the participants during study participation. Subject characteristics are presented in Table 1.

2.3.1 *Heart rate and blood pressure*

Resting hemodynamic variables were not different between testing sessions. Heart rate was significantly elevated above baseline immediately post-exercise ($p \leq 0.001$) and 1 hour post-exercise ($p \leq 0.05$) for both HIT and END exercise conditions. SBP was significantly attenuated immediately post-exercise ($p \leq 0.05$), while DBP and MAP did not differ from baseline values at any time point for either exercise condition. Hemodynamic measures during baFMD assessments can be found in Tables 2 and 3.

2.3.2 *Endothelium-dependent function: flow-mediated dilation*

Day-to-day baseline baFMD ICC values were 0.47 for %FMD and 0.62 for AbsFMD, indicating a moderate and substantial correlation respectively [65]. Our CV was 26.7 for %FMD and 26.2 for AbsFMD. There was no interaction or main effect found for the effect of exercise condition on either absolute or relative FMD; however, main effects for time were observed. Immediately post-exercise, baFMD values were not different from baseline; however, 1 hour post-exercise %FMD and AbsFMD were significantly reduced compared to all other time points ($p \leq 0.001$, $p \leq 0.05$ respectively) (Figure 2). %FMD and AbsFMD values at 24-hours post-exercise were not significantly different from baseline values. There was no effect found for SR_{AUC} and no correlation was found between SR and %FMD. All baFMD values for END and HIT are presented in Tables 2 and 3 respectively.

Mauchly's test of sphericity was violated when comparing pre-occlusion diameters, indicating non-uniform variance among groups. Greenhouse-Geisser and Huynh-Feldt corrections were considered accordingly. END and HIT elicited similar changes in pre-occlusion diameter. Immediately post-exercise, pre-occlusion diameter was significantly larger than baseline pre-occlusion diameter ($p \leq 0.001$). One hour post-exercise, pre-occlusion diameter had further increased and was significantly different from both baseline and immediately post-exercise ($p \leq 0.05$) (Figure 3). Larger peak diameters were found immediately post-exercise and 1 hour post exercise compared to baseline peak diameters for both END and HIT ($p \leq 0.001$) (Figure 3). Time to peak baFMD diameter following occlusion was not different between groups or at any time point.

2.3.3 *Endothelium-independent function: nitroglycerin administration*

%NTG demonstrated main effects for exercise condition and time point. %NTG dilation values associated with the HIT exercise bout were significantly greater than those associated with END ($p \leq 0.05$). For both END and HIT, %NTG decreased immediately post-exercise compared to baseline ($p \leq 0.05$) and returned to baseline values at the 24-hour time point. AbsNTG revealed no main effect for condition and demonstrated a similar response as %NTG for time point, with depressed AbsNTG immediately post-exercise ($p \leq 0.05$) and return to baseline values by 24 hours (Figure 4).

Pre-NTG diameters were significantly elevated immediately post-exercise in both the END and HIT conditions ($p \leq 0.05$); however, there was no significant difference found for maximum NTG diameter at any time points or between exercise conditions (Figure 5). Time to peak NTG diameter was not different between groups or at any time point. All NTG values are presented in Tables 4 and 5.

2.4 DISCUSSION

Currently there is no consensus on the time course of acute changes in endothelial function following a bout of exercise. Over a range of time points from 0 to 60 minutes following dynamic lower limb exercise, there is reported evidence of improvements [28-36], no change [31-33, 39, 40, 42, 44-46] and impairments [36-43] in baFMD values. Comparing these results collectively presents many challenges as a result of inconsistencies with 1) FMD protocol used, 2) time points of assessment, 3) study populations, 4) exercise dose and 5) diurnal variation of FMD tests. While comparisons between different exercise intensities have recently been completed [32, 39], there is yet

to be an investigation comparing the acute baFMD response between high-intensity interval exercise and continuous moderate-intensity exercise in healthy males. Considering that HIT may improve exercise adherence [66, 67] and confer greater cardiovascular benefits than END [56, 57], such an investigation is warranted. In the present study, we examined the baFMD response to acute bouts of HIT and END immediately post-exercise, 1 hour post-exercise and 24 hours post-exercise in young, healthy males.

2.4.1 *Endothelium-dependent function: flow-mediated dilation*

In the current study, we found similar responses in relative and absolute baFMD at each time point for END and HIT, indicating that HIT and END evoke similar acute responses in vascular function. This finding supports previous investigations that have demonstrated HIT and END result in comparable vascular adaptations over several weeks of training in young healthy individuals [51], CAD patients [53] and individuals with CHF [56]. This result is significant simply because HIT requires less training time and is reported as more enjoyable than END exercise by young healthy individuals [54] and CAD patients [55]. Insufficient time [47-49] and enjoyment [47, 68] are frequently cited barriers to habitual exercise, thus prescribing an exercise program that requires less time and is more enjoyable represents a unique opportunity to improve adherence to exercise programs both in prevention of and rehabilitation from CVD.

Immediately post-exercise

Recently, it was reported that exercise intensity is an independent predictor of baFMD values immediately post-exercise [39]; a theory supported by a review of the literature. Immediately post-exercise, enhanced baFMD values have been reported in

response to low-intensity exercise [28, 29], while unchanged or reduced baFMD values have been found in response to more intense exercise bouts [32, 38, 39]. The relationship between exercise intensity and baFMD values appears to involve reactive oxygen species (ROS) generated during exercise and the resulting oxidative stress on the endothelium. Elevated levels of oxidative stress are frequently reported following higher-intensity exercise, compared to low- or moderate-intensity exercise, which do not cause significant elevations in ROS [32, 69, 70]. Furthermore, multiple studies have demonstrated the involvement of ROS in impairing dilatory capacity, using ROS scavengers to abolish reductions in baFMD values post-exercise [29, 44, 71].

The present study found no change in baFMD values immediately following 30 minutes of cycling at 55% PPO. In a similar population of healthy, untrained men, Birk *et al*. found no change in baFMD values following 30 minutes of cycling at 50% maximum HR. However, when Birk *et al*. increased exercise intensity to 70 or 85% of maximum HR, baFMD values immediately post-exercise were reduced [39]. Conversely, our HIT intervention included 10 1-minute bouts at 80% PPO, but did not evoke a reduction in baFMD values, as reported by Birk *et al*. at comparable intensities. A possible explanation for this finding is that the periods of intermittent rest during our HIT protocol allowed brief recovery of the antioxidant capacity of the system, preventing the oxidative stress and subsequent vascular impairment associated with strenuous exercise [32, 69-71]. Our findings suggest that HIT exercise may represent a novel stimulus that prevents the transient impairment in dilatory capacity seen immediately following a bout of sustained intense exercise in untrained men [38, 39].

It has been suggested that assessing baFMD immediately post-exercise may be affected by arterial dilation that occurs in response to augmented blood flow during exercise [72]. When the arterial diameter is elevated post-exercise, shear stress is reduced and - because shear stress is the stimulus for dilation - results in lower baFMD values [63, 72, 73]. Additionally, the calculation for %FMD is inherently biased toward large pre-occlusion diameters, resulting in smaller %FMD values. The present investigation found increased pre-occlusion diameter immediately post-exercise for both HIT and END; however, this did not result in attenuated baFMD values, but unchanged %FMD and AbsFMD. It is possible that elevated pre-occlusion diameter masked what would have otherwise been an increase in baFMD values immediately post-exercise, as maximum diameter was increased compared to baseline.

Similar to baFMD values, previous investigations suggest pre-occlusion diameter immediately post-exercise is a function of exercise intensity. No change in pre-occlusion diameter has been reported following low intensity exercise [28, 29], while moderate and high-intensity exercise evokes an increase in pre-occlusion diameter [38, 39]. Moreover, Birk *et al*. found that in untrained men, pre-occlusion diameter increased in an intensity-dependent fashion with higher intensity exercise resulting in greater diameter [39]. Contrary to this finding, our results demonstrated similar elevations in pre-occlusion diameter for HIT and END immediately post-exercise.

In contrast to our finding of unchanged baFMD values immediately following both END and HIT, Johnson *et al*. reported increased baFMD values following 30 minutes treadmill running at 50% VO_2 peak or an individually calorie matched protocol at 80% VO_2 peak (approximately 20 minutes) [32]. This discrepancy is likely the result

of Johnson's use of trained men (VO_2 peak: 64.0 versus 46.6 $ml/kg^{-1}/min^{-1}$ in the present study), as trained individuals demonstrate a greater resistance to exercise-induced oxidative stress [74]. Additionally, Johnson *et al*. found that larger exercise doses resulted in unchanged baFMD values, contrary to previous reports of reduced baFMD values following intense or long-duration exercise in untrained men [38, 39]. Additional studies have found that individuals with greater baseline activity levels are – depending on the exercise intensity - more likely to demonstrate increased baFMD values [36] and more resistant to possible reductions in baFMD values [43]. Collectively, these studies indicate that trained individuals are less likely to demonstrate reductions in baFMD values post-exercise.

1 Hour post-exercise

One hour following both HIT and END, we found baFMD values were significantly attenuated. Previous investigations have reported similarly attenuated baFMD values approximately 30 minutes post-exercise [40, 41, 43]; however, only one study found reduced baFMD still evident 1 hour following exercise cessation [42]. A return to baseline baFMD values 1 hour post-exercise is more common throughout the literature [32, 33, 39, 42, 46].

In contrast to immediately post-exercise, baFMD values 1 hour post-exercise do not appear to be affected by intensity. Reports of baFMD values that are similar to baseline 1 hour following different exercise intensities exist for overweight [36], untrained [39] and trained [32] men. Collectively, these studies report various baFMD responses immediately post-exercise and a return to baseline values 1 hour later, indicating that both improvements and impairments are short-lived in these populations.

While the present study found no effect of intensity on the baFMD values at 1 hour post-exercise, our finding of reduced baFMD values 1 hour post-exercise for both exercise conditions is uncommon.

A possible explanation for the attenuated baFMD values 1 hour post-exercise is the dramatically elevated pre-occlusion diameter, elevated above both baseline and immediately post-exercise values. While elevated pre-occlusion diameter 1 hour post-exercise is not novel [34, 42], it is in contrast to the more commonly reported return to baseline diameter at 30 [40, 41] and 60 minutes [32, 33] post-exercise. As previously discussed, vessel diameter and baFMD values are inversely related; thus, markedly elevated pre-occlusion diameters may explain the attenuated baFMD values 1 hour post-exercise.

Llewellyn *et al*. offered an alternative explanation for reduced baFMD values post-exercise. They theorized that relative increase in shear stress and not absolute shear stress is the primary determinant of flow-mediated dilation. Thus, the increased shear stress resulting from exercise hyperemia desensitizes the endothelium to the increased shear stress during a baFMD assessment, resulting in reduced vascular responsiveness [41]. In support of this theory, they demonstrated a negative relationship between pre-occlusion SR and baFMD values from pre- to post-exercise [41]. This explanation seems plausible for their data, in which pre-occlusion diameter was unchanged post-exercise and SR was increased. This is an unlikely explanation for the attenuated baFMD in the present study however, as pre-occlusion diameter was elevated post-exercise and the companion pre-occlusion shear rates were not different from baseline.

Additionally, it is possible that a 'ceiling effect' occurred, in which the increased pre-occlusion diameter was so close to maximal endothelium-dependent dilation capacity that it appeared as if dilatory capacity was impaired, when in reality, it had simply reached its threshold. In support of this theory, peak baFMD diameters were similar immediately post-exercise and 1 hour post-exercise.

Interestingly, in addition to increased pre-occlusion diameter, heart rate remained elevated above baseline 1 hour following exercise cessation. Perhaps the cumulative effects of two doses of NTG (baseline and immediately post-exercise) combined with the exercise intervention caused the marked increase in pre-occlusion diameters. Brachial artery dilation does exhibit a dose-response with sublingual NTG administration [75]; however, the duration of these effects is not well documented. Furthermore, it is possible that the marked elevation in pre-occlusion diameters caused a reflexive increase in sympathetic output, resulting in elevated HR at this time point. It has previously been demonstrated that HR does increase mildly in a dose-dependent fashion in response to NTG administration in healthy older subjects [76]. In addition to increasing HR, enhanced sympathetic activity may also account for the blunted baFMD response 1 hour post-exercise, as enhanced sympathetic activity reduces baFMD values [77]. Interestingly, in previous investigations that reported elevated pre-occlusion diameters 1 hour post-exercise, participants were exposed to a baFMD assessment, a dose of NTG and an exercise bout, prior to baFMD assessment at the 1 hour time point [34, 42]. Alternatively, when NTG was not administered prior to exercise, a return to baseline pre-occlusion diameter has been reported 30 minutes [40, 41] and 60 minutes [32] post-exercise in trained males; however, this finding is not universal [30, 43]. The time course

of NTG-induced dilation, as well as the combined effects of NTG and exercise on arterial diameter warrants further investigation.

It is clear that pre-occlusion diameter can significantly alter baFMD values post-exercise. Harris *et al.* determined pre-occlusion diameters to be intensity dependent, after finding increased pre-occlusion diameters 1 hour following high-intensity exercise and no change following moderate-intensity exercise in overweight men [36]. Other investigations have reported increased pre-occlusion diameters 1 hour following intense exercise [42] and unchanged pre-occlusion diameters 1 hour following moderate-intensity exercise [40, 41]. In contrast to the findings of Harris *et al*. in overweight men, trained men were found to return to baseline pre-occlusion diameters 1 hour following four exercise bouts of varying intensity and duration [32]. This data suggests that, in addition to exercise intensity, training status may affect the duration of changes in arterial diameter post-exercise. In opposition of both of these investigations, the present study found markedly increased pre-occlusion diameters 1 hour following both HIT and END in untrained subjects. Our pre-occlusion diameters did not appear to be intensity-dependent as reported by Harris *et al*. or, independent of intensity, returned to baseline values 1 hour post-exercise as reported by Johnson *et al*. The effect of exercise intensity and training status on resting arterial diameter 1 hour following exercise cessation remains unclear.

A final possible explanation for the reduced baFMD values 1 hour post-exercise is that oxidative stress impaired endothelial function. While oxidative stress is typically thought to affect vascular function for only a short time post-exercise, the time course is highly variable and dependent on several factors, including exercise intensity and

duration. Some indices of oxidative stress remain elevated several hours post-exercise [78]; however, their effect on vascular function remains unclear. Rognmo *et al*. reported a reduced %FMD 1 hour following a bout of high-intensity exercise in a group of 'highly endurance-trained' men and attributed the reduction to 'oxidative radicals caus(ing) local vascular stress in the vessel'. Surprisingly, they reported elevated NO bioavailability and total antioxidant status in conjunction with depressed baFMD, leading them to conclude that additional mechanisms were involved in determining vascular tone post-exercise [42]. Additionally, a group of sedentary young men also demonstrated increased NO bioavailability and total antioxidant status 1 hour post-exercise, coinciding with unchanged %FMD [42]. In trained young men, Johnson *et al*. found unchanged levels of oxidative stress 1 hour post-exercise with unchanged baFMD values [32]. Based on the investigations to date, we feel it is unlikely that our exercise bouts were strenuous enough to increase oxidative stress and impair vascular function 1 hour post-exercise, especially considering that baFMD values were unchanged immediately post-exercise.

All of the previously discussed studies report unchanged or reduced baFMD values 1 hour post-exercise in subjects devoid of risk factors for endothelial dysfunction [32, 33, 39-43, 45, 46], with one report of increased baFMD values in active overweight men [36]. Conversely, in a recent study from our lab, significant improvements in %FMD and AbsFMD were reported 1 hour post-exercise in a group of CAD patients, in spite of increased pre-occlusion diameters [34]. Improved baFMD values have also been reported 45 and 60 minutes following exercise in postmenopausal women [33, 35] and 75 minutes post-exercise in obese individuals [30]. CAD [79], post-menopause [80] and obesity [81, 82] have all been identified as risk factors for endothelial dysfunction,

indicating that individuals with antecedent dysfunction are more likely to experience longer duration acute vascular improvements in response to exercise. This finding parallels reports of chronic improvements in endothelial function seen with habitual exercise training in patients with a priori endothelial dysfunction.

It has been suggested that the absence of improved baFMD values in healthy populations post-exercise may be the result of a 'ceiling effect'. Simply, that in young healthy individuals, NO availability and utilization are near optimal levels at baseline resting conditions and that NO increases from exercise are surplus and therefore have no impact on dilatory capacity [33]. Rognmo *et al*. demonstrated that sedentary young men and highly endurance-trained men have comparable %FMD at rest, revealing negligible increases in vascular function despite intense exercise training [42]. In contradiction of this theory, Johnson *et al*. demonstrated that with the correct dose of exercise, even trained men could experience acute improvements in vascular function [32]. In further opposition of the 'ceiling effect' theory, is that subjects in our study experienced greater peak baFMD diameters immediately and 1 hour post-exercise, demonstrating that maximal endothelium-dependent dilation was not achieved at baseline. Multiple investigations have reported similar increases in maximum diameter 1 hour post-exercise [30, 32, 34, 46] and Johnson *et al*. found elevated peak diameters after high-intensity exercise bouts only, with no change after moderate intensity sessions.

While our study failed to see improvements in baFMD values at any time point, it is important to interpret these results in the context of absolute diameters. One hour post-exercise we found attenuated baFMD values with parallel increases in pre-occlusion and

maximum diameters. Although the overall dilatory capacity of the vessel may have been reduced, the capacity of the vessel to transport blood was actually increased.

24 Hours post-exercise

In the present study, baFMD values returned to baseline 24 hours following exercise cessation, in accordance with previous findings in sedentary and trained young men following high-intensity exercise [42]. This is somewhat in contrast to the findings of Tyldum *et al*. who reported elevated baFMD values 16-18 hours post-exercise for both HIT and END exercise conditions, with a markedly greater improvement for HIT that lasted an additional four hours, despite the introduction of a high-fat meal – a stimulus known to induce endothelial dysfunction [57]. These contrasting findings may be the result of different study populations as Tyldum *et al*. used participants with an average age of 42 years, while return to baseline baFMD values 24 hours post-exercise were reported in trained and untrained men in their mid twenties [42]. This is significant because it has been demonstrated that advancing age is an independent risk factor for endothelial dysfunction, with evidence for declining function prior to age 40 [83-86]. These findings may further indicate that individuals with a priori endothelial dysfunction stand to experience long-duration improvement in baFMD values post-exercise.

Normalized FMD

It has previously been suggested that normalized FMD should only be considered when there is a clear relationship between SR_{AUC} and %FMD [87, 88]. At rest, Llewellyn *et al*. found a strong correlation between SR_{AUC} and %FMD, that was abolished post-exercise, in association with a highly variable SR during reactive hyperemia [41]. The present investigation failed to demonstrate the correlation between

SR_{AUC} and %FMD at rest (Figure 6). Post-exercise, our results were similar to Llewellyn *et al*., as we found highly variable normalized FMD values and no association between SR_{AUC} and %FMD (Figure 6). Thus, based on these recommendations, normalized FMD will not be discussed further.

2.4.2 Endothelium-independent function: nitroglycerin administration

The current study found AbsNTG and %NTG both significantly decreased immediately post-exercise; however, we do not believe this to be indicative of reduced smooth muscle function. It is important to consider that exercise hyperemia causing arterial dilation was apparent during the NTG assessments performed immediately post-exercise. As a result, pre-NTG diameters at this time point were significantly increased compared to baseline, and likely contributed to the blunted NTG response. While %NTG and AbsNTG were diminished, peak NTG diameters post-exercise were identical to those achieved at baseline, indicating that peak dilation capacity of the vascular smooth muscle was not functionally impaired immediately post exercise, but that a 'ceiling effect' was likely the cause of the reduction in %NTG and AbsNTG values.

NTG assessments performed immediately following dynamic lower-limb exercise are rare in the literature. Tinken *et al*. reported no change in %NTG five minutes following a low-intensity cycling intervention [28] and in multiple studies examining acute response to handgrip exercise, %NTG was not altered immediately following exercise [26, 28, 37]. None of these studies reported an increase in pre-NTG diameters post-exercise, likely as a result of considerably less-intense exercise interventions, compared with the present study [26, 28, 37]. This supports our theory that vascular smooth muscle was not impaired post-exercise, but that reduced values in the current

study were the result of an elevated pre-NTG diameter. While we did not assess NTG dilation 1 hour post-exercise, there is reliable evidence that endothelium-independent function is unaffected 30-60 minutes post-exercise in healthy subjects [26, 33, 41, 42].

Several investigations have reported peak NTG dilation 3-4 minutes following administration in healthy subjects [89-91], leading to a recommendation that arterial images be collected for four minutes [92]. Hence, 4-5 minutes of NTG image-collection has become prevalent throughout the literature [33, 37, 44, 93, 94]. In the current study, NTG-induced dilation occurred consistently at an average of minute 8 (8 ± 2 minutes) for all time points. Our finding reaffirms results from Akamatsu *et al.,* who found peak dilation around 7 minutes [95] and Bressler *et al.*, who found that peak dilation was significantly greater from dilation achieved at 3 and 5 minutes [96] following NTG administration. As a result, some laboratories have began to measure dilation for 10 minutes following NTG administration [28, 34, 41]; which appears to be sufficient, as Bressler *et al*. reported 90% of their 52 participants having reached peak dilation by 8 minutes [96]. Our investigation supports the adoption of a ten-minute measurement period following NTG administration in determination of endothelium-independent dilation.

2.4.4 Limitations

Interpreting post-exercise FMD values may be complicated by several factors, including alterations in shear stress and pre-occlusion diameter, as well as altered sympathetic output [72]. Larger pre-occlusion diameters are associated with smaller baFMD values [72, 97]; an effect evident both immediately and 1 hour post-exercise in the present study. The dramatically elevated pre-occlusion diameter 1 hour post-exercise

led us to speculate that the cumulative effect of two doses of NTG, two baFMD assessments and an exercise bout may have had an effect on resting arterial diameter. A previous investigation reported a dose-response relationship between sublingual NTG and baFMD [75], which could possibly explain why pre-occlusion diameter was greater 1 hour post-exercise than immediately post-exercise.

While training status of subjects has shown to be an important determinant in baFMD responses post-exercise [32, 39, 43], activity levels of participants were not recorded. Based on participants' VO_2 peaks, it appears that the group was similar with regard to exercise capacity (range: 40.2 – 52.6, average: 46.4 ± 4.7 ml $O_2 \bullet kg^{-1} \bullet min^{-1}$)

2.4.5 Future directions

A study comparing longer duration and more strenuous bouts of HIT with different doses of sustained exercise is needed to determine if the intermittent rest periods during HIT prevent the transient reduction in vascular function immediately following sustained high-intensity exercise described elsewhere [38, 39]. While the present study supports this theory, it is possible that our bout of HIT simply did not include enough intervals to elicit this reduction.

The recurrent finding of improved baFMD values 1 hour post-exercise in populations with risk factors for endothelial dysfunction [30, 33, 34], while healthy populations consistently fail to show improvements at this time point is interesting because it mirrors reports of chronic adaptations to exercise training [25]. A direct comparison of the acute vascular response in healthy individuals and a population with endothelial dysfunction may improve understanding of the mechanisms governing chronic vascular adaptations to exercise.

Recently, several investigations have examined baFMD immediately post-exercise in young healthy and trained individuals [29, 32, 38, 39, 98]; however, minimal data exists for this time point in clinical populations. While increased oxidative stress post-exercise is well documented [32, 69, 74], how this affects populations with diminished baseline function remains to be seen. Perhaps oxidative stress-induced endothelial dysfunction plays a role in the poorly understood mechanism behind exertion related cardiovascular events [99-101].

Multiple investigations have demonstrated that attenuated baFMD values immediately following exercise can be prevented through antioxidant administration [29, 44] or remote ischemic preconditioning [38]. If oxidative stress does seriously contribute to increased cardiovascular risk post-exercise, there may be a role for prophylactic antioxidant supplementation with exercise in clinical populations. Conversely, some reports indicate the elevated levels of ROS post-exercise are essential for chronic adaptations. Further research in this area is warranted to determine if health promoting vascular adaptations still present in the face of antioxidant therapy with exercise.

2.4.6 Conclusions

The present study demonstrated that high-intensity interval exercise and endurance exercise result in similar acute baFMD responses in healthy young males. This finding is noteworthy because our bout of high-intensity exercise did not evoke a reduction in baFMD values immediately post-exercise, as previously reported [39]. HIT may offer a unique stimulus compared to sustained exercise and avoid this transient reduction in vascular function. The effect this may have on chronic adaptations to exercise remains to be seen.

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Table 1. Subject characteristics

	Mean ± SD
Age (years)	23.3 ± 2.1
Height (cm)	179 ± 8
Weight (kg)	80.1 ± 11.8
Body Mass Index (kg m^{-2})	25.0 ± 2.7
Resting Hemodynamics	
Heart Rate (beats per minute)	57.5 ± 7.9
Systolic Blood Pressure (mmHg)	118.0 ± 8.9
Diastolic Blood Pressure (mmHg)	66.6 ± 5.6
Mean Arterial Pressure (mmHg)	83.7 ± 6.5
VO_2 Peak (mL kg^{-1} min^{-1})	46.4 ± 4.7

Values presented as mean ± standard deviation

Table 2. END baFMD and hemodynamic values

	Baseline	Post-ex	1 h post-ex	24 h post-ex
HR (bpm)	56.5 ± 8.9	97.3 ± 8.8*	67.4 ± 9.9**	56.3 ± 9.2
SPB (mmHg)	120.0 ± 11.5	105.1 ± 7.9**	110.9 ± 12.6	115.8 ± 9.0
DBP (mmHg)	67.3 ± 7.5	63.6 ± 6.8	64.3 ± 8.2	66.1 ± 6.3
MAP (mmHg)	84.9 ± 8.6	77.4 ± 7.1	79.9 ± 9.5	82.6 ± 7.0
%FMD (%)	6.8 ± 2.4	6.9 ± 2.7	2.6 ± 1.9*	5.0 ± 2.7
AbsFMD (mm)	0.27 ± 0.10	0.29 ± 0.10	0.12 ± 0.09**	0.2 ± 0.11
NormFMD ($x10^{-4}$)	4.8 ± 5.8	2.4 ± 1.9	0.92 ± 0.7	2.5 ±2.8
Pre-occ diam (mm)	4.0 ± 0.4	4.3 ± 0.5*	4.6 ± 0.4*	4.1 ± 0.4
Max diam (mm)	4.3 ± 0.4	4.6 ± 0.4*	4.7 ± 0.4*	4.3 ± 0.4
Time to peak (s)	61 ± 22	85 ± 26	71 ± 28	65 ± 23

Values presented as mean ± standard deviation
significant from baseline at *P≤0.001 or **P≤0.05

Table 3. HIT baFMD and hemodynamic values

	Baseline	Post-ex	1 h post-ex	24 h post-ex
HR (bpm)	58.6 ± 7.3	94.4 ± 5.5*	65.6 ± 6.7**	59.2 ± 11.2
SPB (mmHg)	116.0 ± 8.5	105.0 ± 8.7**	110.3 ± 10.5	117.2 ± 8.9
DBP (mmHg)	65.8 ± 4.8	62.5 ± 5.8	63.5 ± 7.1	66.3 ± 5.6
MAP (mmHg)	83.7 ± 7.3	77.0 ± 6.5	79.5 ± 8.6	83.2 ± 6.5
%FMD (%)	5.9 ± 2.3	8.3 ± 2.8	2.5 ± 1.5*	4.6 ± 1.6
AbsFMD (mm)	0.24 ± 0.11	0.35 ± 0.10	0.11 ± 0.07**	0.18 ± 0.07
NormFMD ($x10^{-4}$)	6.8 ± 13.0	3.4 ± 2.7	2.0 ± 2.0	2.5 ± 1.7
Pre-occ diam (mm)	4.0 ± 0.4	4.2 ± 0.4*	4.5 ± 0.5*	4.0 ± 0.3
Max diam (mm)	4.2 ± 0.4	4.6 ± 0.4*	4.6 ± 0.5*	4.2 ± 0.4
Time to peak (s)	56 ± 15	79 ± 23	52 ± 21	60 ± 24

Values presented as mean ± standard deviation
significant from baseline at *P≤0.001 or **P≤0.05

Table 4. END NTG values

	Baseline	Post-ex	1 h post-ex	24 h post-ex
%NTG (%)	18.3 ± 3.1	10.9 ± 4.9**	--	17.3 ± 3.5
AbsNTG (mm)	0.76 ± 0.12	0.47 ± 0.19**	--	0.72 ± 0.12
Pre-NTG diam (mm)	4.2 ± 0.3	4.4 ± 0.5**	--	4.2 ± 0.4
Max NTG diam (mm)	4.9 ± 0.4	4.9 ± 0.4	--	4.9 ± 0.4
Time to Peak (min)	9 ± 1	8 ± 2	--	8 ± 2

Values presented as mean ± standard deviation
significant from baseline at *P≤0.001 or **P≤0.05

Table 5. HIT NTG values

	Baseline	Post-ex	1 h post-ex	24 h post-ex
%NTG (%)	18.8 ± 4.4	12.3 ± 3.1**	--	20.9 ± 6.2
AbsNTG (mm)	0.77 ± 0.14	0.54 ± 0.12**	--	0.84 ± 0.22
Pre-NTG diam (mm)	4.2 ± 0.4	4.5 ± 0.4**	--	4.1 ± 0.4
Max NTG diam (mm)	4.9 ± 0.4	5.0 ± 0.4	--	4.9 ± 0.5
Time to Peak (min)	8 ± 2	8 ± 2	--	8 ± 2

Values presented as mean ± standard deviation
significant from baseline at *P≤0.001 or **P≤0.05

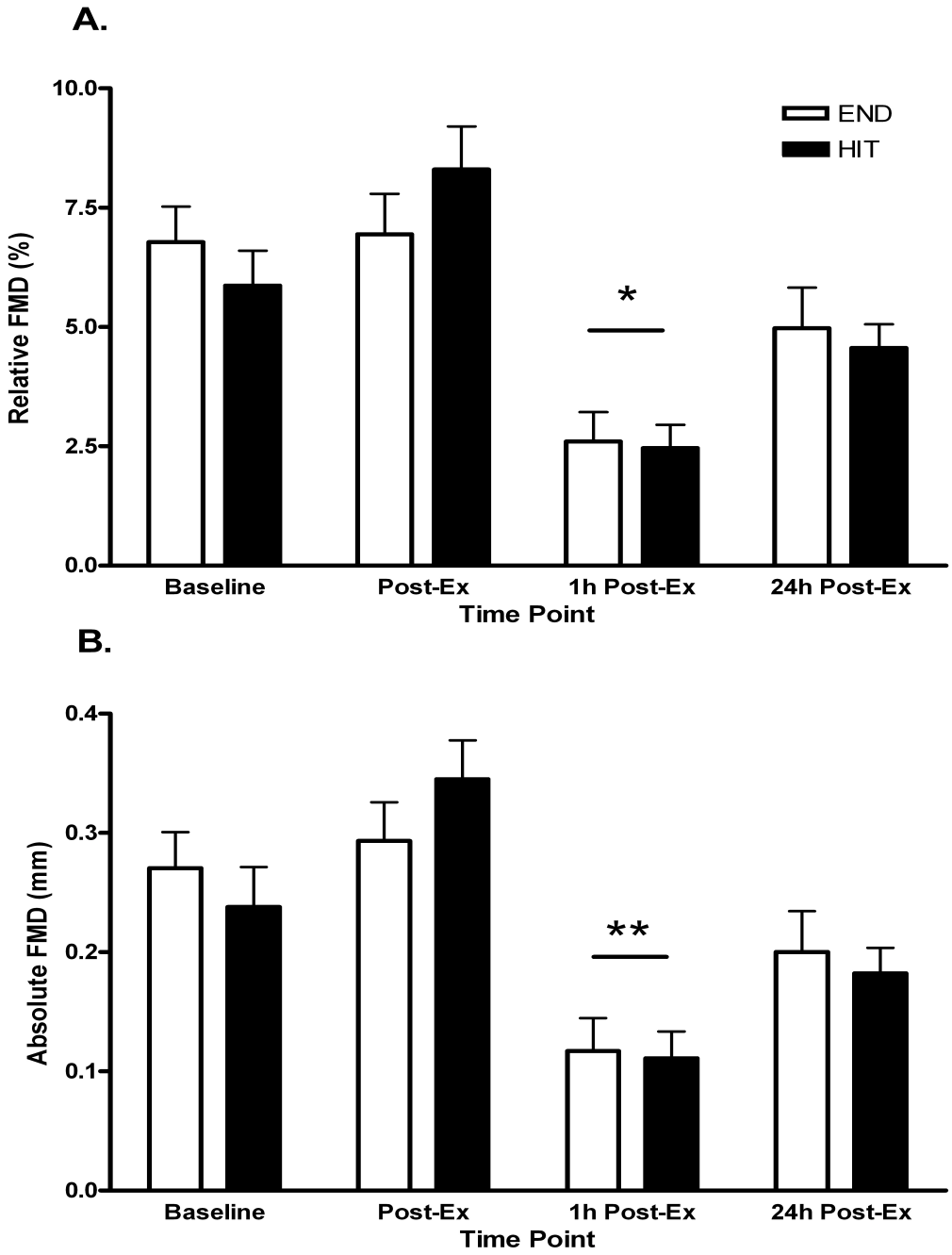


Figure 2. A) Relative FMD B) Absolute FMD
*p<0.001 significant from Baseline; **p<0.05 significant from Baseline

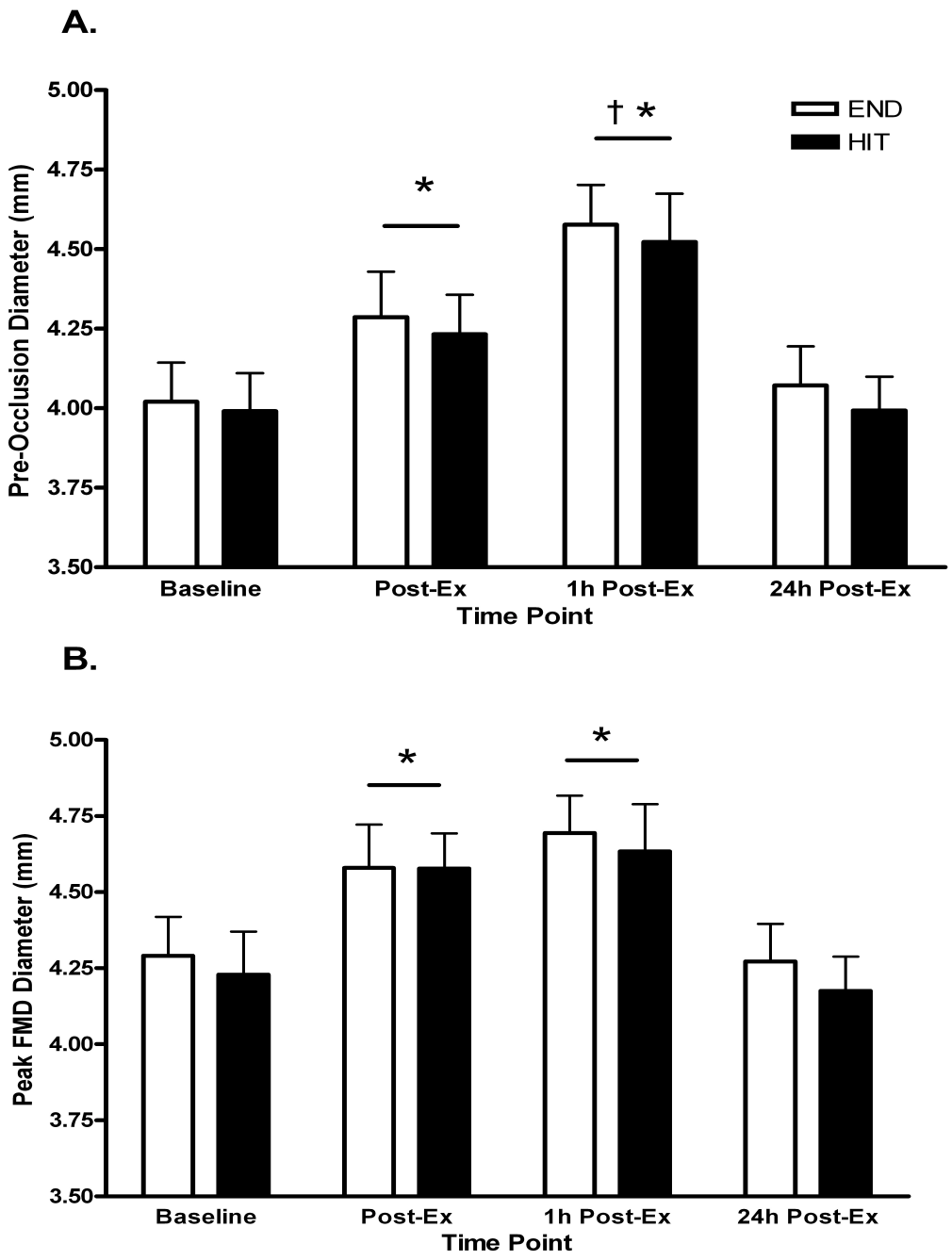


Figure 3. A) Pre-occlusion diameter B) Peak FMD diameter
*p<0.001 significant from Baseline; † p<0.01 significant from Post-ex

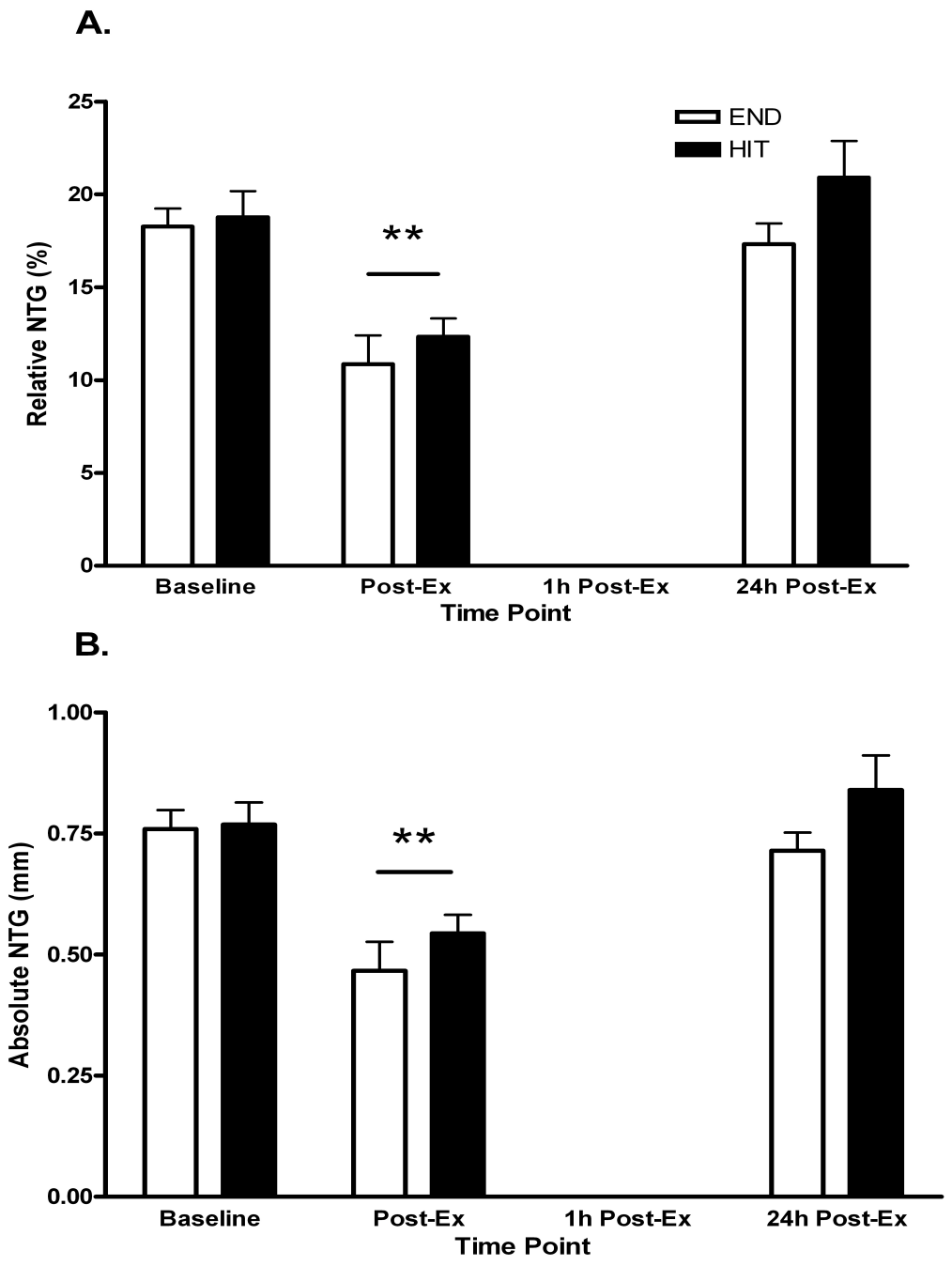


Figure 4. A) Relative NTG B) Absolute NTG
**p<0.05 significant from Baseline
Relative NTG Main Effect for exercise condition HIT > END (p<0.05)

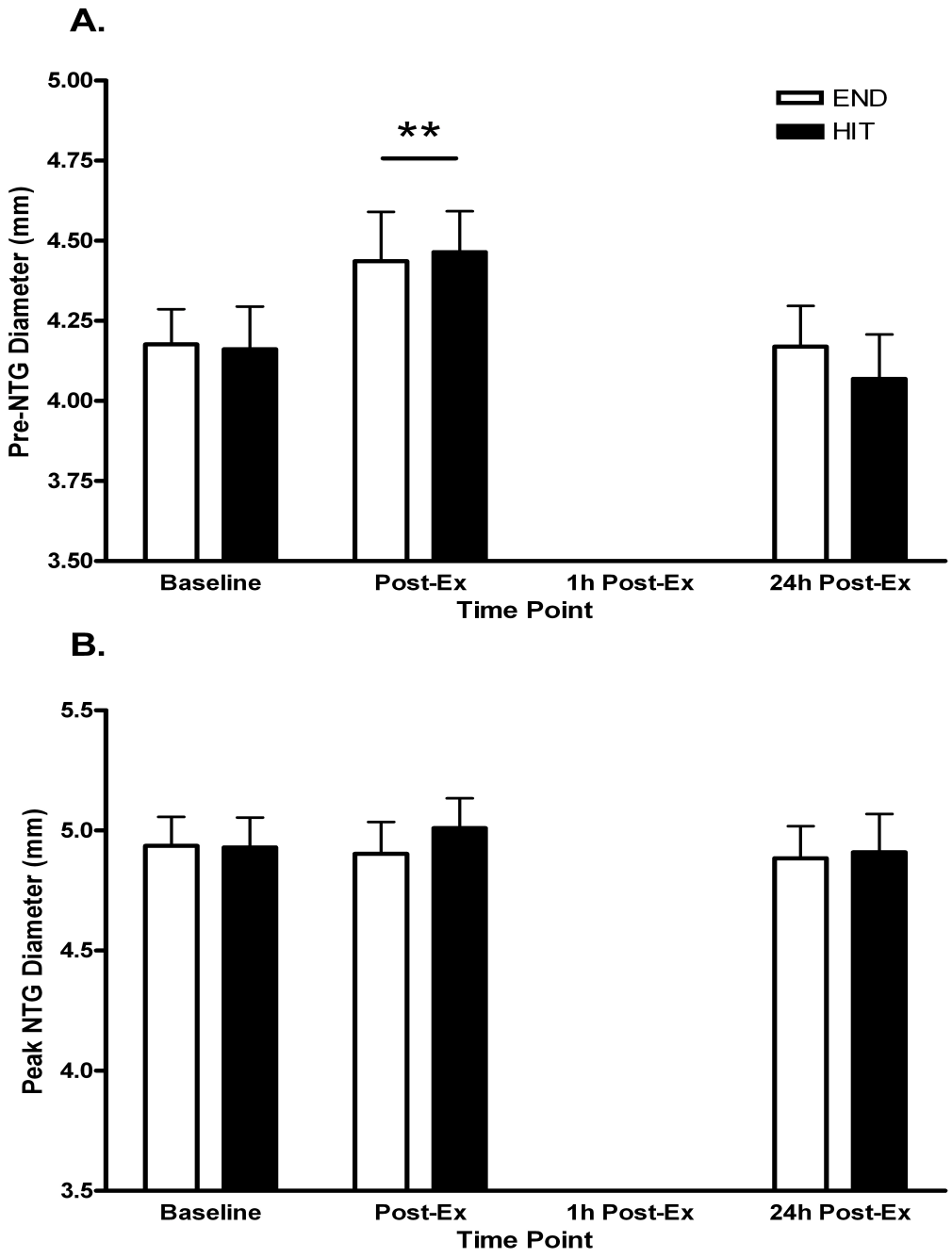
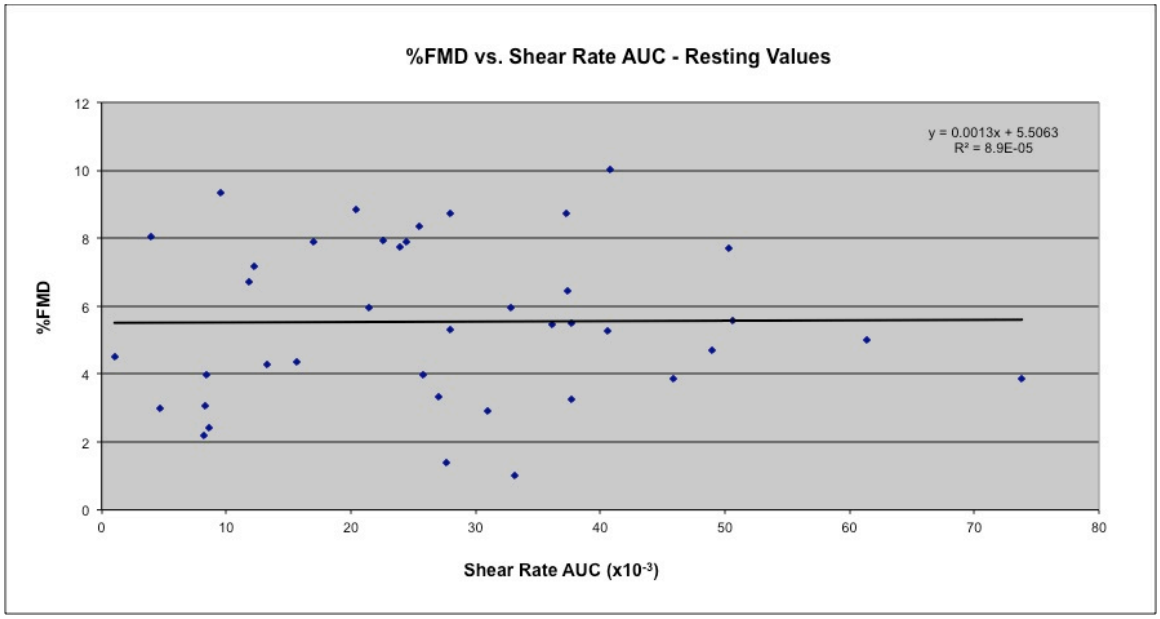


Figure 5. A) Pre-NTG Diameter B) Peak NTG Diameter
**p<0.05 significant from Baseline

A.



B.

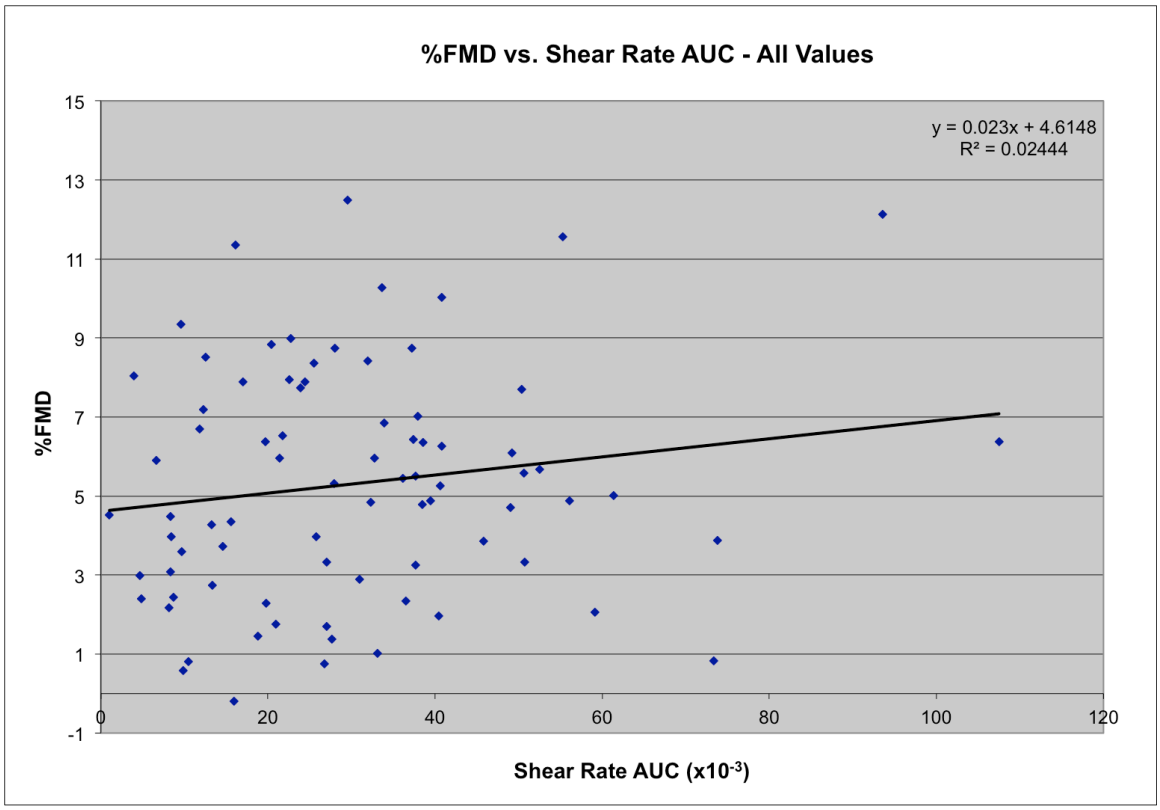


Figure 6. A) %FMD vs. Shear Rate AUC at Rest (Baseline and 24 hours post-exercise), B) %FMD vs. Shear Rate AUC at All Time Points

APPENDIX A – Participant information and consent form

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EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

PARTICIPANT INFORMATION SHEET - YOUNG

TITLE: Examining Changes in Endothelial Function and Arterial Stiffness following Acute Bouts of Exercise

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Sponsors: Natural Sciences and Engineering Research Council

You are being invited to participate in a research study conducted by the Department of Kinesiology at McMaster University. Before agreeing to participate in this study, it is important that you read and understand the following explanation of the proposed study. This form outlines the purpose and testing procedures used in this study. It also describes your right to refuse to participate or withdraw from the study at any time, the time commitment and the potential risks and benefits, so that you can make an informed decision. This is known as the informed consent process. Please ask the study investigators to explain anything you do not understand before signing this consent form.

Participant Initials: _____

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I. STUDY PURPOSE

It has long been established that chronic exercise training can improve quality of life and cardiovascular health across many variables. However, the acute effects of exercise on the cardiovascular system following a single bout of exercise are not as well understood. The purpose of this study will be to examine changes in blood vessel function immediately following two different exercise conditions: high-intensity interval exercise and continuous aerobic exercise.

II. DESCRIPTION OF TESTING PROCEDURES:

What is the time commitment?

You will be asked to visit the Exercise and Metabolism Research Laboratory (IWC E102, McMaster University) on five separate occasions. Your first visit will last approximately 45 minutes. Following that, two primary visits will last approximately 3 hours, and two follow-up visits, held 24 hours after each primary visit, will last approximately 45 minutes. All visits will be scheduled to take place at the same time of day. Before each visit you should abstain from exercise for at least 48 hours, avoid alcohol and caffeine consumption for at least 12 hours, and any vasoactive medications (i.e. nitrates) for 4 hours prior to each laboratory visit. You will be asked to arrive at the laboratory having fasted for a minimum of 8 hours. You should also bring appropriate exercise attire including loose fitting shorts.

On your first visit to the laboratory you will be introduced to the laboratory environment and testing procedures. Following this, an investigator will take you through the consenting process, where you will be given an opportunity to ask questions before deciding whether or not you wish to participate. Upon reciept of informed consent, you will complete session 1 of 5, which will include a maximal exercise test to determine the workloads for the given exercise interventions. .

The second visit to the laboratory will include one exercise bout, a blood sample and multiple measures of blood vessel structure and function. The next visit to the laboratory (follow-up visit #1) will occur 24 hours after your initial visit and will last approximately 45 minutes. It will include two of the same measures of vessel structure and function from the initial visit, but no exercise or blood sample. The fourth and fifth visits will be identical to the second and third respectively, except that the type of exercise will be different and no blood will be drawn. The order of interventions will be randomized and you will be informed of which intervention you will be performing when you arrive at the laboratory for testing. A description of each intervention and measurement technique is provided below.

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HEART MEASURES

a) Heart Rate: Heart rate will be measured with 6 electrodes positioned on the surface of the skin on your chest.

BLOOD VESSEL MEASURES

a) Blood Pressure: Blood pressure will be monitored non-invasively using Nexfin Finapres methodology. An inflatable cuff will be placed around the middle finger and a small monitor will be placed around the wrist. This technique will allow us to measure continuous blood pressure and heart rate throughout the protocol.

b) Pulse Wave Velocity: This is a measure of the speed of the blood between two different points along the blood vessels. It is calculated from measurements of heart rate and data collected from the blood vessels. The blood vessel data are collected by applanation tonometry, a small device placed on the surface of the skin at the site of an artery. Tonometry uses a force transducer to provide data that is then used to calculate pulse wave velocity. Tonometry will be conducted at your carotid artery (neck), femoral artery (groin), radial artery (wrist) and dorsalis pedis artery (foot). The values obtained from these measurements will allow for the calculation of central (chest) and peripheral (leg) pulse wave velocities.

c) Endothelial Function: The endothelium is a thin layer of cells that lines the inside of all blood vessels and moderates arterial size. To measure endothelial function, baseline measurements of blood vessel diameter are compared to diameters obtained following an experimental condition. The increase in the blood vessel diameter (dilation) following each condition gives an indication of the health of the endothelium in that vessel (endothelial function).

- **Endothelium-dependent flow-mediated dilation:** This technique involves the placement of a blood pressure cuff around the forearm, approximately 10cm below the elbow. The cuff is inflated to a pressure where it stops all blood flow to your arm (commonly 200mmHg) and will be held at this pressure for a period of five minutes. Continual measures of blood vessel diameter and the speed of blood flow will be obtained using Doppler ultrasound. A probe will be placed on the upper arm (below the biceps) at the site of the brachial artery. Images will be taken at rest (before cuff inflation), prior to cuff deflation (end of five minute period), and following cuff deflation for five minutes. These measurements can be used to determine endothelial function.

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- **Endothelium-independent dilation:** This technique involves the administration of a 0.4 mg dose of sublingual (under the tongue) nitroglycerin (NTG). Measures of vessel diameter and the speed of blood flow will be taken at rest (before NTG administration) and for ten minutes following NTG administration.

Maximal Exercise Test (VO_2 Max Test)

The VO_2 max test will take place on a cycle ergometer. The test will consist of a 5 minute warm-up at 50 watts whereafter resistance will be increased 1 watt every 2 seconds until volitional exhaustion is reached or until pedal cadence drops below 40 revolutions per minute. Values obtained during this test will be used to set the workload during the two exercise interventions explained below

Blood Sample

A single 10mL tube of blood will be drawn during baseline at your first visit to the laboratory. A small butterfly needle will be inserted at the anticubital vein in the elbow. A trained technician will collect all blood samples.

Interventions

All exercise sessions will be performed on a stationary bike and will include a standardized 3-minute warm up and 3-minute cool down at 30% of your maximal capacity (VO_{2max}). All exercise sessions will be supervised by a member of the research team who will ensure that you are using proper techniques and exercising at the correct intensities.

a) Traditional Cardiac Rehabilitation Exercise = 30 minutes
- Continuous cycling for 30 minutes at 55% of VO_{2max}

b) High Intensity Exercise = 20 minutes
- 10 x 1 min intervals at 80% VO_{2max} separated by 1 min intervals at 30% VO_{2max}

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III. POTENTIAL RISKS AND DISCOMFORTS:

All Doppler ultrasound, blood pressure, heart rate and tonometer procedures are non-invasive and offer minimal risk. In rare instances, you may experience a minor skin irritation from the electrode adhesive and/or conducting gel.

With respect to the measure of endothelial-dependent vasodilation, there are no known risks associated with the five-minute period of blood flow stoppage to one's arm. Nevertheless, during this five-minute period you may feel brief numbness and/or tingling in the arm when blood flow is stopped and a 'pins and needles' sensation upon cuff release. These sensations should only last a few minutes and there are no lasting effects of this procedure on the function of the arm.

The reported side effects of nitroglycerin administration include dizziness or lightheadedness especially upon standing, sensation of burning or tingling upon sublingual administration, and headache (http://media.pfizer.com/files/products/uspi_nitrostat.pdf). The most likely side effect will be dizziness or lightheadedness; however, the administration of nitroglycerin while you are lying down should minimize this risk. If dizziness or lightheadedness does occur, it should quickly subside.

Exercise alone briefly increases your risk of cardiovascular complication; however, the risks associated with maximal exercise are low (less than 0.1%). All exercise sessions included in this study will involve submaximal exercise; therefore there are no known risks other than feelings of cramping or fatigue in your legs or buttocks from the exercise bout. These feelings should subside within a few days.

There is minimal risk associated with giving a blood sample. You may feel mild discomfort in the arm when the butterfly needle is inserted, however, the procedure is brief and is rarely associated with pain. The insertion of needles for blood sampling is a common medical practice and involves few risks if proper precautions are taken. The needle is inserted under completely sterile conditions; however, there is a theoretical risk of infection. There is also a chance of internal bleeding if adequate pressure is not maintained upon removal of the needle. This may cause some minor discomfort and could result in bruising/skin discoloration, which could last for up to a few weeks. In very rare occasions, trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication after having taken blood several thousand times.

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IV. BENEFITS AND REMUNERATION:

As a participant in this study you will gain an increased knowledge of the health of your blood vessels while contributing to the scientific community. You will be provided a $20 Tim Hortons gift card for your participation in the study.

V. CONFIDENTIALITY:

Any personal information revealed and experimental data collected will be treated as confidential and will not be revealed to anyone other than the study investigators. All data from this study will be stored in offices and secured on computers to which only the investigators will have access. You should be aware that results of this study will be made available to the scientific community, through publication in a scientific journal, although neither your name nor any reference to you will be used in compiling or publishing these results. Data will be retained for ten years after the date of publication, at which time all information will be destroyed. In addition, you will have access to your own data, as well as the group data when it becomes available and if you are interested.

VI. PARTICIPATION AND WITHDRAWAL:

You can choose whether to participate in this study or not. As a participant in this study, you have the option of removing your data from the study, the right to refuse to participate in any component of the study, and you may also withdraw your consent to participate all together without prejudice or consequence to any other interaction that you may have with the study investigators including future care and/or treatment. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen which warrant doing so.

VII. INQUIRIES:

Study Procedures
If you have any questions about the study please feel free to contact Dr. Maureen MacDonald at 905-525-9140, extension 23580.

Rights as a Research Participant
If you have any questions about your rights as a research participant, please contact the Office of the Chair of the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at 905-521-2100, extension 42013.

Participant Initials:

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CONSENT STATEMENT

SIGNATURE OF RESEARCH PARTICIPANT

I have had the opportunity to read the preceding information and I fully understand the risks associated with the experimental procedures. I have been able to discuss this study with the investigators and all of my questions have been answered to my satisfaction. I consent to take part in the study with the understanding that I may withdraw at any time without affecting me in anyway. I understand that I will receive a signed copy of this form.

I voluntarily consent to participate in this study.

_____
PRINTED NAME OF PARTICIPANT

_____ _____
SIGNATURE DATE

Consent form administered and explained in person by:

_____
PRINTED NAME AND TITLE

_____ _____
SIGNATURE DATE

Signature of Principal Investigator:

_____
PRINTED NAME AND TITLE

_____ _____
SIGNATURE DATE

Participant Initials:

APPENDIX B – VO_2 peak data collection form

File Name: **Participant #**

Acute Exercise & Endothelial Function

Visit: VO_2 Peak Test **Cadence: _____** **Seat Height: _____**

Date: _____ Time: _____ DOB: _____

Initials: _____ Height: _____ Weight: _____

Room Temp: _____ Humidity: _____ Last Meal: _____

BP/MAP/HR #1: _____ #2: _____ #3: _____

Time	PO (W)	HR	VO_2
WU	_____	_____	_____
1min	_____	_____	_____
2min	_____	_____	_____
3min	_____	_____	_____
4min	_____	_____	_____
5min	_____	_____	_____
6min	_____	_____	_____
7min	_____	_____	_____
8min	_____	_____	_____
9min	_____	_____	_____
10min	_____	_____	_____
11min	_____	_____	_____
12min	_____	_____	_____
13min	_____	_____	_____
14min	_____	_____	_____
15min	_____	_____	_____
16min	_____	_____	_____

APPENDIX C – Data collection form

File Name: **Participant #**

Acute Exercise & Endothelial Function

Visit #: ____ Date: _____ Time: _____ Exercise Bout: HIT / END

Participant follow pre-visit instructions: Y / N Cuff Size: _____

If no, explain:

DOB: _____ **(Y / O)** Initials: _____

Height: _____ Room Temp: _____

Weight: _____ Humidity: _____

Medications: _____

Blood Draw completed by: _____ Time: _____

Blood Prepared by: _____

Eppendorf Label: _____

Pulse Wave Velocity

Distances

Carotid – sternal notch: _____cm Sternal notch – umbilicus: _____cm

Sternal notch-radial: _____cm Umbilicus – femoral: _____ cm

Femoral – posterior tibialis: _____cm

1

File Name: **Participant #**

FMD #1 – Baseline

FILE NAME: _____ **NEXFIN FILE NAME: _____**

Ultrasound Probe: _____ PW Frequency Setting: _____

Multigon Range and Setting: _____ Doppler Probe Angle: _____

Cuff Inflation Pressure: _____ FPS: _____

Depth: _____ Volume: _____

Sample Volume: _____ Scale: _____

Image #'s	Comments

Nitroglycerin #1 – Baseline

FILE NAME: _____ **NEXFIN FILE NAME: _____**

Image #'s	Comments

File Name: **Participant #**

Exercise: HIT / End **Ex. Completed at: _____ Seat Height: _____**

Workloads: PPO: _____ 30% PPO: _____ END: 55% PPO: _____

HIT: 80% PPO: _____ **Warm-Up/Cool Down: 180s @ 30%**

Complete Exercise: YES / NO **End: 999+801s @ 55%**

Reason: **HIT: 60s @ 80%, 60s @ 30% x 10**

FMD #2 – Immediately Post-Exercise

FILE NAME: _____ NEXFIN FILE NAME: _____

Image #'s	Comments

Nitroglycerin #2 – Immediately Post-Exercise

FILE NAME: _____ NEXFIN FILE NAME: _____

Image #'s	Comments

3

File Name: **Participant #**

COMMENTS:

FMD #3 – 1 Hour Post-Exercise

FILE NAME: _____ **NEXFIN FILE NAME: _____**

Image #'s	Comments

Comments:

4

File Name: **Participant #**

24 Hour Follow-Up

Follow-up #: _____ Date: _____ Time: _____ Follow-up for: HIT / END

Participant follow pre-visit instructions: Y / N

If no, explain:

Initials: _____

Room Temp: _____

Humidity: _____

Comments:

5

File Name: **Participant #**

FMD #4 – 24 Hours Post-Exercise

FILE NAME: _____ **NEXFIN FILE NAME: _____**

Ultrasound Probe: _____ PW Frequency Setting: _____

Multigon Range and Setting: _____ Doppler Probe Angle: _____

Cuff Inflation Pressure: _____ FPS: _____

Depth: _____ Volume: _____

Sample Volume: _____ Scale: _____

Image #'s	**Comments**

Nitroglycerin #3 – 24 Hours Post-Exercise

FILE NAME: _____ **NEXFIN FILE NAME: _____**

Image #'s	**Comments**

APPENDIX D – Raw Data

Table 1. Subject characteristics

ID	Age	Height (cm)	Weight (kg)	BMI	VO_2 Peak ($ml\ kg^{-1}\ min^{-1}$)
1	25	185	91	26.6	43.3
2	22	181	91.5	27.9	42.4
3	25	179	67.5	21.1	52.6
4	22	163	71.5	26.9	51.6
5	21	169	65.5	22.9	42.1
6	21	183	84.5	25.2	43.4
7	25	183	75	22.4	49.6
8	21	177	86.5	27.6	46.4
9	25	177	69	22.0	51.9
10	26	189	99	27.7	40.2
Mean	**23.30**	**179**	**80.10**	**25.04**	**46.4**
SD	**2.06**	**0.08**	**11.84**	**2.67**	**4.7**

Table 2. Resting hemodynamic variables

ID	HR (bpm)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
1	58.6	118	69.2	85.5
2	53.5	126.8	71.4	89.9
3	49.7	120.8	70.7	87.4
4	46.7	118.5	69.2	85.7
5	53.4	125.7	73.3	90.8
6	74.3	131.6	68.0	89.2
7	61.2	102.6	55.7	71.3
8	60.4	106.6	59.7	75.3
9	54.5	114.9	64.5	81.3
10	63.1	114.8	64.0	81.0
Mean	**57.5**	**118.0**	**66.6**	**83.7**
SD	**7.9**	**8.9**	**5.6**	**6.5**

Table 3. Heart rate (bpm)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	58.3	103.1	77.5	56.0
	2	51.0	82.5	61.7	54.2
	3	47.7	102.4	64.1	55.7
	4	43.2	91.8	54.3	49.8
	5	54.0	110.7	64.7	51.4
	6	74.0	101.6	78.9	79.3
	7	63.6	89.0	68.2	51.8
	8	55.4	98.8	69.4	63.4
	9	53.5	89.2	53.2	46.9
	10	64.1	104.3	82.1	54.8
	Mean	**56.5**	**97.3**	**67.4**	**56.3**
	SD	**8.9**	**8.8**	**9.9**	**9.2**
HIT	1	58.9	96.0	72.6	59.9
	2	56.0	87.7	59.6	56.3
	3	51.8	92.3	66.3	42.4
	4	50.1	87.6	55.3	51.4
	5	52.8	99.9	59.5	52.2
	6	74.5	103.2	72.7	77.7
	7	58.8	91.5	63.8	59.6
	8	65.3	97.0	71.5	76.2
	9	55.6	89.7	61.1	51.2
	10	62.1	99.4	73.6	65.7
	Mean	**58.6**	**94.4**	**65.6**	**59.2**
	SD	**7.3**	**5.5**	**6.7**	**11.2**
Mean		57.5	95.9	66.5	57.8
SD		8.0	7.3	8.3	10.1

Table 4. Systolic Blood Pressure (mmHg)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	124.2	-	101.7	131.1
	2	127.3	102.9	108.2	107.7
	3	133.3	114.3	122.8	128.1
	4	115.1	106.1	116.8	105.7
	5	130.8	111.2	108.8	124.5
	6	132.6	116.5	135.1	107.8
	7	99.4	98.4	88.2	111.9
	8	106.5	99.5	113.9	116.8
	9	115.6	104.7	103.6	113.3
	10	116.0	92.0	109.3	111.2
	Mean	**120.1**	**105.1**	**110.9**	**115.8**
	SD	**11.5**	**7.9**	**12.6**	**9.0**
HIT	1	111.9	93.9	106.6	113.1
	2	126.3	104.1	103.6	112.0
	3	108.3	112.0	124.7	130.0
	4	122.0	102.8	110.7	123.0
	5	120.7	114.5	113.3	121.3
	6	130.6	110.9	127.0	131.5
	7	105.7	93.6	91.1	106.5
	8	106.6	96.8	101.9	107.6
	9	114.2	118.2	110.6	117.0
	10	113.6	103.0	113.5	109.9
	Mean	**116.0**	**105.0**	**110.3**	**117.2**
	SD	**8.5**	**8.7**	**10.5**	**8.9**
Mean		118.0	105.0	110.6	116.5
SD		10.1	8.1	11.3	8.8

Table 5. Diastolic blood pressure (mmHg)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	72.7	-	63.8	75.2
	2	70.1	59.1	59.6	56.7
	3	77.9	69.6	77.2	74.7
	4	68.4	67.6	68.3	63.6
	5	77.0	70.8	63.1	72.8
	6	66.6	70.3	77.1	63.2
	7	54.6	58.8	49.5	63.5
	8	58.7	59.7	62.3	62.2
	9	63.7	65.0	61.5	61.0
	10	63.8	51.1	61.1	67.6
	Mean	**67.3**	**63.6**	**64.3**	**66.1**
	SD	**7.5**	**6.8**	**8.2**	**6.3**
HIT	1	65.7	59.4	63.4	63.4
	2	72.8	57.8	55.0	62.2
	3	63.5	65.0	74.4	75.0
	4	70.1	64.9	66.3	72.5
	5	69.6	70.1	69.6	68.0
	6	69.4	60.9	69.9	70.0
	7	56.7	55.4	51.1	57.5
	8	60.7	62.9	58.8	64.1
	9	65.3	72.7	63.8	69.5
	10	64.3	55.6	63.2	60.5
	Mean	**65.8**	**62.5**	**63.5**	**66.3**
	SD	**4.8**	**5.8**	**7.1**	**5.6**
Mean		66.6	63.0	63.9	66.1
SD		6.2	6.1	7.5	5.8

Table 6. Mean arterial pressure (mmHg)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	89.9	-	76.4	93.8
	2	89.1	73.7	75.8	73.7
	3	96.3	84.5	92.4	92.5
	4	83.9	80.4	84.5	77.7
	5	94.9	84.2	78.3	90.1
	6	88.6	85.7	96.4	78.1
	7	69.6	72.0	62.4	79.6
	8	74.6	73.0	79.5	80.4
	9	81.0	78.2	75.5	78.4
	10	81.2	64.7	77.2	82.2
	Mean	**84.9**	**77.4**	**79.9**	**82.6**
	SD	**8.6**	**7.1**	**9.5**	**7.0**
HIT	1	81.1	70.9	77.8	80.0
	2	90.6	73.2	71.2	78.8
	3	78.5	80.7	91.1	93.3
	4	87.4	77.5	81.1	89.3
	5	86.7	84.9	84.2	85.8
	6	89.8	77.6	88.9	90.5
	7	73.0	68.2	64.4	73.9
	8	76.0	74.2	73.1	78.6
	9	81.6	87.9	79.4	85.3
	10	80.7	71.4	80.0	77.0
	Mean	**82.5**	**76.6**	**79.1**	**83.2**
	SD	**5.9**	**6.3**	**8.1**	**6.5**
Mean		83.7	77.0	79.5	82.9
SD		7.3	6.5	8.6	6.6

Table 7. Absolute FMD (mm)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	0.286	0.216	0.150	0.265
	2	0.187	0.091	0.078	0.157
	3	0.358	0.323	0.302	0.061
	4	0.273	0.226	0.136	0.196
	5	0.043	0.359	0.039	0.090
	6	0.270	0.303	0.040	0.155
	7	0.290	0.365	0.026	0.107
	8	0.373	0.270	0.115	0.376
	9	0.293	0.470	0.212	0.360
	10	0.332	0.310	0.071	0.233
	Mean	**0.271**	**0.293**	**0.117**	**0.200**
	SD	**0.095**	**0.102**	**0.087**	**0.108**
HIT	1	0.272	0.300	0.117	0.233
	2	0.167	0.240	0.095	0.151
	3	0.387	0.236	0.108	0.132
	4	0.106	0.417	0.026	0.146
	5	0.150	0.364	0.195	0.093
	6	0.182	0.272	-0.010	0.135
	7	0.168	0.252	0.107	0.197
	8	0.427	0.372	0.183	0.330
	9	0.297	0.461	0.210	0.192
	10	0.223	0.538	0.081	0.212
	Mean	**0.238**	**0.345**	**0.111**	**0.182**
	SD	**0.106**	**0.103**	**0.071**	**0.067**
Mean		0.254	0.319	0.114	0.191
SD		0.100	0.103	0.077	0.088

Table 8. Relative FMD (%)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	7.19	4.88	3.32	6.44
	2	4.70	2.06	1.71	3.88
	3	8.05	6.84	6.36	1.38
	4	8.36	6.37	3.73	5.96
	5	1.02	8.52	0.83	2.18
	6	5.96	6.37	0.80	3.34
	7	7.90	10.27	0.58	2.90
	8	8.74	6.08	2.35	8.83
	9	7.89	11.56	4.88	9.35
	10	7.95	6.52	1.46	5.45
	Mean	**6.78**	**6.95**	**2.60**	**4.97**
	SD	**2.36**	**2.68**	**1.94**	**2.71**
HIT	1	6.70	7.01	2.74	5.58
	2	4.27	5.68	1.96	3.86
	3	8.74	4.79	2.29	2.98
	4	3.25	12.13	0.76	4.35
	5	3.98	8.99	4.48	2.43
	6	3.97	5.91	-0.19	3.08
	7	4.52	6.27	2.39	5.32
	8	10.02	8.42	3.59	7.74
	9	7.70	11.35	4.83	5.01
	10	5.50	12.49	1.75	5.25
	Mean	**5.87**	**8.30**	**2.46**	**4.56**
	SD	**2.31**	**2.84**	**1.56**	**1.57**
Mean		6.32	7.63	2.53	4.77
SD		2.32	2.78	1.71	2.17

Table 9. Pre-occlusion diameter

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	3.97	4.42	4.52	4.12
	2	3.98	4.39	4.56	4.05
	3	4.45	4.73	4.75	4.42
	4	3.27	3.54	3.65	3.30
	5	4.17	4.22	4.71	4.11
	6	4.54	4.76	5.03	4.66
	7	3.67	3.55	4.42	3.69
	8	4.26	4.44	4.91	4.26
	9	3.71	4.06	4.35	3.85
	10	4.18	4.75	4.87	4.27
	Mean	**4.02**	**4.29**	**4.58**	**4.07**
	SD	**0.39**	**0.45**	**0.39**	**0.39**
HIT	1	4.06	4.28	4.28	4.17
	2	3.91	4.23	4.84	3.90
	3	4.43	4.91	4.72	4.43
	4	3.27	3.44	3.46	3.37
	5	3.78	4.05	4.34	3.81
	6	4.58	4.61	5.11	4.40
	7	3.71	4.03	4.45	3.70
	8	4.26	4.42	5.09	4.27
	9	3.86	4.06	4.35	3.84
	10	4.05	4.30	4.60	4.04
	Mean	**3.99**	**4.23**	**4.52**	**3.99**
	SD	**0.38**	**0.39**	**0.48**	**0.33**
Mean		4.01	4.26	4.55	4.03
SD		0.37	0.41	0.43	0.35

Table 10. Maximum baFMD diameter

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	4.26	4.63	4.67	4.39
	2	4.17	4.48	4.64	4.21
	3	4.81	5.05	5.05	4.49
	4	3.54	3.77	3.79	3.49
	5	4.21	4.58	4.75	4.20
	6	4.81	5.06	5.07	4.81
	7	3.96	3.92	4.44	3.79
	8	4.64	4.72	5.03	4.63
	9	4.00	4.53	4.56	4.21
	10	4.51	5.06	4.95	4.50
	Mean	**4.29**	**4.58**	**4.69**	**4.27**
	SD	**0.40**	**0.45**	**0.39**	**0.39**
HIT	1	4.34	4.58	4.39	4.41
	2	4.07	4.47	4.93	4.05
	3	4.81	5.15	4.83	4.56
	4	3.38	3.85	3.48	3.51
	5	3.93	4.41	4.54	3.90
	6	4.76	4.88	5.10	4.53
	7	3.88	4.28	4.56	3.90
	8	4.69	4.80	5.27	4.60
	9	4.15	4.52	4.56	4.03
	10	4.27	4.84	4.68	4.26
	Mean	**4.23**	**4.58**	**4.63**	**4.18**
	SD	**0.45**	**0.36**	**0.49**	**0.36**
Mean		4.26	4.58	4.66	4.22
SD		0.42	0.40	0.43	0.37

Table 11. Normalized FMD ($x10^4$)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	5.86	1.24	0.66	1.72
	2	0.96	0.35	0.63	0.53
	3	20.6	2.02	1.65	0.50
	4	3.28	0.59	2.55	1.82
	5	0.31	6.81	0.11	2.66
	6	2.79	3.23	0.76	1.23
	7	4.65	3.05	0.59	0.94
	8	2.35	1.24	0.64	4.33
	9	3.23	2.09	0.87	9.78
	10	3.52	3.00	0.78	1.51
	Mean	**4.75**	**2.36**	**0.92**	**2.50**
	SD	**5.78**	**1.87**	**0.69**	**2.80**
HIT	1	5.68	1.85	2.05	1.10
	2	3.22	1.08	0.48	0.84
	3	3.12	1.25	1.16	6.34
	4	0.86	1.30	0.28	2.79
	5	4.72	3.95	5.40	2.81
	6	1.54	8.85	-0.12	3.71
	7	43.6	1.54	4.95	1.90
	8	2.46	2.64	3.70	3.23
	9	1.53	7.03	1.50	0.82
	10	1.46	4.23	0.84	1.29
	Mean	**6.82**	**3.37**	**2.02**	**2.48**
	SD	**13.0**	**2.68**	**1.98**	**1.71**
Mean		5.79	2.87	1.47	2.49
SD		9.86	2.31	1.55	2.26

Table 12. Time to peak baFMD diameter (sec)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	37.1	103	90.2	61.6
	2	90.7	80.6	68.7	114
	3	22.1	88.7	108	83.6
	4	57.5	145	40.0	64.4
	5	95.1	44.6	122	29.3
	6	61.0	65.2	42.6	63.7
	7	57.8	86.0	49.0	71.0
	8	67.8	71.8	68.0	52.4
	9	54.2	87.7	67.2	44.3
	10	67.6	77.8	55.9	68.8
	Mean	**61.1**	**85.1**	**71.1**	**65.3**
	SD	**21.8**	**26.3**	**27.5**	**22.7**
HIT	1	33.4	91.1	39.4	79.7
	2	47.9	106	96.9	87.3
	3	63.8	73.5	56.8	20.9
	4	60.6	116	66.2	45.9
	5	28.8	67.7	24.0	34.0
	6	63.7	33.2	53.2	38.3
	7	57.8	86.0	30.7	71.6
	8	71.6	70.0	34.0	55.5
	9	63.3	72.4	64.1	89.9
	10	72.2	77.0	54.3	72.1
	Mean	**56.3**	**79.2**	**52.0**	**59.5**
	SD	**15.0**	**22.7**	**21.4**	**24.0**
Mean		82.2	61.5	62.4	58.7
SD		24.1	25.9	22.9	18.4

Table 13. Absolute NTG (mm)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	0.762	0.226		0.626
	2	0.916	0.611		0.818
	3	0.746	0.116		0.665
	4	0.640	0.532		0.756
	5	0.497	0.356		0.435
	6	0.835	0.641		0.821
	7	0.753	0.638		0.817
	8	0.812	0.568		0.744
	9	0.905	0.616		0.693
	10	0.729	0.365		0.774
	Mean	**0.759**	**0.467**		**0.715**
	SD	**0.124**	**0.189**		**0.119**
HIT	1	0.725	0.418		0.654
	2	1.006	0.455		1.088
	3	0.783	0.436		1.187
	4	0.789	0.590		0.903
	5	0.570	0.494		0.410
	6	0.561	0.696		0.935
	7	0.806	0.530		0.685
	8	0.867	0.730		0.936
	9	0.918	0.673		0.821
	10	0.664	0.420		0.785
	Mean	**0.769**	**0.544**		**0.840**
	SD	**0.144**	**0.120**		**0.224**
Mean		0.762	0.506		0.778
SD		0.131	0.159		0.186

Table 14. Relative NTG (%)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	18.3	5.0		15.5
	2	22.5	14.4		19.7
	3	17.7	2.3		15.8
	4	18.5	15.1		22.6
	5	11.4	7.8		10.3
	6	18.1	13.3		17.4
	7	19.6	16.0		21.9
	8	18.1	12.6		16.2
	9	22.3	14.9		16.5
	10	16.1	7.2		17.3
	Mean	**18.3**	**10.9**		**17.3**
	SD	**3.1**	**4.9**		**3.5**
HIT	1	17.7	9.2		15.2
	2	25.3	10.1		26.8
	3	17.8	8.7		29.0
	4	23.6	16.3		30.2
	5	14.2	11.4		10.4
	6	11.5	14.6		21.2
	7	20.8	12.7		17.9
	8	19.4	15.6		20.5
	9	22.7	15.9		20.0
	10	14.7	8.7		17.9
	Mean	**18.8**	**12.3**		**20.9**
	SD	**4.4**	**3.1**		**6.2**
Mean		18.5	11.6		19.1
SD		3.7	4.0		5.3

Table 15. Pre-NTG diameter (mm)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	4.16	4.48		4.05
	2	4.06	4.23		4.16
	3	4.21	5.06		4.21
	4	3.47	3.52		3.34
	5	4.34	4.56		4.23
	6	4.61	4.82		4.72
	7	3.84	3.98		3.73
	8	4.49	4.50		4.58
	9	4.06	4.14		4.21
	10	4.53	5.07		4.47
	Mean	**4.18**	**4.44**		**4.17**
	SD	**0.35**	**0.49**		**0.40**
HIT	1	4.10	4.52		4.29
	2	3.98	4.50		4.05
	3	4.39	5.03		4.09
	4	3.35	3.61		2.99
	5	4.01	4.34		3.95
	6	4.89	4.77		4.41
	7	3.88	4.17		3.83
	8	4.46	4.67		4.56
	9	4.04	4.22		4.11
	10	4.51	4.82		4.38
	Mean	**4.16**	**4.47**		**4.07**
	SD	**0.42**	**0.40**		**0.44**
Mean		4.17	4.45		4.12
SD		0.38	0.44		0.41

Table 16. Maximum NTG diameter (mm)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	4.92	4.70		4.67
	2	4.98	4.84		4.97
	3	4.96	5.17		4.87
	4	4.11	4.05		4.10
	5	4.84	4.91		4.66
	6	5.44	5.47		5.54
	7	4.59	4.61		4.55
	8	5.30	5.07		5.33
	9	4.96	4.76		4.90
	10	5.26	5.44		5.24
	Mean	**4.94**	**4.90**		**4.88**
	SD	**0.38**	**0.42**		**0.42**
HIT	1	4.83	4.94		4.95
	2	4.99	4.96		5.14
	3	5.18	5.46		5.27
	4	4.13	4.20		3.90
	5	4.58	4.84		4.36
	6	5.45	5.46		5.35
	7	4.69	4.70		4.52
	8	5.33	5.40		5.50
	9	4.96	4.89		4.94
	10	5.17	5.24		5.17
	Mean	**4.93**	**5.01**		**4.91**
	SD	**0.39**	**0.40**		**0.50**
Mean		4.93	4.96		4.90
SD		0.38	0.40		0.45

Table 17. Time at peak NTG dilation (min)

	ID	Baseline	Post-ex	1 h post-ex	24 h post-ex
END	1	6	10		4
	2	10	5		5
	3	9	8		10
	4	10	8		7
	5	8	8		10
	6	10	10		8
	7	9	10		10
	8	8	10		10
	9	9	9		8
	10	8	5		7
	Mean	**9**	**8**		**8**
	SD	**1**	**2**		**2**
HIT	1	6	3		9
	2	10	9		10
	3	6	8		10
	4	8	5		5
	5	9	10		7
	6	5	10		8
	7	6	10		6
	8	9	10		7
	9	10	10		10
	10	9	9		9
	Mean	**8**	**8**		**8**
	SD	**2**	**2**		**2**
Mean		8	8		8
SD		2	2		2