

**EVALUATION OF GENDER DIFFERENCES IN ENDOTHELIUM-
INDEPENDENT DILATION IN HEALTHY ADULTS USING PERIPHERAL
ARTERIAL TONOMETRY**

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ABSTRACT

Peripheral arterial tonometry (PAT) is a non-invasive method used to evaluate vascular function. PAT is often used to measure digital pulsatile volume changes in response to reactive hyperemia, which provides a measure of endothelium-dependent dilation (EDD). Reactive hyperemia does not allow one to quantify endothelium-independent dilation (EID), which is typically measured using sublingual nitroglycerin (NTG) mediated dilation. Though most research examining vascular function and cardiovascular disease has focused on EDD, there is evidence that cardiovascular risk factors may impair EID. To our knowledge, PAT has not been used with NTG to determine EID. The purpose of this study was to examine the microvascular vasodilation response to nitroglycerin (NTG) in healthy adults using PAT. Microvascular responses to reactive hyperemia and NTG were evaluated in 86 (41 F, 45 M) healthy subjects (age 37 ± 5 yrs). Beat-to-beat plethysmographic measurements of finger arterial pulse waves were recorded for 5-min following reactive hyperemia. After a 10-min rest period, sublingual NTG (0.4 mg) was administered and PAT signal changes were measured for 10-min. Peak reactive hyperemic index (RHI) and peak NTG-mediated index (NMI) were determined in all subjects. Though there were no significant gender differences in peak RHI (2.07 ± 0.56 F vs. 1.91 ± 0.58 M, $P=0.20$), peak NMI was significantly greater in females (3.11 ± 1.59 F vs. 2.50 ± 1.34 M, $P=0.05$). Time to peak NMI was not significantly different between genders (7-min, 28-s [± 1 -min, 47-s] M, vs. 7-min, 14-s [± 1 -min, 49-s] F, $P=0.58$). In this population of healthy adults, RHI did not differ by gender. However, we observed a significantly greater microvascular vasodilation response to NTG using PAT in females than in males. Significance of this finding is unclear, but may indicate the beginning of cardiovascular changes in adult males, as detected with lower peak NMI at the microvascular level. Future studies are needed to determine the exact mechanism underlying the reported gender differences in EID.

Key Words: Peripheral Vascular, Endothelium, Microcirculation, Vasodilation, Nitric Oxide, Gender.

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CHAPTER 1. INTRODUCTION

Endothelial dysfunction is thought to be a predictor of the development of atherosclerosis (Kunsch & Medford, 2000; Lieberman et al., 1996; Ludmer et al., 1986; Ross, 1993) and is an independent predictor of cardiovascular disease in various populations (Anderson et al., 1995; Dengel et al., 2011; Gokce et al., 2002; Halcox et al., 2002; Schindler et al., 2003; Yoshida et al., 2006). If endothelial dysfunction can be identified prior to the development of atherosclerosis, interventions to prevent atherosclerosis may be used before the onset of the clinical disease (Dengel & Bronas, 2010; Faizi et al., 2009).

The most widely used noninvasive technique for measuring peripheral endothelial function is ultrasound imaging of the brachial artery during flow-mediated dilation (FMD) (Agewall et al., 2001; Corretti et al., 2002). Brachial FMD measures one dimension of endothelial health – the ability of the artery to increase blood flow and dilate in response to ischemia. Recently, it has been established using peripheral arterial tonometry (PAT) that abnormalities in pulse wave signal changes are significantly associated with FMD (Kuvin et al., 2003). PAT is a non-invasive method of evaluating vascular function via finger plethysmography. Typically, PAT is used to measure digital pulsatile wave changes in response to reactive hyperemia, providing a measure of endothelium-dependent dilation (EDD). Reactive hyperemia, however, does not allow one to quantify endothelium-independent dilation (EID), which is a vital part of overall vascular health (Ducharme et al., 1999; Pepe et al., 1999; Roman et al., 2006; Thelen et al., 2008).

Both PAT and FMD are noninvasive techniques that attempt to assess peripheral vascular endothelial function. Over the last decade FMD has become a standard noninvasive measure of endothelial function (Corretti et al., 2002). Unfortunately, this technique requires highly-specific technician training and specialized equipment, along with specific knowledge for interpretation of the measurement, so it is not widely applicable, even in the research setting. Furthermore, results can be highly variable due to a high degree of operator dependence and natural intra-individual physiological variations in vascular function. Peripheral arterial tonometry potentially offers a more simplified, noninvasive measurement that may be more generalizable in both the clinical and research arenas. Mechanistic studies assessing vascular function based on the response to nitroglycerin (NTG) (or other drugs that result in increased levels of nitric oxide), as well as studies utilizing drugs that inhibit nitric oxide (NO) (including N-monomethyl-L-arginine, L-NMMA), clearly demonstrate the effect of the NO pathway in EID (Lieberman et al., 1996; Thijssen et al., 2010). These factors may also influence pulse wave amplitude (PWA) measurements, as assessed with PAT, in a similar manner as observed with brachial measurements of FMD (Kuvin et al., 2003), suggesting that PWA of the small arteries of the finger may be influenced by endothelial function in a magnitude and direction similar to that of larger conduit arteries.

Previous research has demonstrated that females have greater EDD (using FMD) when compared to males, even when correcting for baseline diameter, but there is less of

a consensus with regards to EID according to gender (Celermajer et al., 1994; Jensen-Urstad and Johansson, 2001; Kaplon et al., 2011; Pierce et al., 2011). The influence of gender on EID has been studied in some detail; however, to date, obtaining measures of EID with finger plethysmography has not been widely researched, to our knowledge. If EID in the microvasculature can be better characterized, it could be more widely utilized by researchers, and possibly clinicians, as a metric of overall vascular function. Therefore, the purpose of the present study was to examine the microvascular vasodilation responses to NTG-mediated dilation in healthy adults using PAT, according to gender.

A complete literature review, as well as a subsequent methodology, results, discussion, and conclusion surrounding the present study are detailed in the following chapters:

Chapter two summarizes the current literature related to mechanisms of vascular function and EID as a measure of the exogenous effect of NTG on smooth muscle relaxation. Gender differences in EID will be discussed. Additionally, the potential of utilizing EID as a metric of overall vascular function will be discussed.

Chapter three details the methodology used in this study. Background information on the study population is stated. Data abstraction and measurement techniques,

including anthropometric, blood pressure, and vascular assessments, as well as statistical analyses are discussed.

Chapter four addresses the findings of this study, by examining the time to peak dilation, maximal dilation, difference between NMI response at different time points along the time course, as well as possible age, race, and gender differences in this response in the study population.

Chapter five summarizes and discusses the implications of these findings with reference to the current literature. Results of gender differences in NMI response are discussed. Discussion of time-course of microvascular vs. macrovascular response is also reviewed.

Chapter six offers conclusions of the resulting study, as well as states the necessity for further research to determine the exact mechanism underlying the reported gender differences in EID, as well as to assess how CVD risk factors in other populations affect the time course of microvascular dilation in response to NTG mediated dilation.

CHAPTER 2. REVIEW OF LITERATURE

Endothelial Dysfunction Can be used as a Predictor of Overall Cardiovascular Health

The noninvasive assessment of endothelial function is important in the early detection and diagnosis of the onset of cardiovascular disease (CVD) (Anderson et al., 1995; Dengel et al., 2011; Gokce et al., 2002; Halcox et al., 2002; Schindler et al., 2003; Yoshida et al., 2006) and atherosclerosis in various populations (Kunsch and Medford, 2000; Lieberman et al., 1996; Ludmer et al., 1986; Ross, 1993). Evidence for this relationship lies in studies that have demonstrated that subjects with known CVD risk factors have attenuated EDD and EID. Recent work has found that endothelial dysfunction predicts long-term atherosclerosis progression and the risk of cardiovascular and cerebrovascular events (Blum et al., 2012; Faizi et al., 2009; Suwaidi et al., 2000; also reviewed by Dengel and Bronas, 2010).

Endothelial-dependent flow-mediated dilation can be used to assess the contribution of naturally occurring bioavailability of NO in the vasculature (Corretti et al., 2002; Olesen et al., 1988; Thijssen et al. 2010). To determine EID, the administration of NTG (or other exogenous drugs/interventions that result in elevated levels of NO in the blood) can be used. In addition, the NO pathway can be inhibited using interventions such as L-NMMA to determine the relative contribution of NO versus other vasodilatory factors. Endothelial independent dilation measures are a means of establishing cardiovascular health in addition to EDD, thus establishing the relative contribution of the vascular endothelium. Gokce et al. (2002) demonstrated lower EID in patients with hypertension as compared to healthy control subjects. Dengel et al. (2008) found that

patients with acute lymphoblastic leukemia (ALL) who were treated with chemotherapy during childhood demonstrated impaired FMD but found no difference in nitrate-mediated EID between healthy subjects and ALL survivors. However, the same study reported that females had greater EID when compared to males within the patient population of adult survivors of ALL (Dengel et al., 2008).

Several studies have been performed to establish a relationship between vascular function in the heart and that of the periphery. Anderson et al. (1995) performed a study where they sought to determine the relationship between coronary vascular tone and EDD. This study compared coronary vascular response to acetylcholine in a catheter laboratory setting, using angiography, to peripheral vascular response, using brachial FMD. Findings of this study indicated that patients with coronary vascular dysfunction also had reduced FMD when compared to normal, healthy subjects, suggesting that peripheral vascular function is related to coronary vascular function. Chen et al. (2011) demonstrated that young individuals with ST-elevated myocardial infarction (STEMI) had decreased brachial FMD when compared to age- and gender-matched healthy control subjects. All patients were <40 years of age and each group was comprised of 29 individuals. Regression analysis revealed that only EDD and age were predictive of STEMI. This is an important study because these results confirmed that, even in relatively young patients, the relationship between coronary endothelial dysfunction, which has previously been shown to predict susceptibility to acute coronary events (Halcox et al., 2002), and brachial vasodilatory response exists. These studies provide

further evidence that EDD is likely a useful surrogate for the reliable, non-invasive, assessment of vascular function at the level of the heart.

There is a relatively clear relationship between peripheral vascular function and risk of CVD (i.e. vascular function at the level of the heart). More recent work has also demonstrated a possible relationship between peripheral vascular function and vascular function in the brain. Blum et al. (2012) studied systemic vascular function (using EDD) and acute ischemic stroke. Forty-three patients participated in this study, and acute stroke patients had severe endothelial dysfunction post-event. These results suggest that peripheral vascular function is also correlated with cerebral vascular function.

Though most research examining vascular function and CVD has focused on EDD, there is evidence that assessing EID provides important information regarding the cause of dysfunction. Lieberman et al. (1996) looked at the difference in EDD between young, healthy, male subjects (<40 years of age) and matched young males with known coronary artery disease (CAD). For this study, investigators also looked at EID using sublingual nitroglycerine. Endothelial dependent dilation was lower in CAD subjects than in healthy controls, but there was no difference between the subjects in EID, suggesting that the impaired vascular function likely occurred in the endothelium.

To further support the concept that endothelial mediated dysfunction is primarily related to vascular function at the level of the heart, Gokce et al. (2002) investigated

brachial artery EDD on post-operative outcomes in 187 subjects undergoing cardiac vascular surgery. Subjects who had a post-operative event had lower EDD than patients without a post-operative event. In contrast, EID in response to nitroglycerine administration was similar between the groups, indicating that dysfunction of the endothelium, specifically, that is related to cardiac vascular function. Of interest, when the investigators set a change in EDD of 8.1% as a cut-off, the EDD test had a sensitivity of 95%, a specificity of 37%, and a negative predictive value of 98% for post-operative events, again, suggesting that EDD is a useful surrogate for non-invasive assessment of coronary vascular function.

Mechanisms of Endothelium Dependent Dilation and Endothelium Independent

Dilation

The vessels of the human body have the capacity to dilate in response to shear stress, likely in order to meet the demands of downstream vascular beds (Furchgott and Zawadzki, 1980). Flow-mediated dilation typically describes the phenomenon of the vascular response (dilation) to an increase in shear stress. Although the mechanisms were unclear at the time, Furchgott and Zawadzki (1980) demonstrated that the endothelial cells were imperative for dilation to occur in response to acetylcholine. Follow-up studies determined that the vasoactive substance resulting in dilation was nitric oxide (NO). Further support of the role of NO in vasodilation was confirmed with studies using NO blockade with L-NMMA which reduced FMD from approximately 5% to approximately 1% (Joannides et al., 1995; Mullen et al., 2001).

Although the exact mechanisms of the downstream signaling response to shear stress continue to be elucidated, calcium activated potassium channels seem to play a key role (Cooke et al., 1991). With increasing levels of calcium, endothelial nitric oxide synthase (eNOS) is activated, resulting in the generation of NO, which causes vasodilation (Cooke et al., 1991; Joannides et al., 1995; Pohl et al., 1985). Of interest, the vessels of eNOS knockout mice still respond to shear stress with dilation (Sun et al., 1999) suggesting other important pathways for dilation in response to the increase in shear stress. Endothelial nitric oxide synthase increases differently in the acute and short-term/prolonged conditions of shear stress. Acutely, eNOS bioavailability increases in response to calcium activated potassium channels (Betik et al., 2009). With slightly more prolonged shear stress, eNOS is phosphorylated, thus reducing degradation and increasing its bioavailability (Corretti et al., 2002). With even longer exposure to shear stress, eNOS transcription increases, leading to large amounts of eNOS.

Effect of Gender on Vascular Function

Although it is clear that both EDD and EID are altered in diseased states, most previous research suggests EDD, but not EID, is different (at the macrovascular level) between men and women (Celermajer et al. 1994; Jensen-Urstad and Johansson, 2001; Kaplon et al., 2011; Moreau et al., 2012; Pierce et al., 2011;). Celermajer et al. (1994) sought to determine why there were differences in coronary and cerebrovascular disease between men and women according to age. They assessed EDD and EID at the brachial

artery in a total of 103 men and 135 women with an age range from 15-72 years. EDD was stable in men <40 years, but declined as they aged. In women, there was no decline until after the age of 50, suggesting gender-specific hormones (i.e., estrogen or estrogen receptors) may play a role in the vascular response to shear stress. In this study the investigators reported no difference in EID according to age between genders.

In an attempt to further elucidate the role of gender and age on vascular endothelial function, Jensen-Urstad and Johansson (2001) sought to determine differences between EED and EID between men and women at the age of 55 when compared to a previously studied group of 35 year-old men and women. Endothelial dependent dilation was similar between men and women at the age of 55. Fifty-five year-old women had smaller EDD as compared to 35 year-old women, even after correcting for baseline brachial artery diameter. Endothelial dependent dilation was similar in 55 year-old and 35 year-old men, again, suggesting the role of gender-specific hormones.

Although exercise is generally believed to improve vascular function, this may differ according to gender. Pierce et al. (2011) sought to determine the influence of aerobic exercise (8 weeks brisk walking, 6 days/week for approximately 50 min/day) on EDD between men and women. Endothelial dependent dilation increased in men (55-79 years of age) with exercise training, but did not change in post-menopausal women. The investigators also used a non-exercising control group and found no change over time in EDD. In this study, EID did not change with exercise in either men or women.

The sympathetic nervous system (SNS) regulates several cardiovascular parameters at rest and during exercise. Kaplon et al. (2011) sought to determine the relationship between SNS activity and EDD between men and women, using plasma norepinephrine concentration as a surrogate for SNS activity. Endothelial dependent dilation was inversely related to norepinephrine concentration. Using multiple regression analysis, the authors corrected for differences in norepinephrine activity and found that EDD was related to age in men but not women, possibly suggesting the role of decreased estrogen levels with age in women. Norepinephrine concentration was the strongest factor that was related to EDD, even when compared to CVD risk factors. Endothelial independent dilation was not related to age in men or women. In another study, Moreau et al. (2012) also sought to determine the influence of age and menopausal status on EDD in women. Endothelium dependent dilation was highest in premenopausal women, lower in perimenopausal women, and lowest in postmenopausal women. Estrogen promotes endothelial NO production through increases in e-NOS. Additionally, estrogen activates prostacyclin and thromboxane A₂ release that are both NO independent regulators of vascular function which could explain differences in vascular function according to gender (Novella et al., 2012).

Peripheral Arterial Tonometry (PAT) as a Technique to Assess Vascular Function

Recently, it has been established using PAT that abnormalities in pulse wave signal changes are significantly associated with FMD (Kuvin et al., 2003). Peripheral

arterial tonometry is a non-invasive method of evaluating vascular function via finger plethysmography. Typically, PAT is used to measure digital pulsatile volume changes in response to reactive hyperemia, providing a measure of EDD. Reactive hyperemia, however, does not allow one to quantify EID, which is a vital part of overall vascular health (Ducharme et al., 1999; Pepe et al., 1999; Roman et al., 2006; Thelen et al., 2008). Therefore, the focus of this study was the assessment of EID between males and females using PAT.

CHAPTER 3. METHODOLOGY

The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board (IRB). Procedures of the present study were followed in accordance to the University of Minnesota's IRB and the Health Insurance Portability and Accountability Act (HIPAA) guidelines.

Study Population

Individuals from a previous longitudinal cohort were invited to participate in the present study. Participants with chronic diseases (e.g., type 1 diabetes, kidney dialysis patients, and cancer patients) were excluded from the study. Microvascular responses to reactive hyperemia and NTG were evaluated in 86 (41 female, 45 male) healthy subjects (mean age 37 ± 5 yrs, \pm SD). All subjects submitted written informed consent for participation in the study. Testing was performed in the morning, following an overnight fast, at the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All studies were conducted in a quiet setting of constant temperature (22°C-23°C). A study physician and/or certified nurse practitioner reviewed with the subject the study procedures and plans for the evaluation, reviewed prescription medications, and conducted a comprehensive medical examination including current and past medical history (with particular attention to cardiovascular disease), review of symptoms, and a physical examination.

Measurements

Anthropometric and Blood Pressure Assessments

Height and weight were obtained using a standard stadiometer (Ayrton Stadiometer, Model S100, Prior Lake, MN, USA) and electronic scale (ST Scale-Tronix, Serial No. 5002-8893, White Plains, NY, USA), respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Subjects were asked to fast for 10-h, withhold from taking medications, and abstain from smoking, caffeine, and vigorous exercise 24-h prior to testing. Testing began after a 5-min seated rest period. Blood pressure was obtained on the right arm using an automatic sphygmomanometer (Colin Press-Mate, Model BP-8800C, San Antonio, TX, USA).

Vascular Assessment

Endothelial function was measured non-invasively by digital reactive hyperemia using peripheral arterial tonometry (PAT) (EndoPAT2000, Itamar Medical, Caesarea, Israel). After 10-min of quiet rest in the supine position, one PAT finger probe was placed on the index finger of the hand undergoing hyperemia testing (left hand), and a second PAT probe was placed on the contralateral index finger (right hand). The probes inflate to apply a uniform pressure (10 mmHg less than diastolic blood pressure) on the fingers and detect small pulse volume changes throughout the cardiac cycle. Following the collection of 5-minutes of baseline data, a blood pressure cuff on the upper left forearm (just below the elbow) was inflated to a suprasystolic level for 5-minutes. Following cuff release, the change in pulse amplitude during reactive hyperemia was measured for 5-minutes. The ratio of the hyperemic and the baseline pulse amplitude (corrected for the same ratio on the control finger) was calculated and expressed as the

reactive hyperemic index (RHI). After a 10-minutes rest period, sublingual NTG (0.4-mg) was administered, and PAT signal changes were measured for 10-minutes. The peak NTG mediated index (NMI: the calculated ratio of the post-NTG PAT signal relative to baseline signal, indexed to the contra-lateral finger) was determined in all subjects.

Statistical Analysis

SPSS version 17.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses. An independent samples t-test was used to compare demographic characteristics by gender. Analysis also included time-course data for vascular reactivity response. Multivariate analysis of co-variance (MANCOVA) with Bonferroni *post hoc* tests was used to compare NMI and RHI by gender. Time to peak NMI and RHI were calculated by identifying the time point at which each individual reached maximal change in NMI and RHI, and then averaged among the subject population. Pearson correlations were used to assess the relationship between subject characteristic data and RHI, peak NMI, and percent change in NMI. Data are presented as mean \pm standard deviation. An alpha value of 0.05 was used to signify statistical significance.

CHAPTER 4. RESULTS

Mean demographic data of the study population (n=86) are presented in Table 1. Age, weight, body mass index (BMI), and blood pressure (BP) were not significantly different between males and females. Peak RHI did not significantly differ between genders (females: 2.07 ± 0.56 vs. males: 1.91 ± 0.58 , $P=0.20$) (Figure 1). For the entire study population, peak RHI was not correlated with age ($r=0.12$, $P=0.35$), height ($r=0.07$, $P=0.66$), weight ($r=-0.06$, $P=0.69$), BMI ($r=-0.18$, $P=0.90$), SBP ($r=-0.09$, $P=0.48$), or DBP ($r=-0.65$, $P=0.61$). In females, peak RHI was not correlated with age ($r=0.10$, $P=0.54$), height ($r=-0.04$, $P=0.66$), weight ($r=-0.21$, $P=0.628$), BMI ($r=-0.17$, $P=0.39$), SBP ($r=-0.18$, $P=0.29$), or DBP ($r=-0.14$, $P=0.40$). In males, peak RHI was, again, not related to age ($r=0.16$, $P=0.45$), height ($r=-0.10$, $P=0.70$), weight ($r=0.12$, $P=0.69$), BMI ($r=0.21$, $P=0.38$), SBP ($r=0.10$, $P=0.64$), or DBP ($r=0.06$, $P=0.79$).

Average time course and peak NTG mediated response of the study population are presented in Figure 2. Peak NMI was significantly greater in females than males (3.11 ± 1.59 vs. 2.50 ± 1.34 , for females and males, respectively, $P=0.05$), whereas no significant difference in time to peak NMI was observed according to gender (females: 7-min, 28-s vs. males: 7-min, 14-s, $P=0.58$) (Figure 2). In the entire study population, peak NMI was not related to age ($r=0.07$, $P=0.50$), height ($r=-0.07$, $P=0.58$), weight ($r=-0.12$, $P=0.37$), SBP ($r=0.12$, $P=0.33$), or DBP ($r=0.17$, $P=0.16$). When assessed according to gender, there was no relationship between peak NMI and our primary subject characteristics (females: age $r=0.06$, $P=0.71$, height $r=0.06$, $P=0.73$, weight $r=-0.18$,

$P=0.32$, SBP $r=0.11$, $P=0.32$, DBP $r=0.19$, $P=0.24$; males: age $r=0.76$, $P=0.62$, height $r=0.14$, $P=0.45$, weight $r=0.10$, $P=0.60$, SBP $r=0.17$, $P=0.32$, DBP $r=0.15$, $P=0.41$).

The percent change in peak NMI from baseline was greater in females when compared to males (277 ± 30 vs. $198\pm23\%$ for females and males, respectively, $P<0.05$) (Figure 3). Within the entire group in the present study, there was, again, no relationship between age ($r=0.08$, $P=0.48$), height ($r=-0.07$, $P=0.60$), weight ($r=-0.14$, $P=0.28$), SBP ($r=0.13$, $P=0.28$), or DBP ($r=0.16$, $P=0.19$). As with peak RHI and peak NMI, there was no relationship between percent change in NMI and subject demographics (females: age $r=0.11$, $P=0.51$, height $r=0.05$, $P=0.78$, weight $r=-0.19$, $P=0.32$, SBP $r=0.18$, $P=0.49$, DBP $r=0.20$, $P=0.23$; males: age $r=0.04$, $P=0.78$, height $r=0.22$, $P=0.25$, weight $r=0.07$, $P=0.72$, SBP $r=0.20$, $P=0.25$, DBP $r=0.11$, $P=0.53$).

CHAPTER 5. DISCUSSION

To our knowledge, the present study is the first to examine gender differences in microvascular vasodilation response with PAT following NTG mediated dilation. These findings indicate that females have a higher NMI response compared to males and that peak NMI in males and females occurs at 7-min, 23-s, with an average peak NMI of 2.78 ± 1.49 . Previously reported data on brachial artery EID responses to NTG administration utilizing ultrasound imaging have reported time to peak dilations ranging between 3- and 5-min (Bressler et al., 2000; Ducharme et al., 1999; Kapuku et al., 2004; Pepe et al., 2004; Thelen et al., 2008). Our data suggest that the time-course of peak NMI response is slower in the microvascular compared to the response in the macrovasculature, possibly due to the location and/or size of the vessels.

One potential explanation for the different time-course in the microvasculature may be that changes in blood flow in the brachial artery creates the increase in volume in the microvessels. Therefore, the brachial artery dilates first in response to NTG creating an increase in blood flow that triggers the downstream vasodilation in the microvessels. Another possible explanation, although speculative, is that there may be differences in the ratio of smooth muscle cells in the two arterial beds, which could also influence the time of response to NTG. Finally, it could be speculated that the dose of NTG required to elicit peak NMI may differ from that required for brachial EID.

In this study of healthy adults, we observed a significantly greater microvascular vasodilation response to NTG using PAT in females than in males. The exact mechanism

underlying the difference in EID between males and females is unknown. It is possible that the observed differences in smooth muscle function may be related to differences in the amount of sex hormones and/or the number of sex hormone receptors between males and females. Although testosterone and estrogen have the capacity to increase the bioavailability of NO, estrogen also acts on ancillary pathways, specifically prostacyclin and thromboxane A₂, which may explain why females have a greater EID response to NTG using PAT (Lopes et al., 2012; Novella et al., 2012). Females have higher numbers of arterial estrogen receptors than males, and as a result, may be more sensitive to estrogen as a vasodilator than similarly aged-matched males (Collins et al., 1995). Evidence for the influence of sex-specific hormones in differences between males and females lies in two studies that compared younger to older males and females. Celermajer et al. (1994) demonstrated no decline in vascular function in women until after the age of 50. Moreau et al. (2012) also found that EDD was highest in premenopausal women, lower in perimenopausal women, and lowest in postmenopausal women.

A potential limitation of the present study is that subjects were drawn from a relatively homogenous population, particularly in relation to age. In contrast to previous studies, we found no relationship between age and peak NMI or percent change in NMI within the entire group or according to gender. Because of the homogeneous subject population in the present study the results may not be applicable to the general population across the lifespan or in less-healthy individuals. Another limitation is that we did not account for women's menstrual cycle by testing our female subjects during the same

phase of their menstrual cycle. However, it has been previously reported (Ketel et al., 2009; Williams et al., 1998; Viridis et al., 2003), that there are no significant differences in EID during the different phases of the menstrual cycle. Characteristics of subjects included in this study are similar to those of the previous studies in that they all used healthy, normal-weight, premenopausal women. We also did not control for oral contraceptives. However, the 2003 study by Viridis et al. on the effect of third-generation oral contraceptives on vascular function in healthy young women, reported that endothelial function remained unchanged after six months of oral contraceptive use. Therefore, it is unlikely that either of these two factors would influence the results of the present study.

The data from this cohort did not include any dietary or physical activity measures. While these measures were controlled for acutely at the time of measurement, previous research (Karpoff et al., 2008; Kelly et al., 2004) on peripheral vascular reactivity measured using FMD has shown that chronic exercise can improve vascular reactivity over that of a control population without an exercise intervention. The effect of chronic exercise on PAT is currently unknown.

An additional limitation is that finger plethysmography does not currently exhibit the ability to measure baseline diameter of the microvasculature. Dengel et al. (2011) recently demonstrated a difference in conduit vessel (i.e., brachial artery) size between males and females. It is also possible that resistance arteries (i.e., the microvasculature)

may also differ in diameters between genders. However, because artery diameter is not measured with PAT, we do not have a way to determine vessel size. Flow mediated dilation (FMD) measures the change in vessel diameter while PAT measures pulsatile changes in vascular blood flow, which are different phenomena. Future studies could also examine brachial artery blood flow velocity following NTG mediated dilation in relation to NMI, which would require the use of ultrasound. However, FMD, rather than brachial artery blood flow, has been more widely correlated with endothelial function (Kuvin et al., 2003). Therefore, further research is needed to assess vascular reactivity in response to NTG as it may relate to artery health and function.

CHAPTER 6. CONCLUSION

In the present study, we observed a significantly greater microvascular vasodilation response to NTG using PAT in healthy adult females when compared to males. Time-to-peak NMI occurred between 7- and 8-min post-NTG administration and did not differ by gender. These findings suggest that gender differences exist in the microvascular vasodilation responses to NTG using PAT. Future studies are needed to determine the exact mechanism underlying the reported gender differences in EID, as well as to assess how CVD risk factors in other populations affect the time course of microvascular dilation in response to NTG mediated dilation.

CHAPTER 7. REFERENCES

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Table 1 Mean (\pm S.D.) demographic characteristics

	ALL (n=86)	MALE (n=45)	FEMALE (n=41)	<i>P</i> -value
Age (yrs)	36.9 \pm 4.8	36.7 \pm 5.4	37.1 \pm 4.2	0.581
Height (cm)	169.1 \pm 8.8	174.2 \pm 9.5	165.7 \pm 6.6	0.023
Weight (kg)	82.4 \pm 18.7	84.1 \pm 15.4	80.8 \pm 20.7	0.189
BMI (kg/m ²)	28.8 \pm 6.4	27.7 \pm 4.4	29.4 \pm 7.3	0.804
SBP (mmHg)	123 \pm 12	123 \pm 9	124 \pm 14	0.693
DBP(mmHg)	70 \pm 9	70 \pm 8	71 \pm 10	0.619

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; S.D., standard deviation.

FIGURE CAPTIONS

Figure 1 Peak reactive hyperemia response in males (■) vs. females (□). Data are presented as mean \pm SEM.

Figure 2 Average time course (minutes) of nitroglycerin mediated response in entire study population (n=86) (●) as well as separated between females (▲) and males (■). * $P=0.05$ for peak NMI in males vs. females.

Figure 3 Percent change in nitroglycerine-mediated index from baseline to peak in males (■) vs. females (□). Data are presented as mean \pm SEM.

Figure 1

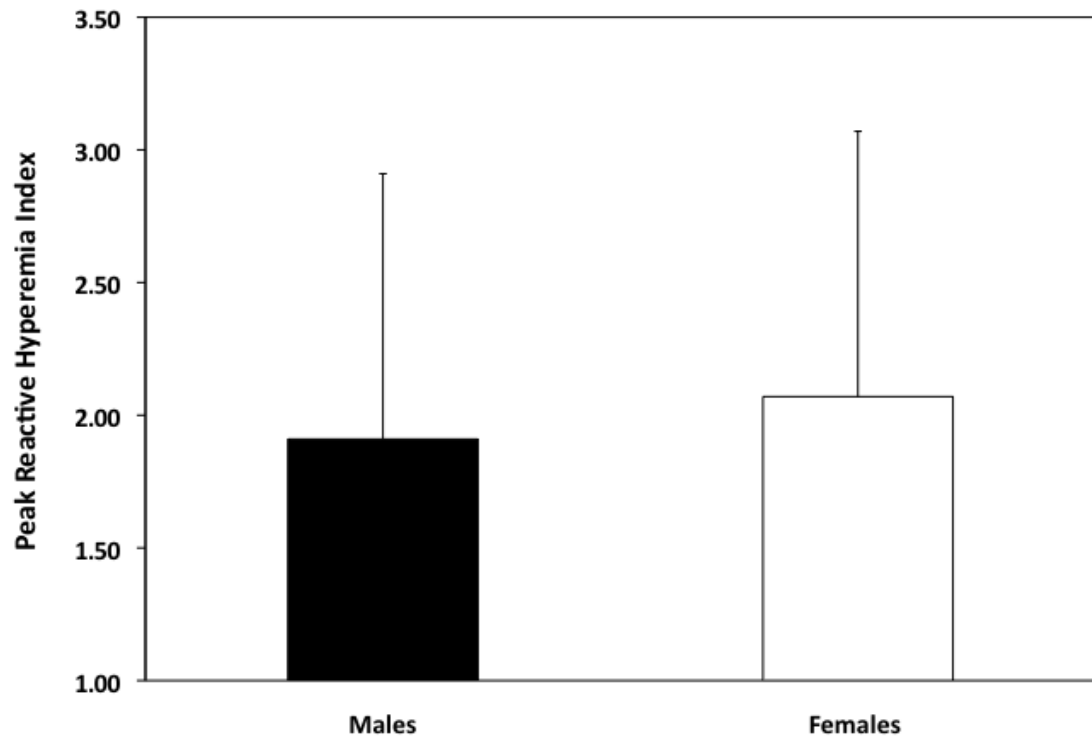


Figure 2

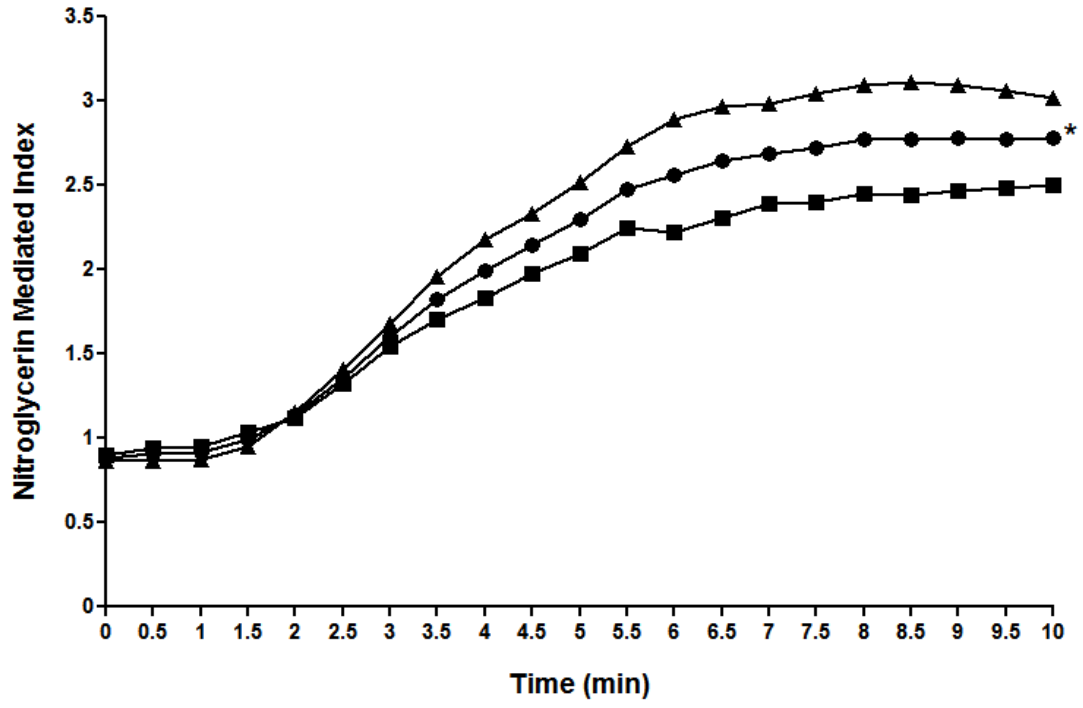


Figure 3

