Evaluation of gender differences in endothelium-independent dilation using peripheral arterial tonometry

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Summary

Background: A change in peripheral arterial tonometry (PAT) in response to reactive hyperaemia is often used to provide a non-invasive measure of endothelium-dependent dilation (EDD). Reactive hyperaemia does not allow one to quantify endothelium-independent dilation (EID), which is part of overall vascular function. Although most research examining vascular function and cardiovascular disease has focused on EDD, there is evidence that cardiovascular risk factors may impair EID.

Purpose: To examine the microvascular vasodilation response to nitroglycerin (NTG) in healthy adults using PAT.

Methods: Microvascular responses to reactive hyperaemia and NTG were evaluated in 86 (41 female and 45 male) healthy subjects (age 37 ± 5 years). Beat-to-beat plethysmographic measurements of finger arterial pulse waves were recorded for 5 min following reactive hyperaemia. After a 10-min rest period, sublingual NTG (0.4 mg) was administered and PAT signal changes were measured for 10 min. Peak reactive hyperaemic index (RHI) and peak NTG-mediated index (NMI) were determined in all subjects.

Results: There were no significant gender differences in peak RHI (females: 2.07 ± 0.56 versus males: 1.91 ± 0.58, P = 0.20). Mean peak NMI for all subjects was 2.78 (±1.49). Peak NMI was significantly greater in females than in males (3.11 ± 1.59 versus 2.50 ± 1.34, P = 0.05). Time to peak NMI was not significantly different between genders (7 min, 28 s [±1 min, 47 s], versus 7 min, 14 s [±1 min, 49 s], P = 0.58).

Conclusion: In this population of healthy adults, peak NMI was significantly greater in females than in males. These findings suggest that gender differences exist in the microvascular vasodilation responses to NTG using PAT.

Introduction

Endothelial dysfunction is thought to be one of the early stages in the development of atherosclerosis (Kunsch & Medford, 1999; Lieberman et al., 1996; Ludmer et al., 1986; Ross, 1993) and is an independent predictor of cardiovascular disease (CVD) 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 non-invasive technique for measuring peripheral endothelial function is ultrasound imaging during flow-mediated dilation (FMD) of the brachial artery (Agewall et al., 2007; Corretti et al., 2002). 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. Interventions used to study vascular function, including reactive hyperaemia and the response to nitroglycerin (NTG), 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). These data suggest that PWA of the small arteries of the finger is influenced by endothelial function in a magnitude and direction similar to that of larger conduit arteries.

Typically, PAT is used to measure digital pulsatile volume changes in response to reactive hyperaemia, providing a
measure of endothelium-dependent dilation (EDD). Reactive hyperaemia, 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., 2004; Roman et al., 2006; Thelen et al., 2008). Nitroglycerin administration supplies the vascular smooth muscle with nitric oxide (NO), causing systemic vasodilation. Use of NTG allows for a measure of EID, in which NO bioavailability and endothelial function are not factors in the dilatory response. Impaired EID is an indication of vascular smooth muscle dysfunction.

While most research concerning vascular dysfunction has focused on EDD as an indicator of cardiovascular disease, there is evidence to support EID as a marker of vascular dysfunction and disease risk (Adams et al., 1998; Heitzer et al., 2001; Jensen-Urstad et al., 1997). Significant EID impairment has been noted in those with known risk factors of atherosclerosis (Adams et al., 1998), those who have suffered a cardiovascular event compared with those who have not (Heitzer et al., 2001). Another study by Jensen-Urstad et al. (1997) indicated that gender was the most significant impact on both EDD and EID. Thus, the measurement of EID in the microvasculature of males and females may be an important indicator of cardiovascular health.

To our knowledge, the present study is the first to examine microvascular vasodilation response with PAT following NTG-mediation dilation. If EID in the microvasculature can be better characterized, it could be more widely utilized by researchers as a metric of overall vascular function. Therefore, the purposes of the present study were to evaluate the time course of microvascular dilatory response to sublingual NTG, identify the time point at which peak dilation occurs in healthy adults and evaluate any gender differences in response.

Methods

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

Study population

Individuals from a previous longitudinal cohort were invited to participate in this study. Participants with chronic diseases (e.g. type 1 diabetes, kidney dialysis patients and patients with cancer) were excluded from the study. Microvascular responses to reactive hyperaemia and NTG were evaluated in 86 (41 female and 45 male) healthy subjects (mean age 37 ± 5 years). 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 (22–23°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, review of symptoms (with particular attention to cardiovascular disease) and a physical examination.

Anthropometric and blood pressure measurements

Height and weight were obtained using a standard stadiometer (Model S100; Ayrton, Prior Lake, MN, USA) and electronic scale (ST Scale-Tronix, 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 (Model BP-8800C; Colin Press-Mate, San Antonio, TX, USA).

Vascular assessment

Endothelial function was measured non-invasively by digital reactive hyperaemia (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 hyperaemia 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 min of baseline data, a blood pressure cuff on the upper left forearm (just below the elbow) was inflated to a suprasystolic level for 5 min. Following cuff release, the change in pulse amplitude during reactive hyperaemia was measured for 5-min. The ratio of the hyperaemic and the baseline pulse amplitude (corrected for the same ratio on the control finger) was calculated and expressed as the reactive hyperaemic index (RHI). After a 10-min rest period, sublingual NTG (0.4 mg) was administered and PAT signal changes were measured for 10 min. The peak NTG-mediated index (NMI: the calculated ratio of the post-NTG PAT signal relative to baseline signal, indexed to the contralateral finger) was determined in all subjects.

Statistical analysis

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

Results

Mean demographic data of the study population (n = 86) are presented in Table 1. Age, weight, BMI and BP were not significantly different between males and females. Peak RHI did not significantly differ between genders (females: 2.07 ± 0.56 versus males: 1.91 ± 0.58, P = 0.20). Average time course and peak NTG-mediated response of the study population are presented in Fig. 1. Peak NMI was significantly greater in females than in males (3.11 ± 1.59 versus 2.50 ± 1.34, P = 0.05), whereas no difference in time to peak NMI was reported between genders (females: 7 min, 28 s versus males: 7 min, 14 s, P = 0.58) (Fig. 1).

Discussion

To our knowledge, the present study is the first to examine microvascular vasodilation response with PAT following NTG-mediated dilation. These findings indicate that females have a higher NMI response compared with 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 (Ducharme et al., 1999; Pepe et al., 2004; Thelen et al., 2008; Bressler et al., 2000; Kapuku et al., 2004). Our data suggest that the time course of peak NMI response is slower in the microvasculature compared with the response in the macrovasculature.

One potential explanation for the different time course in the microvasculature may be that changes in blood flow in the brachial artery create 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 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 peak NMI was significantly greater in females than in males. The exact mechanism underlying the gender differences in EID is unknown. It is possible that the observed differences in smooth muscle function may be related to the number of sex hormone receptors. Females have higher numbers of arterial oestrogen receptors than males, and as a result, may be more sensitive to vasodilators than similarly age-matched males (Collins et al., 1995).

A potential limitation of the present study is that subjects were drawn from a relatively homogenous population, particularly in relation to age. Therefore, results of this study may not be applicable to the general population across the lifespan and 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 (Virdis et al., 2003; Karpoff et al., 2008; Kelly et al., 2004) 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 use healthy, normal-weight, premenopausal women. We also did not control for oral contraceptives. However, the study by Virdis et al. (2003), on the effect of third-generation oral contraceptives on vascular function in healthy young women, reported that endothelial function remained unchanged after 6 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 (Ketel et al., 2009; Williams et al., 1998) 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 possible that resistance arteries 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. FMD measures the change in vessel diameter, while PAT measures pulsatile changes in vascular blood flow, which are different phenomena. It may be of interest to examine brachial artery blood flow velocity following NTG mediation dilation in relation to NMI. However, FMD, rather than brachial artery blood flow, has been more widely correlated with endothelial function (Kuvun 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.

**Conclusion**

In this study of healthy adults, we observed that peak NMI indicated a strong trend towards a significantly greater difference in females than in males. Time to peak NMI occurs between 7- and 8-min post-NTG administrations and does 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.

**References**


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