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EVALUATION OF GENDER DIFFERENCE IN ENDOTHELIUM-INDEPENDENT DILATION USING PERIPHERAL ARTERIAL TONOMETRY

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Abstract

A change in peripheral arterial tonometry (PAT) in response to reactive hyperemia is often used to provide a non-invasive measure of endothelium-dependent dilation (EDD). Reactive hyperemia 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 hyperemia and NTG were evaluated in 86 (41 female, 45 male) 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.

RESULTS—There were no significant gender differences in peak RHI (females: 2.07 ± 0.56 vs. males: 1.91 ± 0.58 , $P=0.20$). Mean peak NMI for all subjects was $2.78 (\pm 1.49)$. Peak NMI was significantly greater in females than males (3.11 ± 1.59 vs. 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], vs. 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 males. These findings suggest that gender differences exist in the microvascular vasodilation responses to NTG using PAT.

Keywords

Peripheral Vascular; Endothelium; Microcirculation; Vasodilation; Nitric Oxide; Gender

INTRODUCTION

Endothelial dysfunction is thought to be one of the early stages in the development of atherosclerosis¹⁻⁴ and is an independent predictor of cardiovascular disease (CVD) in various populations.⁵⁻¹⁰ 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.^{11,12}

The most widely used noninvasive technique for measuring peripheral endothelial function is ultrasound imaging during flow-mediated dilation (FMD) of the brachial artery.^{13,14} Recently, it has been established using peripheral arterial tonometry (PAT) that abnormalities in pulse wave signal changes is significantly associated with FMD.¹⁵ PAT is a non-invasive method of evaluating vascular smooth muscle function via finger plethysmography. Interventions used to study vascular function, including reactive hyperemia and the response to NTG, influence pulse wave amplitude (PWA) measurements, as assessed with PAT, in a similar manner as observed with brachial measurements of FMD.¹⁵ 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 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.¹⁶⁻¹⁹ Nitroglycerine (NTG) 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.²⁰⁻²² Significant EID impairment has been noted in those with known risk factors of atherosclerosis²⁰, those who have suffered a cardiovascular event compared with those who have not²¹. Another study by Jensen-Urstad et al. indicated that gender was the most significant impact on both EDD and EID²². 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 nitroglycerine (NTG) mediated 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 purpose of the present study was 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 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). 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, 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 (Ayrton Stadiometer, Model S100, 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 (Colin Press-Mate, Model BP-8800C, San Antonio, TX, USA).

Vascular Assessment

Endothelial function was measured non-invasively by digital reactive hyperemia (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-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 hyperemia was measured for 5-min. 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-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 contra-lateral 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 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. Data are presented as mean $\pm$ 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 vs. males: 1.91 ± 0.58 , $P=0.20$). Average time course and peak NTG mediated response of the study population are presented in Figure 1. Peak NMI was significantly greater in females than males (3.11 ± 1.59 vs. 2.50 ± 1.34 , $P=0.05$), whereas no difference in time to peak NMI was reported between genders (females: 7-min, 28-s vs. males: 7-min, 14-s, $P=0.58$) (Figure 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 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.^{16,17,19,23,24} Our data suggest that the time-course of peak NMI response is slower in the microvascular compared to 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 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 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 that peak NMI indicated a strong trend toward a significantly greater difference in females than 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 estrogen receptors than males, and as a result, may be more sensitive to vasodilators than similarly aged-matched males.²⁵

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 reported²⁸⁻³⁰ 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 Viridis et al., (2003)²⁸ 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^{26,27} 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)⁶ 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. Flow mediated dilation (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 mediated dilation in relation to NMI. However, FMD, rather than brachial artery blood flow, has been more widely correlated with endothelial function.¹⁵ 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 toward a significantly greater difference in females than males. Time-to-peak NMI occurs between 7- and 8-min post-NTG administration 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.

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Figure 1. Average time course 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.

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

	ALL (n=86)	MALE (n=45)	FEMALE (n=41)	p-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.