Endothelium-independent dilation in children and adolescents
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Introduction
Brachial artery assessment of vasoreactivity is commonly used to provide a non-invasive index of endothelial health and is often referred to as endothelium-dependent dilation (EDD). The EDD response is a surrogate measure of endogenous nitric oxide (NO) bioavailability within the endothelium layer and can help identify the presence of endothelial dysfunction, a barometer of arterial health. The ultrasound imaging following reactive hyperaemia is widely used and has been well validated (Celermajer et al., 1992; Corretti et al., 1995, 2002; Raitakari & Celermajer, 2000; Romen et al., 2006). Similarly, endothelium-independent dilation (EID), a reflection of smooth muscle sensitivity, can be assessed by tracking the change in brachial artery diameter following administration of nitroglycerin (NTG), an exogenously administered NO donor. The technique utilizes NTG to facilitate vascular smooth muscle relaxation. EID is frequently used to ensure the EDD response assessed via reactive hyperaemia is truly a result of NO-bioavailability and not necessarily a reflection of smooth muscle function.

A previous study in adults reported that a single dose (0.4 mg) of NTG, sublingual or spray, stimulates a maximal vasodilator response during EID testing (Ducharme et al., 1999). In this study, brachial artery dilation was assessed at 3-min post-NTG administration. In another adult study, Bressler et al. (2000) found that peak dilation occurred at approximately 5 min in coronary artery disease patients, with many individual peak responses occurring between 3 and 5 min. Finally, in a sample of healthy young adults, we reported the peak EID response occurred at 5-min post-NTG administration and that reporting per cent dilation at 3 min significantly underestimates true EID (Thelen et al., 2008).

To our knowledge, no studies to date have described the time course of the EID response in children and adolescents. It is possible that the time course of peak EID in youth could be different than adults. Indeed, differences in EDD time course in youth have been described by Järvisalo et al. (2002) who observed that peak response occurs later in children and adolescents (79 s) when compared with adults (Bressler et al., 2000). Therefore, the purpose of this study was to describe the time course of brachial artery dilation response to a fixed dose of sublingual NTG, identify the time point at which peak dilation occurs in children and adolescents, and assess any gender differences in response. We hypothesized that the time course of EID would be similar in children as has been described in adults and that smaller resting brachial artery diameter in females would result in higher peak EID response compared males.

Methods
The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board (IRB).

Summary
Peak brachial artery dilation post-nitroglycerin (NTG) administration occurs between 3 and 5 min in adults. The purpose of this study was to identify the time to peak dilation response to sublingual NTG (0.3 mg) in youth. Endothelium-independent dilation (EID) was measured in 198 healthy (113 males, 85 females) youth (6–18 years) via ultrasound imaging of the brachial artery following NTG administration. Time to peak EID was 268 s following NTG administration, with no significant (P = 0.6) difference between males and females. There was a significant (P<0.001) difference between EID post-NTG at the 3 versus 4 min, 4 versus 5-min, and 3 versus 5 min time points. Peak EID (males: 24.8 ± 0.5 versus females: 25.3 ± 0.6%, P = 0.6) was not significantly different after accounting for baseline diameter. Peak response to NTG administration occurs between 4 and 5 min. The results demonstrate the importance of measuring EID up to 5-min post-NTG administration in youth.
Additionally, the procedures followed in the study adhered to the University of Minnesota’s IRB and the Health Insurance Portability and Accountability Act (HIPAA) guidelines.

**Study population**

One hundred ninety-eight healthy children and adolescents (113 males, 85 females) between the ages of 6 and 18 years (mean age 13.9 ± 0.2 years) were assessed for EID up to 5 min. Subject data was previously collected within a University of Minnesota IRB-approved study of healthy siblings of cancer survivors, and another University of Minnesota IRB-approved study of adiposity amongst healthy children. All subjects submitted written informed assent and consent for participation in the study. Subjects were taken from community-based study protocols and were otherwise considered healthy individuals. Prior to vascular testing, subjects were asked to fast for 12 h prior to testing as well as abstain from caffeine ingestion leading up to study commencement. Subjects were instructed not to take their usual morning medications until testing was complete. A study physician and/or certified nurse practitioner was present to review study procedures and evaluation plans, prescription medications, and conduct comprehensive medical examinations including current and past medical history, review of systems (with particular attention to cardiovascular and endocrine issues), family history (with particular attention to cardiovascular disease and diabetes), and a physical examination. A urine-based pregnancy test was completed for female participants when applicable.

**Measurements**

**Anthropometric and blood pressure assessments**

Height and weight were obtained using a standard stadiometer (Model S100; Ayrton, Prior Lake, MN, USA) and electronic scale (Serial No. 5002-8893; ST Scale-Tronix, White Plains, NY, USA), respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Tanner ‘pubertal’ stage was determined by trained paediatricians (Tanner, 1962). Body composition was obtained using dual energy X-ray absorptiometry (DXA) (Versions 10.5; Prodigy, 3M, Madison, WI, USA). Seated blood pressure was obtained on the control arm using an automatic sphygmomanometer (Model BP-8800C; Colin Press-Mate, San Antonio, TX, USA) after 5 min of quiet rest in the supine position.

**Vascular assessment**

Testing was performed in the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All the vascular studies were performed in a quiet, temperature-controlled environment (22–23°C). Endothelium-independent dilation was assessed using 0.3 mg sublingual NTG, the dose considered appropriate for children by the University of Minnesota Institutional Review Board.

Following 15 min of quiet rest in the supine position, vascular images were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512; Siemens Medical Solutions USA, Inc. Mountain View, CA, USA) with an 8.0-MHz linear array probe held in place by a stereotactic device. This system was interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. All arterial images were triggered and captured at the R wave of the electrocardiogram (end-diastolic diameter), then digitized and stored on a personal computer for later off-line analysis using electronic wall-tracking software (Vascular Research Tools 5; Medical Imaging Application, LLC, Iowa City, IA, USA).

Brachial artery diameter was assessed continuously for 5-min post-NTG administration. All image files were averaged over 10-s periods and peak dilation during the study was defined as the greatest per cent change from resting baseline brachial artery diameter. Digital image analysis was performed by the same trained reader blinded to group assignments.

**Statistical analysis**

SPSS version 17.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Results are expressed as mean ± standard error of the mean (SEM). An independent sample t-test was used to compare demographical characteristics by gender. Univariate analysis of co-variance (ANCOVA) with Bonferroni post hoc tests was used to compare per cent dilations by genders whilst accounting for baseline diameter differences and Tanner ‘pubertal’ stage, as well as age differences. A repeated measures analysis of variance with a Bonferroni post hoc test was also used to compare gender differences in percent dilation at different time points.

An alpha value of 0.05 was used to signify statistical significance. Time to peak dilation was calculated by identifying the time point at which each individual reached maximal change in per cent dilation, and then averaged amongst the subject population.

**Results**

Mean demographical data amongst the study population (N = 198) are presented in Table 1. A significant difference in height, per cent body fat, total lean mass, and baseline brachial diameter was reported between genders. Time to peak dilation after 0.3 mg sublingual NTG administration was approximately 4 min, 28 s (males: 268±2 ± 2.6 versus females: 266±2 ± 3.1 s, P = 0.6), with peak dilation of 24±9 ± 0.4% (males: 24±8 ± 0.5 versus females: 25±3 ± 0.6%, P = 0.6) for subjects reporting data up to 5 min. A significant (P<0.001) difference in peak brachial artery dilation response to NTG was reported at 3 versus 4 min, 4 versus 5 min, and 3 versus 5 min (Fig. 1).
Table 1  Mean (± standard error) demographical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All (N = 198)</th>
<th>Male (n = 113)</th>
<th>Female (n = 85)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.9 ± 0.2</td>
<td>13.8 ± 0.2</td>
<td>14.1 ± 0.3</td>
<td>0.394</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.1 ± 0.9</td>
<td>163.3 ± 1.4</td>
<td>160.2 ± 1.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.7 ± 1.4</td>
<td>63.0 ± 2.1</td>
<td>60.0 ± 1.7</td>
<td>0.297</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>23.1 ± 0.4</td>
<td>23.0 ± 0.5</td>
<td>23.2 ± 0.5</td>
<td>0.763</td>
</tr>
<tr>
<td>Per cent fat (%)</td>
<td>26.8 ± 0.8</td>
<td>23.4 ± 1.1</td>
<td>32.0 ± 1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>17.5 ± 0.9</td>
<td>15.7 ± 1.2</td>
<td>20.1 ± 1.1</td>
<td>0.011</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>44.3 ± 0.9</td>
<td>47.3 ± 1.4</td>
<td>40.2 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline brachial diameter (mm)</td>
<td>3.3 ± 0.0</td>
<td>3.5 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BMI, body mass index.

Figure 1  Average time course of endothelial-independent dilation (EID) for the entire study population (N = 198) (●), as well as separated between males (●) and females (▲) from 0 to 5 min. Significant (P<0.05) differences were reported at 3 versus 4 min (*), 3 versus 5-min (**), and 4 versus 5 min (†) within the entire study population when adjusted for differences in baseline diameter.

There was a significant (P<0.05) difference in EID between males and females; however, significance was lost (24.7 ± 0.5 versus 25.2 ± 0.6%; P = 0.6) after accounting for baseline artery diameter. Tanner ‘pubertal’ stage was not significantly different between males and females (3.7 ± 0.1 versus 4.3 ± 0.1, P = 0.3). Additionally, age was not significantly different between males and females (13.7 ± 0.2 versus 14.0 ± 0.3 years, P = 0.6).

Discussion

The findings indicate that, amongst healthy children and adolescents, maximal brachial artery dilation following the administration of 0.3 mg NTG occurs at 4 min, 28 s, with an average change in peak dilation of 24.9 ± 0.4%. EID at 5 min significantly differed from dilation response at both 3-min and 4-min time points, suggesting that reporting values prior to 5 min may underestimate the true EID response. These findings are similar to our previous report of EID in young adults (Thelen et al., 2008), which demonstrated EID response to NTG in a healthy adult population occurred approximately 5 min following post-NTG administration. A smaller subset (n = 20; 12 males, eight females) reported average time to peak dilation of approximately 4 min, 24 s, with an average change in peak dilation of 25.8 ± 1.2% (male: 24.4 ± 1.6 versus females: 25.8 ± 1.8%). A significant (P<0.05) difference was reported at 3 versus 4 min, 3 versus 5 min, 3 versus 6 min, 3 versus 7 min, and 3 versus 8 min over an 8-min total time course. Our findings are in line with a previous report of EID in adult subjects with clinical evidence of coronary artery disease (Bressler et al., 2000), in which peak dilations ranging between 3 and 5 min were observed.

The present study also supports previous observations regarding the role of brachial artery diameter in peak dilation response (i.e., smaller baseline diameter generally equals higher peak dilation response) and its impact on vascular function. In general, females tend to have higher EID compared with males. The practical implication is that larger baseline diameter, as is commonly seen in men, results in reduced dilation responses compared with the relatively smaller arteries of women (Adams et al., 1995, 1998; Kapuku et al., 2004; Juonala et al., 2008; Dengel et al., 2010). We found a significant difference in peak EID between males and females; however, significance disappeared upon adjusting for differences in baseline brachial diameters. Furthermore, the reported difference supports our hypothesis that smaller resting brachial artery diameter in females results in larger dilation response compared with males. Similar responses to resting brachial artery diameter are reported in adult populations as well. These findings underscore the importance of adjusting for baseline brachial artery diameter when comparing response by gender in children and adolescents. Similarly, no significant differences between Tanner ‘pubertal’ stage, as well as age, were reported.

Strengths of the study include the relatively large sample size, balance between genders, and the uniform approach and analysis of the vascular data. A potential limitation of the following study is that subjects were drawn from a relatively homogenous population; therefore, posing a potential problem in generalizing these findings to a heterogeneous population including less healthy subjects. Also, we used a smaller dose of NTG (0.3 mg) than is commonly used in the adult population, so we cannot rule out the time course of dilation may differ at a dose of 0.4 mg.

Conclusion

In this study population of healthy children and adolescents, maximal EID response was observed at approximately 5-min post-NTG administration. Significant differences in EID were observed at 3 versus 4 min, as well as 4 versus 5 min. Reporting EID at 3 or 4 min may underestimate the true EID peak in children and adolescents. Future research
is needed to assess how cardiovascular disease risk factors in less healthy children and adolescent populations affect the time course of brachial artery dilation in response to NTG administration.

References


