Peak Shear and Peak Flow Mediated Dilation:

A Time Course Relationship

A THESIS

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Dedication

This thesis is dedicated to my loving and unconditionally supportive family. Without them, my return to academia would have been impossible. Thank you all for providing balance to my chaotic journey! I appreciate you more than words can explain.

ABSTRACT

Flow-mediated dilation (FMD) is a measure of endothelial function and is widely used to assess cardiovascular disease risk. This study aimed to explore the temporal relationship of time to peak FMD (FMD_{TTP}) as well as time to peak shear stress ($Shear_{TTP}$) between children and adults. Shear stress and the change in brachial artery diameter were tracked following reactive hyperemia in 122 children and 350 adults using conventional ultrasound. Peak FMD (7.25 ± 0.28 vs. 6.47 ± 0.18%, p=0.022) and Peak Shear rate (314 ± 8 vs. 278 ± 4 sec^{-1}, p=0.0002), and were significantly larger in children than adults. FMD_{TTP} was significantly slower (p=0.027) in children (57.2 sec 95%CI=53.3-61.4 sec.) vs. adults (52.8sec. 95%CI=50.5-55.1sec.). $Shear_{TTP}$ was significantly slower in children than in adults (11.8 sec., 95% CI=11.1-12.6 sec. vs. 10.6 sec., 95% CI=10.2-11.0, p=0.004). There was no significant difference (49.4 ± 2.6 vs. 46.7 ± 1.5 sec., p=0.27) in time from $Shear_{TTP}$ to FMD_{TTP} between children and adults. In the present study we observed that children not only displayed a larger peak FMD, but also the time it takes the shear stimulus to reach its peak is significantly slower in children than adults and that the slower time to peak shear resulted in slowing of the peak dilation of the brachial diameter in children. However, the time from peak Shear to peak FMD was similar. Associations between FMD_{TTP} and $Shear_{TTP.}$in children and adults were mostly explained by baseline diameter.

Key Words: Ultrasound, Flow-mediation Dilation, Shear stress, Time to Peak, Children, Adults

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CHAPTER 1. INTRODUCTION

Ultrasound imaging of the brachial artery following reactive hyperemia, or flow mediated dilation (FMD), is the most widely accepted non-invasive technique for measuring endothelial function (Celemajer, Sorensen & Gooch, 1992). Furthermore, endothelial dysfunction is known to represent atherosclerotic risk in both adults (Bonetti, Lerman & Lerman, 2003; Dengel & Bronas, 2010) and children (Meyer, Kundt, Steiner, Schuff-Werner, & Kienast, 2006). A number of other variables, including peak shear stress can also be measured during FMD studies.

Shear stress (τ) is recognized as the stimulus that elicits the FMD (Gnasso et al., 2001) and is directly related to fluid viscosity (η) and flow rate (*du/dz*) (Chandran, Rittgers, & Yoganathan, 2012). According to Poiseuille's Law, wall shear stress (τ_w) is further defined as a function directly proportional to blood flow (q) and viscosity while inversely proportional to arterial diameter (Parker, Trehearn, & Meendering, 2009): $\tau = 4\eta q/\pi r^3$. The vast majority of studies examining the relationship of shear stress and FMD have focused on the magnitude of brachial artery dilation in response to reactive hyperemia in adults (Black, Cable, Thijssen, & Green, 2008; Pyke, Dwyer, & Tschakovsky, 2004; Pyke & Tschakovsky, 2005; Pyke & Tschakovsky, 2007; Thijssen et al., 2009; Widlansky, 2009) and children (Järvisalo et al., 2002; Thijssen et al., 2009). However, two studies included time to peak data within the analyses.

Thijssen et al. (2009) investigated the relationship of arterial shear stress and the magnitude of FMD in children and adults and found significant differences in shear rate

area under the curve (AUC) while finding no differences in FMD time to peak. Black et al. (2008) sought to explain the importance of measuring the time course of FMD. They found time to peak FMD differences in young versus older adults.

To our knowledge, no studies have specifically explored the time-course relationship between arterial shear stress and FMD in a population of children and adults. Therefore, the purpose of the present study was to examine this temporal relationship between time to peak shear stress ($Shear_{TTP}$) and time to peak FMD (FMD_{TTP}) in children and adults.

A comprehensive review of relevant literature, followed by methodology, results, discussion, and conclusion relating to the examination of above-mentioned study are detailed in the following chapters:

Chapter two summarizes the current literature as well as pioneering studies related to the endothelium, hematological shear stress and FMD. Current knowledge about FMD and shear stress differences in children and adults are addressed.

Chapter three addresses this study's methodology. Descriptions of the study population (anthropometrics, blood laboratory values and vascular data) are outlined and measurement techniques as well as the statistical analysis strategy are detailed.

The results of this study are presented in chapter four. The differences in FMD and shear stress measures including: baseline values, peak responses, time to peak, as well as area under the curve, in children and adults are detailed. Associations of relevant independent variables to time to peak values are also set forth.

Chapter five includes discussion of the findings. Both agreements as well as differences of time to peak and maximal responses in relation to similar studies are considered and associations of age, gender, baseline diameter, as well as other variables are discussed.

Chapter six summarizes implications and conclusions based on the results of this study. The importance of reporting both FMD and shear stress time course data and future experimental directions are presented.

Chapter seven lists all works cited in this thesis.

CHAPTER 2: REVIEW OF LITERATURE

The Endothelial Cell

The innermost layer of the cardiovascular system is composed of endothelial cells. Together, endothelial cells constitute a functioning organ: The endothelium (Fishman, 1982), contributing 6×10^{13} cells; weighing 1 kilogram; and having a surface area of 7 meters^2 to the adult human body (Cines et al, 1998). Not only do these remarkable cells provide a selective barrier between the blood and tissue, but also facilitate processes ranging from angiogenesis and anticoagulation to smooth muscle regulation. They have inspired the work of noted physiologists such as Frank Starling (1896) and August Krough (1929). Among the many functions of endothelial cells, their paracrine role in vasodilation and vasoconstriction is pivotal in regulating blood flow.

The Discovery of Endothelium-Derived Relaxing Factor

Dusting, Moncada, and Vane (1977) presented the first evidence that endothelial cells play a role in vascular tone. They discovered prostacyclin (PGI_2), a metabolite of arachidonic acid, relaxed bovine coronary arteries *in vitro*. Furthermore, they observed vasoconstriction upon indomethacin (a prostaglandin inhibitor) application. This indicated PGI_2 was endogenous to the vessel. Three years later, a technical mistake led to an epic discovery (Henderson, 1998). When endothelial cells were progressively rubbed off the intimal surface during *in vitro* vessel preparation, the dilation response to acetylcholine was reduced in a somewhat linear fashion (Furchgott & Zawadzki, 1980). Though the exact vasodilator was not elucidated, it was determined not to be any known substance (i.e. PGI_2, bradykinin, cyclic AMP/GDP). It was not until 1986 that Furchott

and Ignarro independently proposed a nitrogen-based compound, which was named endothelium-derived relaxing, factor (EDRF) (Boulanger & Vanhoutte, 1998). Soon after, EDRF was correctly identified as nitric oxide (NO) by chemilumnincence and mass spectroscopy (Palmer & Moncada, 1998). Since then, much work has been done to elucidate the mechanisms in which endothelial cells contribute to vascular dynamics.

The Biofluid Mechanics of Shear Stress

Blood flow within the conducting vessels creates force against the endothelium. This force is known as shear stress (Dewey, Bussolari, Gimbrone, & Davies, 1981; Gnasso et al., 2001; Pyke &, Tschakovsky, 2005; Parker, Trehearn, & Meednderling, 2009). In fact, a fluid is defined as a material that "deforms continuously under the action of a *shear stress* produced by a force that acts parallel to the line of motion" (Chandran, Rittgers, & Yoganathan, 2012, p.3). Shear stress is calculated according to Poiseuille's law: the function directly proportional to blood flow velocity and viscosity while inversely proportional to arterial diameter ($\tau = 4\eta q/\pi r^3$) expressed in units of dynes per centimeter squared (dyne/cm^2) (Chandran et al., 2012). Since collecting blood viscosity is a somewhat invasive procedure, shear rate is commonly used to estimate shear stress and is expressed as flow/diameter (s^{-1}) (Parker et al., 2009). The way the endothelium reacts to shear stress lies in the specialized cell structure of endothelial cells.

Endothelial Cell response to Shear Stress

The inner (luminal) surface of endothelial cells is rich in protein-carbohydrate polymers that form a negatively charged glycocaylx (Levick, 2010). In addition to providing a semipermeable barrier, portions of the glycocalyx are connected to kinase proteins via the cytoskeleton and activate eNOS independently of Ca^{2+} concentration (Fisslthaler et al., 2000). When specific glycocalyx proteins are removed, endothelial cells response to shear stress is markedly reduced (Kumagai & Kassab, 2009). This cytoskeletal dynamic was first demonstrated by an elegant *in vivo* experiment by Dewey, Bussolari, Gimbrone, and Davies (1981). They observed endothelial cell functions such as fluid endocytosis and cytoskeletal arrangement was directly influenced by differing shear stresses. The interaction of shear stress and endothelial cells underlies the physiology of flow mediated dilation.

Flow-Mediated Dilation

Over a decade ago Celermajer et al. (1992) described a method to non-invasively detect endothelial function. This method used ultrasound imaging to record the reaction of the femoral and brachial artery upon tourniquet occlusion and subsequent release. The resultant vasodilation was directly related to blood flow. They named this technique flow-mediated dilation (FMD) (Celermajer et al., 1992). Additionally, they found dilation differences upon nitroglycerine administration and FMD in subjects with known coronary artery disease (CAD). Those with CAD displayed no FMD while maintaining nitroglycerin dilation reinforcing the role of endothelial cells in response to flow. Since

this paramount study, these differences have been well established (Anderson et al., 1995; Lieberman et al., 1996; Neunteufl et al., 1997).

The development of atherosclerosis is known to depend on endothelial cell (Dengel & Bronas, 2010). The NO produced by endothelial cells not only provides vasodilation, but also plays a protective role by inhibiting the progression of atherosclerotic processes such as leucocyte adhesion (Kubes et al, 1991), platelet aggregation (Riddell & Owen, 1997), and smooth muscle proliferation (Ignarro et al, 2001). Because FMD has been found to be an indispensable tool to assess the endothelium, the intricacies of both technique and interpretation have been widely studied.

Shear and FMD in Vascular Literature

A number of studies have examined the magnitude of shear stress and its effect on dilation on the FMD response (Mullen et al., 2001; Agewall et al, 2002; Pyke, Dwyer, & Tschakovsky, 2004; Pyke, & Tschakovsky, 2007; Wray, 2010). However, very few groups have focused on the time course of shear stress and FMD (Black, Cable, Thijssen, & Green, 2008; Thijssen et al. 2008).

Black et al. (2008) established the importance of measuring the time course of FMD. They found time to true peak FMD after a standard 5-minute occlusion differed between young and older adults. Time to peak FMD (FMD_{TTP}) was significantly faster in the younger than older adults ($p < 0.001$) and shear area under the curve ($Shear_{AUC}$) was

similar between the two groups (no *p*-value given). Thijssen et al. (2008) measured shear stress over four different timeframes in children, and adults. They found that children exhibited a significantly larger $Shear_{AUC}$ from adults for all time points measured (0-30 sec, 0-60 sec, and 0-time to peak FMD, $p < 0.001$) and the change from baseline FMD was greater in children than adults ($p < 0.001$) while FMD_{TTP} was similar ($p = 0.47$). Both Black et al. (2008) and Thijssen et al. (2008) concluded that more work needs to be done to determine the relationship between shear and FMD.

The complex nature of the endothelial cell and its important role in vascular homeostasis is paramount to maintain human health. Though many of these intricacies have been explored, there are disagreements as well as gaps in the literature relating to shear stress and vasodilation. To our knowledge there are no published studies specific to temporal relationship differences of shear stress and FMD in both adult and adolescent populations. Therefore, the purpose of the present study was to examine the time course relationship between shear stress and FMD in adults and children. In doing so, a better understanding of vascular physiology may be revealed.

CHAPTER 3: METHODS

Study population

A total of one hundred twenty two healthy children (mean ± SEM age: 10.7 ± 0.2 yrs.; range 6-15 years old; 48 female, 74 male) and three hundred fifty healthy adults (age: 39.2 ± 0.1 range 33-57 years old; 195 female, 155 male) who participated in a longitudinal cardiovascular risk study were used in this analysis. The University of Minnesota Institutional Review Board (IRB) approved the study design and protocol. All adult participants gave written consent; parental consent and child assent was obtained from the child/adolescent participants. All information regarding participant's information was protected in accordance with the Health Insurance and Accountability Act (HIPAA).

Physical and Vascular Assessments

Height was measured with a wall-mounted stadiometer (Ayrton, Model S100, Prior Lake, MN, USA), and weight was measured using an electronic scale (ST Scale-Tronix,(White Plains, NY, USA). Body mass index (BMI) was calculated as the weight (kg) divided by the height (meters) squared. Waist-hip ratio (WHR) was calculated as the ratio of the smallest circumference (cm) between the last palpable rib and iliac crest to the largest circumference over the gluteus maximus.

Seated blood pressure was measured two separate times with a random-zero sphygmomanometer on the right arm. The average of two systolic blood pressures (SBP) and the fifth phase Korotkoff diastolic blood pressure (DBP) measurements were analyzed and recorded.

Venous blood was drawn from an antecubital vein for lipid studies [Low density lipoprotein (LDL), High density lipoprotein (HDL), total cholesterol, triglycerides] and analyzed by the University of Minnesota Fairview laboratory, as previously described (Sinaiko et al., 2001). Fasting insulin and glucose levels were measured in the University of Minnesota Fairview laboratory by a chemiluminescence solid phase immunometric and enzymatic assays respectively.

Vascular testing was conducted in the Clinical and Translational Science Institute (CTSI) of the University of Minnesota. Vascular structure and function were measured in a quiet, temperature-controlled environment (22-23 ^{o}C). Vascular images were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc. Mountain View, CA) with an 8.0-15.0 mHz linear array transducer held in place by a stereotactic arm. This system is 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). All image files were averaged over a 20 sec period and peak dilation during the each study was defined as the greatest percent change from resting baseline brachial artery diameter. A trained sonographer performed all digital image analysis.

Statistical analysis

Data were analyzed using R (version 2.15.1, Vienna Austria) and recorded as mean ± standard error (SEM). A two-sided alpha level of 0.05 was used to define statistical significance. Variables showing a non-normal distribution were log-transformed prior to analysis. *T*-tests were performed to compare baseline differences between age groups.

Main study variables (age and gender) and all independent variables were added to regression models in order to test correlates. Interaction statements for baseline diameter and age and gender were added to the model for proper adjustment. Models for each age group were constructed for each dependent variable (FMD_{TTP} and $Shear_{TTP}$). Stepwise multiple regression analysis was implemented to indicate significant associations. The initial *p-value* cut-off for eliminating independent variables from the model was 0.50. As further reductions were considered a lower *p*-value was used until model fit was significantly compromised.

CHAPTER 4: RESULTS

Table 1 shows the physical characteristics for both the adults and children. As expected, adults were significantly taller and weighed more than children (p=0.0001). Similarly, adults WHR, BMI, SBP and DBP were also significantly different (p=0.0001). Blood variables are shown in Table 2. Fasting insulin, fasting glucose, total cholesterol, triglycerides, and low-density lipoproteins were significantly higher in adults compared to children (p=0.0001) while there was no difference in high-density lipoprotein levels (p=0.1).

Baseline diameter was significantly smaller in children compared to adults (3.07 ± 0.04 vs. 3.79 ± 0.04 mm, p<0.0001). Figure 1 displays greater Peak FMD in children compared to adults (7.25 ± 0.28 vs. 6.47 ± 0.18%, p=0.022). Peak Shear rate was also significantly greater in children than adults (314 ± 8 vs. 278 ± 4 sec^{-1}, p=0.0002) (Figure 2). FMD area under the curve (FMD_{AUC}) and shear rate area under the curve from cuff release to peak FMD ($Shear_{AUC}$) were greater in children than adults (715 ± 35 vs. 602 ± 20 sec^{-1}, p=0.008 and 13231 ± 531 vs. 9907 ± 237 sec^{-1}, p<0.0001 respectively (Figures 3 and 4).

$Shear_{TTP}$ was significantly (p=0.004) slower in children (11.8, 95% CI=11.1-12.6sec.) than adults (10.6, 95% CI=10.2-11.0 sec) and FMD_{TTP} was also significantly slower (p=0.03) in children than adults (57.2, 95% CI=53.3-61.4 sec. vs. 52, 95% CI=50.5-55.1 sec). Interestingly the time from $Shear_{TTP}$ to FMD_{TTP} was the same (45.4, 95% CI=42.4-48.8; 42.3 sec., 95%CI=40.3-44.1 sec) between children and adults respectively (p=0.27) (Figure 5).

Regression models

The variables added to the FMD_{TTP} stepwise regression model for both age groups were age, gender, BMI, SBP, DBP, WHR, HDL), LDL, triglycerides, glucose, insulin, baseline diameter as well as age and gender interactions with baseline diameter. Upon reduction, the emergent associated variables in the adult model were gender (β=1.23, *P*=0.007) and baseline diameter (β=0.42, *p*=0.02). A test for interaction yielded a significant interaction effect for gender and baseline diameter (*p*=0.001). The reduced FMD_{TTP} model for children revealed BMI (β=0.03, *p*=0.009) and gender (β=-0.18, *p*=0.009) to be significantly associated. Baseline diameter interaction terms (age and gender) added to the child model showed no significant interactions.

$Shear_{TTP}$ models started with the same independent variables as the FMD_{TTP} models. No significant predictors were found in the reduced adult model. Similarly, a test for interaction showed no significant age and gender interactions with baseline diameter. The reduced child model indicated age (β=-0.26, *p*=0.02) to be the only significant predictor upon adjustment, however an interaction of age and baseline diameter was evident (β=0.07, *p*=0.04).

Based on the regression models, gender was significantly associated in both FMD_{TTP} models. These models show that the female children had a faster FMD_{TTP} (β = -0.18, *p*=0.009) whereas adult females a slower FMD_{TTP} (β=1.23, *p*=0.007) compared to adult males (reference group) when adjusted for age and baseline diameter. Tests for interaction yielded no significant interaction between baseline diameter and gender or age within the child model (*p*=0.2 and 0.6 respectively). Since BMI was also significantly

associated in the child FMD_{TTP} model, a post hoc comparison (Two group t-test) was initiated between males and females yielding a non-significant difference (p=0.2).

CHAPTER 5: DISCUSSION

The main aim of this study was to compare the temporal relationship of $Shear_{TTP}$ and FMD_{TTP} between children and adults. In this study, children displayed slower $Shear_{TTP}$ and FMD_{TTP} compared to adults. However the time from peak stimulus (shear) and peak response (FMD) was similar in both children and adults. This observation demonstrates that once the peak stimulus is achieved a predictable time to peak FMD may be expected.

Thijssen et al. (2009) reported no significant difference in FMD_{TTP} between children (n=51, age=10 ± 1 yr.) and older adults (n=27, age=58 ± 4 yrs.), which differ from the present finding that FMD_{TTP} is significantly slower in children than adults. These finding may have been influenced by a smaller sample size compared to the present study or the fact that the Thijssen et al. (2009) adult population were older. Black et al. (2008) showed older adults exhibit slower FMD_{TTP} than younger adults (p=0.001). Thijssen et al. (2009) also found that peak FMD was greater in children than adults, which agree with our findings. Though Thijssen et al. (2009) reported FMD_{TTP}, they did not report $Shear_{TTP}$ in absolute terms (only in relation to AUC).

Based on the regression models, baseline arterial diameter is the most common variable associated with the dependent variables (FMD_{TTP} and $Shear_{TTP}$). Arterial diameter is inversely related to FMD in adults (Thijssen et al., 2008) and children (Kapuku et al., 2004). The interaction between baseline diameter and gender or age within both the child and adult FMD_{TTP} models are similar to other FMD related findings (Benjamin et al., 2004; Dengel et al., 2011; Pierce et al., 2011). However, gender was significantly associated with FMD_{TTP} in both models, Female children displayed a 0.18

sec. faster FMD_{TTP} whereas adult females showed a 1.23 sec. slower FMD_{TTP} compared to children and adult males respectively (reference group) when adjusted for age and baseline diameter. These differences could possibly be due to differences in smooth muscle function (Dengel et al., 2011; Mendelsohn & Karas, 2005; Thelen et al., 2008). However, the clinical significance is yet to be determined.

CHAPTER 6: IMPLICATIONS AND CONCLUSION

The most novel finding of this study is that, even though children took longer to reach peak shear as well as peak FMD, there was no difference in the time from peak shear rate to peak FMD compared to adults. This observation demonstrates that once the brachial artery reaches peak hemodynamic stimulus via reactive hyperemia, there may be an expected time to maximal dilation regardless of age. The major implication of this finding is that deviation from this expected time course might indicate vascular abnormalities considering that the participants in this study were healthy. Therefore, future studies examining FMD should not only report $Shear_{TTP}$ and FMD_{TTP}, but also the difference between them (FMD_{TTP}–$Shear_{TTP}$).

Furthermore, the time from $Shear_{TTP}$ to FMD_{TTP} should be studied in groups of children and adults with known cardiovascular risk factors to test the hypothesis that this time course may change as a result of factors such as obesity and type 2 diabetes mellitus. Time course variables in relation to Tanner stages should also be studied to further explore the relationship between development and vascular dynamics.

Strengths of this study are large sample sizes as well as highly developed techniques to capture both FMD and shear in children and adults. The cross sectional design is a limitation (due its inability to determine cause and effect) though is a common method for observational studies such as this. Another limitation is the lack of Tanner stages. The ability to stratify children by pubertal

development may draw better conclusions to developmental effects on FMD and shear stress time courses.

In conclusion, this is the first study to demonstrate that the time course between $Shear_{TTP}$ and FMD_{TTP} are similar in children and adults. However the manner in which children accomplish peak responses is different. Children exhibit significantly slower time to peak shear rate as well as slower time to peak FMD compared to adults. Age, gender, baseline brachial artery diameter, and BMI appear to influence the time course of FMD_{TTP}.

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Table 1– Group comparisons

	Group		*p*-value
Variable	**Children**	**Adults**	
n (Female, Male)	122 (48, 74)	350 (195, 155)	-
% (Female, Male)	(39,61)	(56,44)	-
Age (years)	10.7 ± 0.2	39.2 ± 0.1	< 0.0001
Waist-Hip Ratio	82.5 ± 0.5	88.7 ± 0.5	< 0.0001
Weight (kg)	43.3 ± 1.6	86.0 ± 1.5	< 0.0001
Height (cm)	145.8 ± 1.5	171.5 ± 1.0	< 0.0001
Body mass index (kg/m^2)	19.5 ± 0.4*	29.1 ± 0.4	<0.0001
Systolic blood pressure (mmHg)	113 ± 1	125 ± 1	< 0.0001
Diastolic blood pressure (mmHg)	58 ± 1	70 ± 1	< 0.0001

Data are mean ± SEM. *P*-value < 0.05 demonstrates significant differences between means.

Table 2– Blood Variables

	Group		*p-value*
Variable	**Children**	**Adults**	
High-density Lipoprotein (mmol/L)	0.53 ± 0.01	0.50 ± 0.01	0.1
Low-density Lipoprotein (mmol/L)	0.91 ± 0.02	1.1 ± 0.02	< 0.0001
Triglycerides (mmol/L)	0.79 ± 0.03	1.40 ± 0.06	< 0.0001
Total Cholesterol (mmol/L)	4.1 ± 0.1	4.8 ± 0.1	< 0.0001
Fasting Glucose (mmol/L)	4.2 ± 0.1	5.7 ± 0.1	< 0.0001
Fasting Insulin (pmol/L)	29.4(25.2, 34.2)	33.4(30.6, 37.2)	0.1

Data are mean ± SEM. *P*-value < 0.05 demonstrates significant differences between means. Fasting Insulin was log transformed and values represent back transformed geometric mean (95% Confidence interval).

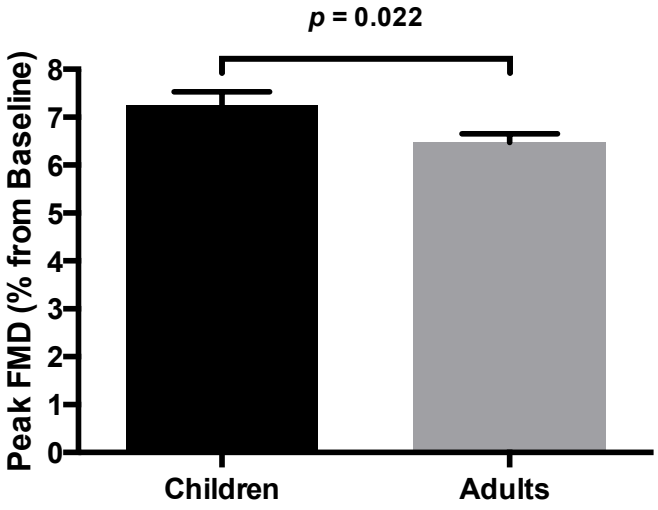

Figure 1. Mean (± SEM) flow-mediated dilation in children and adults.

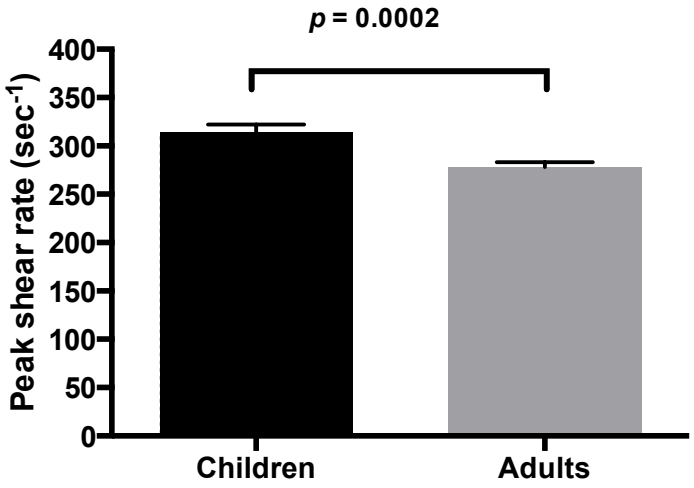

Figure 2. Mean (± SEM) shear rate differences in children and adults.

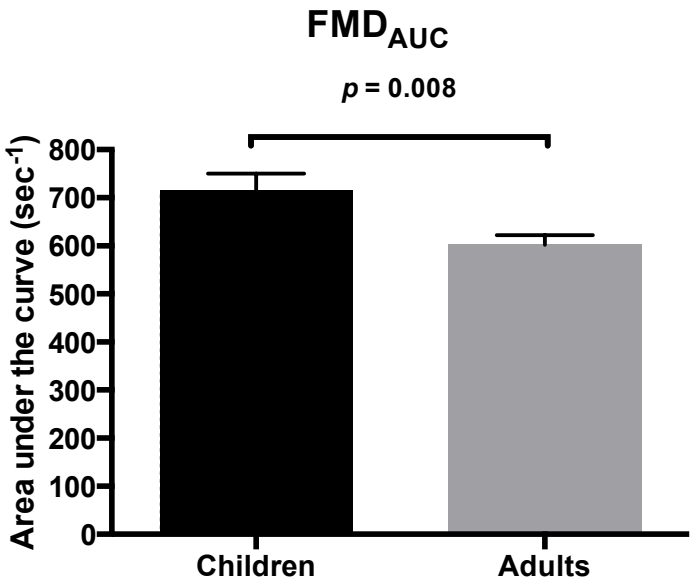

Figure 3. Mean (± SEM) FMD area under the curve in children and adults.

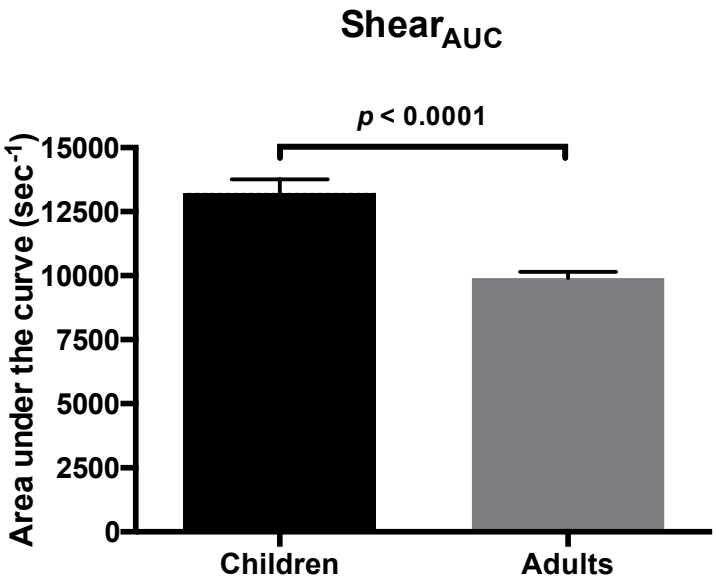

Figure 4. Mean (± SEM) shear area under the curve in children and adults.

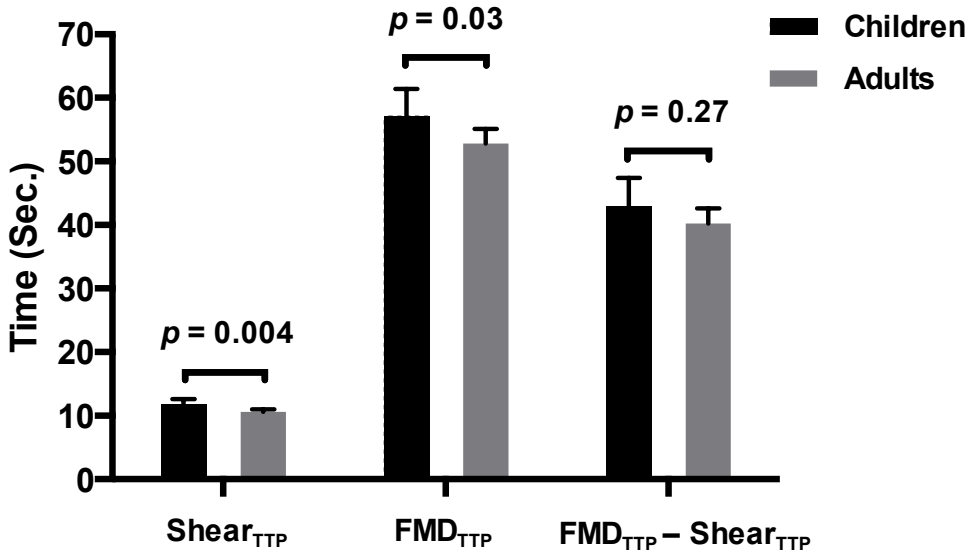

Figure 5. Time course differences between children and adults (GM ± 95% confidence interval). Values represent flow mediated dilation time to peak (FMD_{TTP}), Shear time to peak ($Shear_{TTP}$), and the time from $Shear_{TTP}$ to FMD_{TTP} ($FMD_{TTP} - Shear_{TTP}$).