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Associations of Sex, Age and Adiposity in Endothelium-Independent Dilation in Children

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Abstract

Objective—To examine the association of age, sex, and obesity status on endothelial-independent dilation (EID) among children and adolescents.

Study Design—This study examined 264 children (143 males) between 8 to 18 years old (mean $\pm$ SD: age=14.3 $\pm$ 2.7 years). Endothelial-independent dilation was assessed via ultrasound imaging of the brachial artery following administration of 0.3 milligrams of sublingual nitroglycerin. A one-way analysis of variance with Bonferroni post-hoc comparisons assessed sex-differences in percent peak EID dilation (EID%-peak) and EID area under the curve (EID%-AUC), while analysis of covariance (ANCOVA) adjusted for race, age, body mass index percentile (BMI-percentile), and brachial artery diameter. Multiple linear regression evaluated the association of sex, age, BMI-percentile, percent body fat (%BF), and brachial artery diameter on EID.

Results—Prior to adjustment, EID%-peak was significantly higher among females than males (mean $\pm$ SE: 26.9 $\pm$ 0.5% vs. 22.9 $\pm$ 0.6%, $p<0.001$, respectively); similar findings for EID%-AUC (4214 $\pm$ 105%·sec vs. 3398 $\pm$ 97%·sec, $p<0.001$) were observed. After adjusting for covariates, EID%-AUC remained consistent ($p=0.03$) while EID%-peak was not significantly different between sexes ($p=0.21$). EID%-peak was significantly higher among normal weight compared to obese participants ($p=0.04$), while no differences were observed between obesity status after adjustment

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AUTHOR CONTRIBUTIONS

No payment was received to produce this manuscript. All authors have seen, approved, and take full responsibility for the manuscript. H.Z., J.R.R., and D.R.D. developed the aim, hypothesis, analysis, and interpretation of the data. J.S. and A.R.S. provided the funding and developed the study design. M.M.H. and H.Z. performed the statistical analysis. H.Z. wrote the first draft of the manuscript, and M.M.H. finalized the manuscript for submission. M.M.H. submitted the manuscript for publication. J.R.R., J.S., A.R.S., D.R.J., and D.R.D. provided subject matter expertise and a critical review of the manuscript.

for brachial artery diameter ($p=0.64$). Both unadjusted ($p=0.16$) and adjusted EID%-AUC ($p=0.24$) was not significantly different between obesity status. BMI-percentile was not associated with EID%-peak ($p=0.76$) or EID%-AUC ($p=0.30$). Additionally, %BF was not associated with EID%-peak ($p=0.56$) or EID%-AUC ($p=0.15$). After adjusting for brachial artery diameter, BMI-percentile, and age, males had lower EID%-AUC ($p=0.03$) and lower but not significant EID%-peak ($p=0.21$).

Significance—Vascular smooth muscle function was significantly lower among male children and adolescents, which is suggestive that impaired EID and increased cardiovascular disease risk among males may begin in childhood. Interestingly, obesity status and BMI-percentile was not associated with EID in children and adolescents after adjusting for brachial artery diameter.

Keywords

Vascular smooth muscle function; Endothelial function; Pediatric; Cardiovascular disease risk; Body mass index

INTRODUCTION

One of the primary causes of mortality among adults in developed nations is cardiovascular disease (CVD), and research has shown that vascular disease and endothelial dysfunction are precursors to atherosclerosis (Newman *et al* 1991, Adams *et al* 1998, Rudijanto 2007, Falk 2008, Maruhashi *et al* 2013, Wang *et al* 2013). Atherosclerosis is characterized as a progressive, immunoinflammatory disease with its antecedents beginning early in childhood (Falk 2008). Therefore, evaluating endothelial dysfunction in childhood prior to clinical manifestations of atherosclerotic plaque may be beneficial in early detection and CVD-risk classification (Fernhall *et al* 2008). Characterizations of subclinical atherosclerosis in children and adolescents has focused on endothelial function (Celermajer *et al* 1992; Järvisalo *et al* 2002; Charakida *et al* 2012, Ryder *et al* 2016), with limited information about smooth muscle function.

Endothelium-independent dilation (EID) is reflective of structural malformations within arterial smooth muscles cells that develop secondary to atherosclerosis (Thelen *et al* 2008, Maruhashi *et al* 2013). Nitroglycerine-induced vasodilation, which involves ultrasound imaging of the brachial artery following the exogenous administration of a nitric oxide (NO) donor (e.g., sublingual nitroglycerine), is both a valid and reproducible index of EID (Corretti *et al* 2002). Impaired EID has been associated with several chronic diseases in adults, including coronary artery disease (CAD) (Heitzer *et al* 2001), type 1 (Johnstone *et al* 1993) and type 2 diabetes mellitus (McVeigh *et al* 1992), hypertriglyceridemia (Capell *et al* 2003), and inflammatory bowel disease (Kayahan *et al* 2012). Adult males exhibit a greater proportion of CVD risk factors (Lerner *et al* 1986), and also have reduced vascular smooth muscle function compared to females (Adams *et al* 1998, Thelen *et al* 2008, McCue *et al* 2012). Advanced age is additionally associated with vascular smooth muscle dysfunction, with studies showing impairment of EID with increasing age in asymptomatic adults (Al-Shaer *et al* 2006; Montero *et al* 2015).

Prior studies by our group have shown significantly lower EID in young adult males than females (Dengel *et al* 2011). Because EID has not been studied in childhood, the present study was designed to determine whether there are age-related, sex-related, or obesity-related differences among children and adolescents (Järvisalo *et al* 2002, Pena *et al* 2006). It is hypothesized that a sex difference in EID emerges at the onset of puberty, with females having higher vascular smooth muscle function.

METHODS

Participants

Data were obtained from 264 (143 males) children and adolescents (mean $\pm$ SD: age=14.3 $\pm$ 2.7 years) who participated in two longitudinal studies conducted at the University of Minnesota: (1) a community-based study evaluating cardiometabolic risk in healthy children (2006–2011) (n =166, 90 males), and (2) healthy siblings that served as a control group for a cohort of childhood cancer survivors (2007–2012) (n =98, 53 males). The age range of the participants was 8 to 18 years old, with 31% of the cohort aged 12 years and younger. Participants were normotensive, free from chronic diseases, and were not taking any medications known to influence vascular function. The two studies used the same methods of data collection and were approved by the Institutional Review Board (IRB) at the University of Minnesota. All procedures were followed in accordance with the IRB and HIPAA guidelines. Parents and participants provided informed consent and assent, respectively, for study participation.

Anthropometric Measurements

Height was obtained via a standard stadiometer (Ayrton S100, Prior Lake, Minnesota, USA), and weight was measured by use of an electric scale (ST Scale-Tronix, White Plains, New York, USA). Body mass index (BMI) was calculated as body mass in kilograms (kg) divided by height in meters-squared (m^2). Body mass index percentile (BMI-percentile) was calculated using CDC normality scales and stratified into three categories: normal weight (i.e., >5th percentile to <85th percentile), overweight (i.e., 85th percentile to <95th percentile), and obese (i.e., 95th percentile).

Trained medical providers determined Tanner stage (I–V) by assessing breast development and pubic hair in females and pubic hair in males. Body composition was measured by dual X-ray absorptiometry (DXA: [Lunar Prodigy, General Electric Medical Systems, Madison, WI, USA]) and analyzed using enCore™ software (version 13.6, GE Healthcare, Madison, WI, USA).

Measurement of Endothelium-Independent Dilation

Participants fasted for at least 8 hours and abstained from caffeine and heavy exercise for at least 24 hours prior to their study visit. All data were collected in a quiet, temperature-controlled room (22–23°C). Resting systolic and diastolic blood pressures were recorded using an automated sphygmomanometer (Colin BP-8800, Colin Medical Instruments Corp., San Antonio, TX, USA) on the right arm prior to each vascular assessment.

Vascular images of the left brachial artery were obtained proximal to the antecubital fossa in the longitudinal plane using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with an 8–15 MHz linear array probe. A stereotactic arm maintained the probe with a constant pressure and at a fixed point over the imaged artery. Heart rate (HR) was monitored using a three-lead electrocardiogram (ECG), and end-diastolic images were captured at the R-wave. Images were digitized and stored for off-line analysis using electronic wall-tracking software (Vascular Research Tools 5, Coralville, IA, USA).

Endothelial-independent dilation was performed as previously described; following a 15-minute rest period, participants were given 0.3 milligrams (mg) of sublingual nitroglycerin to assess EID (Celermajer *et al* 1992). Brachial artery diameter was measured continuously for 5-minutes post-nitroglycerin administration. Percent peak EID dilation (EID%-peak) was determined by taking the greatest percent change in artery diameter from baseline. Endothelial-independent dilation area under the curve (EID%-AUC) was defined across the complete relaxation of the brachial artery after nitroglycerin administration. This technique has been previously reported to yield consistent and reproducible measures of EID (Leeson *et al* 1997, Sorensen *et al* 1995).

Statistical Analysis

IBM SPSS Statistics 23 (IBM Corp. Released 2016, IBM SPSS Statistics for Windows, Version 23, Armonk, NY: IBM Corp) was used for statistical analysis. Differences in clinical characteristics between males and females were compared by a one-way analysis of variance (ANOVA), and chi-square tested for differences in demographic characteristics (e.g., race, Tanner stage). A one-way ANOVA with Bonferroni post-hoc pairwise comparisons assessed differences in EID%-peak and EID%-AUC, and analysis of covariance (ANCOVA) adjusted for sex, age, race, BMI-percentile, percent body fat (%BF), and baseline brachial artery diameter. The main effect of sex and Tanner stage, as well as an interaction, was evaluated by a multivariate ANOVA. Multiple linear regression evaluated the association of sex, age, %BF, BMI-percentile, and baseline brachial artery diameter on both EID%-peak and EID%-AUC. To account for potential confounding of adjusting for brachial artery diameter, allometric scaling was performed. Significance was set at $\alpha=0.05$.

RESULTS

Participant demographic and clinical characteristics, divided by sex, are presented in Table 1. Males were significantly taller ($p=0.001$) than females, had a greater amount of lean body mass ($p<0.001$), lower %BF ($p<0.001$), greater systolic blood pressure ($p=0.001$), and larger brachial artery diameter ($p<0.001$). There was a significant relationship between sex and Tanner stage ($\chi^2=13.4$, $p=0.010$), with male sex being associated with lower Tanner stage.

The correlation between EID%-peak and EID%-AUC was 0.87 ($p<0.001$). Male and female brachial artery diameter at baseline were both negatively associated with EID%-peak ($p<0.001$) and EID%-AUC ($p<0.001$). Age was significantly and negatively associated with EID%-peak among males ($p<0.001$), but not females ($p=0.90$) (Figure 1). A similar trend of decreased EID%-AUC with age was observed among males ($p<0.001$), but not females

($p=0.51$) (Figure 1). We performed allometric scaling and the results (not shown) did not differ from non-allometric scaled values. Given that a majority of the literature to date has not utilized allometric scaling, we chose to report our data without allometric scaling.

Results from a multivariate ANOVA depicted that, after adjusting for Tanner stage, females had significantly higher EID%-peak ($p<0.001$). Similar results were observed after adjusting for both age and Tanner stage ($p<0.001$); in particular, females had higher EID%-peak within Tanner stage III (females vs. males: $26.6\pm1.6\%$ vs. $22.2\pm1.4\%$, $p=0.05$), IV ($26.0\pm1.0\%$ vs. $22.7\pm1.1\%$, $p=0.02$) and V ($27.3\pm0.9\%$ vs. $21.0\pm0.9\%$, $p<0.001$) (Figure 2). Multivariate analysis likewise depicted higher EID%-AUC among females after adjustment for Tanner stage ($p<0.001$), with similar results observed after adjusting for age and Tanner stage ($p<0.001$). With adjusting for age and Tanner stage, females had higher adjusted EID %-AUC within Tanner stage III ($4046\pm268\%\cdot\text{sec}$ vs. $3154\pm229\%\cdot\text{sec}$, $p=0.02$), IV ($4083\pm192\%\cdot\text{sec}$ vs. $3286\pm226\%\cdot\text{sec}$, $p=0.05$), and V ($4331\pm156\%\cdot\text{sec}$ vs. $3041\pm160\%\cdot\text{sec}$, $p<0.001$) (Figure 2).

Unadjusted EID%-peak was significantly higher among females compared to males ($p<0.001$), as also was unadjusted EID%-AUC ($p<0.001$) (Table 2). Adjustment for race, age, and BMI-percentile similarly resulted in both significantly higher EID%-peak ($p<0.001$) and EID%-AUC ($p<0.001$) among females (Table 2). After adjusting for race, age, brachial artery diameter, and BMI-percentile, females had a significantly higher EID%-AUC ($3938\pm100\%\cdot\text{sec}$ vs. $3630\pm92\%\cdot\text{sec}$, $p=0.03$), but not EID%-peak ($25.2\pm0.6\%$ vs. $24.3\pm0.5\%$, $p=0.21$) (Table 2). Differences in both EID%-peak ($25.2\pm0.6\%$ vs. $24.3\pm0.5\%$, $p=0.31$) and EID%-AUC ($3883\pm106\%\cdot\text{sec}$ vs. $3679\pm95\%\cdot\text{sec}$, $p=0.19$) were not significant after adjusting for race, age, brachial artery diameter, and %BF (Table 2). Unadjusted EID %-peak was significantly higher among normal weight I compared to obese participants ($p=0.02$), and this persisted after adjusting for race, age, and sex ($p=0.03$) (Table 3). There were no differences in EID%-peak between obesity status after adjustment for race, age, sex, and brachial artery diameter ($p=0.64$) (Table 3). Unadjusted EID%-AUC was not significant across different obesity status ($p=0.16$), and this trend was consistent regardless of adjusting with ($p=0.24$) and without ($p=0.26$) brachial artery diameter (Table 3).

Linear regression analysis displayed a non-significant relationship between BMI-percentile and both EID%-peak ($p=0.76$) (Table 4) and EID%-AUC ($p=0.30$) (Table 5). Additionally, %BF was not significantly associated with EID%-peak ($p=0.56$) (Table 4) or EID%-AUC ($p=0.15$) (Table 5). After adjustment for age, brachial artery diameter, and BMI-percentile, the male sex was significantly and negatively associated with EID%-AUC ($p=0.03$) (Table 5), but not EID%-peak ($p<0.21$) (Table 4).

DISCUSSION

The main findings from the current study are: 1) males have lower vascular smooth muscle function during pubertal development; 2) age was significantly and negatively associated with EID%-peak and EID%-AUC in males, but not in females; 3) EID%-peak, but not EID %-AUC, was significantly lower among obese participants compared to normal weight, and 4) adiposity, as indicated by BMI-percentile, was not associated with EID%-AUC or EID%-

peak after adjusting for brachial artery diameter. Differences in EID%-peak across varying levels of adiposity were minimized following adjustment for brachial artery diameter, potentially illustrating the effect pubertal growth and excess adiposity exerts on the brachial artery. Since brachial artery diameter increases with age in both sexes (Hopkins *et al* 2015) and with obesity (Wildman *et al* 2004), adjusting for brachial artery diameter during growth years may eliminate structural differences between obesity status. The observations from this study also suggest that males may be predisposed to a blunted EID in childhood, and that features captured in EID%-AUC, but not EID%-peak, are important in defining the emerging difference between males and females after adjustments for covariates. The premature onset of impaired vascular smooth muscle function among male youth and adolescents are important to research, and may potentially explain the etiology behind the increased risk of CVD among adult males.

The observed differences in EID among children and adolescents could be attributed to sex-specific differences in brachial artery diameter (Adams *et al* 1998, Fernhall *et al* 2008). Previous studies have reported an inverse relationship between baseline artery diameter and EID (Brown *et al* 1981, Herrington *et al* 2001). In this current study, males had a significantly greater baseline artery diameter compared to females, and this was significantly associated with both a blunted unadjusted EID%-peak and unadjusted EID%-AUC. Thijssen *et al* (2008) observed a strong and significant inverse correlation between artery size and magnitude of glyceryl trinitrate response among healthy adults. Although Thijssen and colleagues did not investigate sex-specific differences in artery size, this research nevertheless provided evidence that differences in EID is a function of artery diameter (Thijssen *et al* 2008). Dengel *et al* (2011) observed that, within a cohort of healthy young adults, males had a blunted flow-mediated vasodilation (FMD) relative to females, and this difference was largely explained by adjustment for brachial artery diameter. However, the lower EID observed in males was not explained by artery diameter. In this present study, we observed that EID%-AUC was significantly lower among males after adjustment for both brachial artery diameter and BMI-percentile, and adjusted EID%-peak was not significantly different between sexes. While EID%-peak measures peak dilation, EID%-AUC quantifies the entire hyperemic stimulus (Pyke *et al* 2005). Such results imply that sex-differences with total hyperemia manifest in children before sex-differences in peak vasodilation during nitroglycerin-induced vasodilation. The significance of this finding is unclear; it is suggestive that other factors besides artery diameter may impact EID.

Another potential contribution to our findings could be the role of sex hormones which regulate vascular function and could account for sex-specific differences in EID. Testosterone has been demonstrated to exert both vasodilatory (Deenadayalu *et al* 2011) and atherogenic (Campelo *et al* 2012) effects on the vascular system, whereas estrogen has cardio-protective properties that directly affect EID and limit cell migration and proliferation (Akishita *et al* 1997, White *et al* 2002). Activation of estrogen receptors located on the smooth muscle causes potassium-channels to open and reduce nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is a free radical that degrades NO (Mügge *et al* 1993, Mendelsohn *et al* 1994, Chambliss *et al* 2002; White *et al* 2002). The influence of estrogen promotes vessel relaxation, which could extend relaxation time by keeping intracellular calcium concentrations low (White *et al* 2002). As such, the observed

association between age and EID%-AUC could be attributed to the combination of a more developmentally advanced female population and higher estrogen levels observed among post-pubertal females. We did not measure sex-specific hormone concentrations; therefore, we can only speculate that estrogen accounted for increased EID among females, and this could be the topic of further investigation.

Age-related vascular endothelial dysfunction and atherosclerotic development is well documented in adults (Herrington *et al* 2001, Falk 2008, Montero *et al* 2015). Research examining EID response in adults have determined that, with increasing age, endothelial function deteriorates (Al-Shaer *et al* 2006, Marlatt *et al* 2013). Donato *et al* (2009) observed that the age-related reduction in endothelium-dependent dilation was due to increased endothelin-1 protein expression among healthy, sedentary older men. However, little is known about the relation between age and EID among children and adolescents, with most studies using age only as a covariate and not as a variable of interest. We observed age to be significantly and negatively associated with EID%-AUC in males, but not in females. Differences between sexes in regard to EID%-AUC could be attributed to differences in ambulatory blood pressure. Aggoun *et al* (2008) researched EID among prepubescent children and observed that both FMD and EID%-peak were negatively correlated with ambulatory systolic blood pressure. Although the subject population was normotensive, males regardless had elevated ambulatory systolic blood pressure relative to females, as of which could potentially contribute to the decreased EID%-AUC observed among males with increasing age. Furthermore, the effect of age on EID could be another function of hormonal differences between males and females during pubertal development.

The male sex was associated with decreased EID%-AUC after adjusting for BMI-percentile with multiple linear regression analysis; regardless, this trend was not observed for both EID %-peak and EID%-AUC after adjusting for %BF. Since sex-differences in EID was eliminated after adjusting for %BF, this suggests that %BF exerts a significant impact on EID among youth and adolescents. Changes in body composition and regional fat deposition that occur with accelerated growth during pubertal maturation could account for differences in EID between males and females with age. Although obesity and a high BMI increases cardiovascular risk, accumulating evidence suggests that regional fat accumulation is more predictive of cardiovascular risk than excess adiposity (Feijoo-Bandin *et al* 2016). Specifically, subcutaneous adipose tissue (SAT) accumulates below the skin in the abdominal, gluteus and femoral areas, and represents about 80% of body fat (Feijoo-Bandin *et al* 2016). Visceral adipose tissue (VAT) is distributed in close proximity to the abdominal cavity, and accounts for up to 10–20% of total fat in men and 5–8% in women, increasing with age in both genders (Feijoo-Bandin *et al* 2016). Several epidemiology studies have demonstrated VAT accumulation to be associated with CVD, while SAT accumulation is cardio-protective. In particular, adiponectin (Acrp30) is primarily produced by the SAT, and its signaling in the peripheral adipose tissue improves insulin sensitivity, prevents atherosclerotic plaque formation, decreases reactive oxygen species production by endothelial cells, and enhances endothelial cell migration and vascularization (Fernández-Real *et al* 2004, Feijoo-Bandin *et al* 2016). Fernández-Real *et al* (2004) observed Acrp30 to be positively associated with EID among adults, and this positive correlation was independent of insulin sensitivity. It is possible that increased Acrp30 with increased SAT

among females contributed to the observed enhanced vascular smooth muscle function and EID%-AUC with age.

Information available on the influence of obesity on both endothelial-dependent and endothelial-independent response is conflicting. Several studies among adults report no difference in EID between normal weight and obese individuals (Schroeder *et al* 2000, Perticone *et al* 2001), while other studies contend that there is a difference in EID between the groups (de Kreutzenberg *et al* 2003, Ayer *et al* 2011, Christou *et al* 2012). This disagreement also exists among pediatric populations (Tounian *et al* 2002, Jarvisalo *et al* 2002, Woo *et al* 2004, Pena *et al* 2006). With regard to the effect of obesity on endothelial-dependent vasodilation, Ryder *et al* (2016) reported that brachial FMD was positively associated with adiposity within the same cohort used in this present study. In particular, obese children had significantly greater FMD and FMD area under the curve (FMD-AUC) compared to overweight and normal weight children and adolescents (Ryder *et al* 2016). However, we observed no significant differences in EID%-peak or EID%-AUC between normal weight, overweight, and obese children and adolescents after adjusting for covariates. This is in agreement with a previous study that found no difference in EID response between obese adult participants and age-and-sex matched controls (Schroeder *et al* 2000). A separate study reported significantly lower EID response in severely obese children compared with normal weight controls (Woo *et al* 2004). However, the latter study did not adjust for baseline artery diameter, which has been shown to be inversely related to EID (Woo *et al* 2004). Furthermore, the mean BMI for obese participants in the study conducted by Woo *et al* (2004) was greater than the mean BMI in this present sample. It is possible that impaired EID response would also have been detected in this study if there had been a significant number of severely obese, as opposed to the moderate levels of obesity and overweight.

Strengths of our study include a large sample of healthy, asymptomatic children, with a range of age and adiposities, and the inclusion of DXA provided more precise information on body composition. Due to the cross-sectional nature of the study we are unable to determine a causal relationship between sex, age, and obesity with EID. While we observed differences in the effect of age on EID between sexes, future studies will be needed to examine the trajectory of change in EID throughout childhood. The predominantly Caucasian population does not allow us to determine the generalizability of our findings to other racial and ethnic groups. In accordance with the current EID guidelines, this study assessed EID following the administration of 0.3 mg of sublingual nitroglycerin (Corretti *et al* 2002). This dosage may be too insensitive to detect differences in EID among children and adolescents, and potentially larger dosages of nitroglycerine should have been administered. Another limitation was that blood pressure was only measured once at baseline.

CONCLUSION

In conclusion, we have observed that sex may be associated with vascular smooth muscle function in children and adolescents, with differences in EID%-AUC present between boys and girls at an early age. Future research should investigate the effect of sex steroid

hormones, body composition, regional fat deposition, and adipokines on vascular smooth muscle function throughout puberty.

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ABBREVIATIONS

%BF	Percent body fat
Acrp30	Adiponectin
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
BMI-percentile	Body mass index percentile
CAD	Coronary artery disease
CVD	Cardiovascular disease
DXA	Dual X-ray absorptiometry
ECG	Electrocardiogram
EID	Endothelial-independent dilation
EID%-AUC	Endothelial-independent dilation area under the curve
EID%-peak	Percent peak endothelial-independent dilation
FMD	Flow-mediated vasodilation
FMD-AUC	Flow-mediated vasodilation area under the curve
HR	Heart rate
IRB	Institutional review board
Kg	Kilograms
Mg	Milligrams
NO	Nitric oxide
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue

References

- Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, Celermajer DS. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *Journal of the American College of Cardiology*. 1998; 32:123–127. [PubMed: 9669259]
- Aggoun Y, Farpour-Lambert NJ, Marchand LM, Golay E, Maggio AB, Beghetti M. Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure. *European Heart Journal*. 2008; 29:792–799. [PubMed: 18245115]
- Akishita M, Ouchi Y, Miyoshi H, Kozaki K, Inoue S, Ishikawa M, Eto M, Toba K, Orimo H. Estrogen inhibits cuff-induced intimal thickening of rat femoral artery: Effects on migration and proliferation of vascular smooth muscle cells. *Atherosclerosis*. 1997; 130:1–10. [PubMed: 9126642]
- Al-Shaer MH, Choueiri NE, Correia ML, Sinkey CA, Barenz TA, Haynes WG. Effects of aging and atherosclerosis on endothelial and vascular smooth muscle function in humans. *International Journal of Cardiology*. 2006; 109:201–206. [PubMed: 16054252]
- Ayer JG, Harmer JA, David C, Steinbeck KS, Seale JP, Celermajer DS. Severe obesity is associated with impaired arterial smooth muscle function in young adults. *Obesity*. 2011; 19:54–59. [PubMed: 20489689]
- Brown B, Bolson E, Petersen R, Pierce C, Dodge H. The mechanisms of nitroglycerin action: Stenosis vasodilatation as a major component of the drug response. *Circulation*. 1981; 64:1089–1097. [PubMed: 6794931]
- Campelo AE, Cutini PH, Massheimer VL. Cellular actions of testosterone in vascular cells: Mechanism independent of aromatization to estradiol. *Steroids*. 2012; 77:1033–1040. [PubMed: 22728893]
- Capell WH, DeSouza CA, Poirier P, Bell ML, Stauffer BL, Weil KM, Hernandez TL, Eckel RH. Short-term triglyceride lowering with fenofibrate improves vasodilator function in subjects with hypertriglyceridemia. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003; 23:307–313.
- Celermajer DS, Sorensen K, Gooch V, Spiegelhalter DJ, Miller OI, Sullivan I, Lloyd J, Deanfield J. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet*. 1992; 340:1111–1115.
- Chambliss KL, Shaul PW. Estrogen modulation of endothelial nitric oxide synthase. *Endocrine Reviews*. 2002; 23:665–686. [PubMed: 12372846]
- Charakida M, Jones A, Falaschetti E, Khan T, Finan N, Sattar N, Hingorani A, Lawlor DA, Smith GD, Deanfield JE. Childhood obesity and vascular phenotypes: A population study. *Journal of the American College of Cardiology*. 2012; 60:2643–2650. [PubMed: 23177297]
- Christou DD, Pierce GL, Walker AE, Hwang MH, Yoo JK, Luttrell M, Meade TH, English M, Seal DR. Vascular smooth muscle responsiveness to nitric oxide is reduced in healthy adults with increased adiposity. *American Journal of Physiology-Heart and Circulatory Physiology*. 2012; 303:H743–H750. [PubMed: 22821988]
- Corretti MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology*. 2002; 39:257–265. [PubMed: 11788217]
- Deenadayalu VP, White RE, Stallone JN, Gao X, Garcia AJ. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *American Journal of Physiology-Heart and Circulatory Physiology*. 2001; 281:H1720–H1727. [PubMed: 11557563]
- de Kreutzenberg SV, Kiwanuka E, Tiengo A, Avogaro A. Visceral obesity is characterized by impaired nitric oxide-independent vasodilation. *European Heart Journal*. 2003; 24:1210–1215. [PubMed: 12831815]
- Dengel DR, Jacobs DR, Steinberger J, Moran AM, Sinaiko AR. Gender differences in vascular function and insulin sensitivity in young adults. *Clinical Science*. 2011; 120:153–160. [PubMed: 20815810]

- Donato AJ, Gano LB, Eskurza I, Silver AE, Gates PE, Jablonski K, Seals DR. Vascular endothelial dysfunction with aging: Endothelin-1 and endothelial nitric oxide synthase. *American Journal of Physiology-Heart and Circulatory Physiology*. 2009; 297:H425–H432. [PubMed: 19465546]
- Falk E. Pathogenesis of atherosclerosis. *Journal of the American College of Cardiology*. 2008; 47:C7–C12.
- Feijoo-Bandin S, Rodriguez-Penas D, Garcia-Rua V, Mosquera-Leal A, Gonzalez-Juanatey JR, Lago F. Adipokines at the cardiovascular system: Role in Health and Disease. *Journal of Endocrinology and Metabolism*. 2016; 2:1–8.
- Fernández-Real JM, Castro A, Vázquez G, Casamitjana R, López-Bermejo A, Peñarroja G, Ricart W. Adiponectin is associated with vascular function independent of insulin sensitivity. *Diabetes Care*. 2004; 27:739–745. [PubMed: 14988295]
- Fernhall B, Agiovlasitis S. Arterial function in youth: Window into cardiovascular risk. *Journal of Applied Physiology*. 2008; 105:325–333. [PubMed: 18450990]
- Fujimoto R, Morimoto I, Morita E, Sugimoto H, Ito Y, Eto S. Androgen receptors, 5 alpha-reductase activity and androgen-dependent proliferation of vascular smooth muscle cells. *The Journal of Steroid Biochemistry and Molecular Biology*. 1994; 50:169–174. [PubMed: 8049146]
- Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001; 104:2673–2678. [PubMed: 11723017]
- Herrington DM, Fan L, Drum M, Riley WA, Pusser BE, Crouse JR, Burke BL, McBurnie MA, Morgan TM, Espeland MA. Brachial flow-mediated vasodilator responses in population-based research: Methods, reproducibility and effects of age, gender and baseline diameter. *Journal of Cardiovascular Risk*. 2001; 8:319–328. [PubMed: 11702039]
- Hopkins ND, Dengel DR, Stratton G, Kelly AS, Steinberger J, Zavala H, Marlatt K, Perry D, Naylor L, Green DJ. Age and sex relationship with flow-mediated dilation (FMD) in healthy children and adolescents. *Journal of Applied Physiology*. 2015; 119:926–933.
- Järvisalo MJ, Rönkämaa T, Volanen I, Kaitosaari T, Kallio K, Hartiala JJ, Irjala K, Viikari JSA, Simell O, Raitakari OT. Brachial artery dilatation responses in healthy children and adolescents. *American Journal of Physiology-Heart and Circulatory Physiology*. 2002; 282:H87–H92. [PubMed: 11748051]
- Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation*. 1993; 88:2510–2516. [PubMed: 8080489]
- Kayahan H, Sari I, Cullu N, Yuksel F, Demir S, Akarsu M, eGoktay Y, Unsal B, Akpinar H. Evaluation of early atherosclerosis in patients with inflammatory bowel disease. *Digestive Diseases and Sciences*. 2012; 57:2137–2143. [PubMed: 22466100]
- Leeson P, Thorne S, Donald A, Mullen M, Clarkson P, Deanfield J. Non-invasive measurement of endothelial function: Effect on brachial artery dilatation of graded endothelial dependent and independent stimuli. *Heart*. 1997; 78:22–27. [PubMed: 9290397]
- Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: A 26-year follow-up of the Framingham population. *American Heart Journal*. 1986; 111:383–390. [PubMed: 3946178]
- Marlatt KL, Steinberger J, Dengel DR, Sinaiko A, Moran A, Chow LS, Steffen LM, Zhou X, Kelly AS. Impact of pubertal development on endothelial function and arterial elasticity. *The Journal of Pediatrics*. 2013; 163:1432–1436. [PubMed: 23968741]
- Maruhashi T, et al. Nitroglycerine-Induced Vasodilation for Assessment of Vascular Function Significance. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2013; 33:1401–1408.
- McCue MC, Marlatt KL, Kelly AS, Steinberger J, Dengel DR. Evaluation of gender differences in endothelium-independent dilation using peripheral arterial tonometry. *Clinical Physiology and Functional Imaging*. 2012; 32:94–98. [PubMed: 22296628]
- McVeigh G, Brennan G, Johnston G, McDermott B, McGrath L, Henry WR, Andrews JW, Hayes JR. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1992; 35:771–776. [PubMed: 1511805]

- Mendelsohn ME, Karas RH. Estrogen and the blood vessel wall. *Current Opinion in Cardiology*. 1994; 9:619–626. [PubMed: 7987043]
- Montero D, Pierce GL, Stehouwer CD, Padilla J, Thijssen DH. The impact of age on vascular smooth muscle function in humans. *Journal of Hypertension*. 2015; 33:445–453. [PubMed: 25479030]
- Mügge A, Riedel M, Barton M, Kuhn M, Lichtlen PR. Endothelium independent relaxation of human coronary arteries by 17 β -oestradiol in vitro. *Cardiovascular Research*. 1993; 27:1939–1942. [PubMed: 8287400]
- Newman WP, Wattigney W, Berenson GS. Autopsy studies in United States children and adolescents. *Annals of the New York Academy of Sciences*. 1991; 623:16–25. [PubMed: 2042824]
- Pena AS, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper H. Vascular endothelial and smooth muscle function relates to body mass index and glucose in obese and nonobese children. *The Journal of Clinical Endocrinology & Metabolism*. 2006; 91:4467–4471. [PubMed: 16895959]
- Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, Mattioli PL. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress protective effect of vitamin C. *Diabetes*. 2001; 50:159–165. [PubMed: 11147782]
- Pyke KE, Tschakovsky ME. Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *Journal of Applied Physiology*. 2007; 102:1510–1519. [PubMed: 17170205]
- Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. *Acta Med Indones*. 2007; 39:86–93. [PubMed: 17933075]
- Ryder JR, Dengel DR, Jacobs DR, Sinaiko AR, Kelly AS, Steinberger J. Relations among adiposity and insulin resistance with flow-mediated dilation, carotid intima-media thickness, and arterial stiffness in children. *The Journal of Pediatrics*. 2016; 168:205–211. [PubMed: 26427963]
- Schroeder S, Enderle MD, Baumbach A, Ossen R, Herdeg C, Kuettner A, Karsch KR. Influence of vessel size, age and body mass index on the flow-mediated dilatation (FMD%) of the brachial artery. *International Journal of Cardiology*. 2000; 76:219–225. [PubMed: 11104877]
- Sorensen KE, Celermajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, Deanfield JE. Non-invasive measurement of human endothelium dependent arterial responses: Accuracy and reproducibility. *British Heart Journal*. 1995; 74:247–253. [PubMed: 7547018]
- Thelen AM, Kelly AS, Williamson EB, Dengel DR. Examining the time course of endothelium-independent dilation by nitroglycerin. *Ultrasound in Medicine & Biology*. 2008; 34:1217–1220. [PubMed: 18420338]
- Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ. Heterogeneity in conduit artery function in humans: Impact of arterial size. *American Journal of Physiology-Heart and Circulatory Physiology*. 2008; 295:H1927–H1934. [PubMed: 18775852]
- Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, Girardet JP, Bonnet D. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: A prospective study. *The Lancet*. 2001; 358:1400–1404.
- Wang W, Lee Y, Lee CH. Review: The physiological and computational approaches for atherosclerosis treatment. *International Journal of Cardiology*. 2013; 167:1664–1676. [PubMed: 23103138]
- White RE, et al. Endothelium-independent effect of estrogen on Ca²⁺-activated K⁺ channels in human coronary artery smooth muscle cells. *Cardiovascular Research*. 2002; 53:650–661. [PubMed: 11861036]
- Wildman RP, Mehta V, Thompson T, Brockwell S, Sutton-Tyrrell K. Obesity is associated with larger arterial diameters in Caucasian and African-American young adults. *Diabetes care*. 2004; 27:2997–2999. [PubMed: 15562225]
- Woo K, Chook P, Yu C, Sung R, Qiao M, Leung S, Metreweli C, Celermajer DS. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. *International Journal of Obesity*. 2004; 28:852–857.

Figure 1.

Graphical Representation of the Association between Age and BMI-Percentile with EID%-peak and EID%-AUC.

R values represent Pearson correlation coefficients

Figure 2.

Graphical Representation of Tanner Stage by Sex on EID%-peak and EID%-AUC.

Reported as mean $\pm$ SE

Adjustments were made for age

*Indicates significance ($p < 0.05$) between sexes and within Tanner stage as determined by Bonferroni post-hoc pairwise comparisons

Tanner stages that share either letters a or b indicates non-significance ($p > 0.05$) within males as determined by Bonferroni post-hoc pairwise comparisons

Tanner stages that share the letter c indicates non-significance ($p > 0.05$) within females as determined by Bonferroni post-hoc pairwise comparisons

Table 1

Cohort Demographics and Anthropometric Characteristics.

	Females (n=121)	Males (n=143)	Range	p-value
Age (years)	14.4±2.6	14.1±2.7	8–18	0.318
Age Category (n[%])				
8–10 years	10(8.3)	13(9.1)		0.094
10–12 years	9(7.4)	24(16.8)		
12–14 years	35(28.9)	27(18.9)		
14–16 years	30(24.8)	40(28.0)		
16–18 years	37(30.6)	39(27.3)		
Race (n[%])				
Caucasian	88(72.7)	107(74.8)		0.925
Black	23(19.0)	23(16.1)		
American Indian	2(1.7)	4(2.8)		
Asian/Pacific Islander	21(7)	2(1.4)		
Other/Mixed	3(2.5)	2(1.4)		
Unknown	3(2.5)	5(3.5)		
Tanner Stage (n[%])				
I	12(9.9)	29(20.3)		0.010 [†]
II	7(5.8)	19(13.3)		
III	14(11.6)	19(13.3)		
IV	36(29.8)	26(18.2)		
V	52(42.9)	50(34.9)		
Obesity Status (n[%])				
Normal weight	81(66.9)	76(53.1)		0.065
Overweight	25(20.7)	40(28.0)		
Obese	15(12.4)	27(18.9)		
Body Mass (kg)	58.3±16.3	63.0±21.3	25.4–146.7	0.050 [*]
Height (cm)	159.7±10.0	164.9±14.5	129.6–189.6	0.001 [*]
BMI (kg/m²)	22.6±4.9	22.6±5.0	14.7–45.5	0.929
BMI-percentile	67.7±24.5	69.9±27.5	7.4–99.8	0.508
BMI z-score	1.02±0.75	0.87±1.32	−0.56–3.01	0.838
Body Fat (%)	31.1±8.8	22.6 ± 10.6	5.9–53.4	<0.001 [*]
Lean Mass (kg)	37.5±7.7	45.9±13.6	19.9–76.5	<0.001 [*]
Fat Mass (kg)	18.2±10.1	14.4±10.8	2.8–62.9	0.003 [*]
Baseline Artery Diameter (mm)	3.06±0.34	3.46±0.56	2.05–5.25	<0.001 [*]
Systolic Blood Pressure (mmHg)	107±9	112±11	84–142	0.001 [*]
Diastolic Blood Pressure (mmHg)	59±8	59±8	37–96	0.950
Mean Arterial Pressure (mmHg)	75±7	77±8	55–101	0.110
High Density Lipoprotein (mg/dL)	49.6±10.1	47.6±11.7	20–86	0.135
Low Density Lipoprotein (mg/dL)	81.8±22.2	84.4±23.4	32–163	0.285

	Females (n=121)	Males (n=143)	Range	p-value
Total Cholesterol (mg/dL)	147±25.2	147.8±26.6	92–220	0.950

Abbreviations: BMI, body mass index

Obesity status is presented in three categories: normal weight (5th to 85th BMI-percentile), overweight (85th to 95th BMI-percentile), and obese (95th BMI-percentile)

Age, height, body mass, BMI, BMI-percentile, BMI Z-score, body fat, lean mass, fat mass, brachial artery diameter, systolic blood pressure, diastolic blood pressure, mean arterial pressure and cholesterol are presented as mean ± SD

Age category, race, Tanner stage, and obesity status are presented as count (%)

* Indicates significance as determined by analysis of variance (ANOVA)

† Indicates significance as determined by Chi-squared test

Table 2

Endothelium-Independent Dilation in Males and Females.

	Females (n=121)	Males (n=143)	p-value
<i>Endothelium Independent Dilation – Peak Dilation (%)</i>			
Model 1	26.9±0.5 (25.7–28.1)	22.9±0.6 (21.7–24.0)	<0.001 [*]
Model 2	26.9±0.6 (25.8–28.1)	22.8±0.5 (21.7–23.9)	<0.001 [^]
Model 3	25.2±0.6 (24.2–26.3)	24.3±0.6 (23.3–25.2)	0.209
Model 4	25.2±20.6 (24.0–26.4)	24.3±0.5 (23.3–25.4)	0.308
<i>Endothelium Independent Dilation – Area Under the Curve (%·sec)</i>			
Model 1	4214±105 (4007–4422)	3398±97 (3207–3588)	<0.001 [*]
Model 2	4222±105 (4017–4428)	3388±96 (3198–3578)	<0.001 [^]
Model 3	3938±100 (3741–4135)	3630±92 (3450–3810)	0.033 [^]
Model 4	3883±106 (3675–4091)	3679±95 (3491–3866)	0.191

Reported as mean ± SE with 95% confidence interval

Model 1: Unadjusted

Model 2: Adjusted for race, age and BMI-percentile by ANCOVA

Model 3: Adjusted for race, age, brachial artery diameter, and BMI-percentile by ANCOVA

Model 4: Adjusted for race, age, brachial artery diameter, and %BF by ANCOVA

^{*} Indicates significance as determined by ANOVA[^] Indicates significance as determined by ANCOVA

Table 3

Endothelium-Independent Dilation by Obesity Status.

	Normal Weight I (n=54)	Normal Weight II (n=101)	Overweight (n=67)	Obese (n=42)	p-value
<i>Endothelium Independent Dilation – Peak Dilation (%)</i>					
<i>Total</i>	Model 1 26.3±1.0 (24.3–28.4)	25.2±0.6 (23.9–26.5)	24.4±0.8 (22.7–26.0)	22.1±1.1 (19.9–24.2) [§]	0.020 [*]
	Model 2 26.3±0.9 (24.6–28.1)	24.9±0.7 (23.6–26.2)	24.5±0.8 (23.0–26.1)	22.5±1.0 (20.4–24.5) [§]	0.039 [*]
	Model 3 25.3±0.8 (23.7–26.8)	24.3±0.6 (23.2–25.5)	25.2±0.7 (23.8–26.6)	24.2±0.9 (22.4–26.0)	0.636
<i>Females</i>	Model 1 28.1±1.3 (25.5–30.8)	27.4±0.8 (25.8–29.9)	26.4±0.9 (24.4–28.4)	24.1±1.8 (20.2–28.0)	0.179
	Model 2 28.2±1.2 (25.8–30.6)	27.4±0.8 (25.7–29.0)	26.5±1.01 (24.2–28.7)	24.0±1.5 (20.9–27.0)	0.163
	Model 3 27.8±1.1 (25.7–29.8)	26.8±0.7 (25.3–28.2)	26.7±1.0 (24.8–28.7)	26.3±1.4 (23.5–29.1)	0.827
<i>Males</i>	Model 1 24.3±1.6 (21.0–27.6)	22.8±0.9 (20.9–24.7)	23.2±1.2 (20.9–25.6)	20.9±1.2 (18.4–23.5)	0.352
	Model 2 24.5±1.3 (21.9–27.1)	22.8±1.0 (20.9–24.8)	22.9±1.1 (20.7–25.1)	21.1±1.3 (18.5–23.6)	0.341
	Model 3 22.9±1.2 (20.5–25.3)	22.3±0.9 (20.6–24.1)	23.7±1.0 (21.7–25.8)	22.4±1.2 (20.1–24.8)	0.745
<i>Endothelium Independent Dilation – Area Under the Curve (%sec)</i>					
<i>Total</i>	Model 1 3982±163 (3655–4309)	3768±123 (3524–4012)	3830±142 (3547–4113)	3423±201 (3015–3830)	0.162
	Model 2 3980±157 (3670–4289)	3713±115 (3487–3937)	3849±141 (3572–4127)	3527±179 (3174–3879)	0.256
	Model 3 3796±142 (3516–4077)	3619±103 (3415–3823)	3959±123 (3706–4207)	3816±165 (3492–4141)	0.235
<i>Females</i>	Model 1 4384±227 (3916–4852)	4251±159 (3921–4560)	4245±176 (3884–4607)	3784±310 (3119–4449)	0.413
	Model 2 4386±225 (3941–4831)	4233±154 (3841–4538)	4262±212 (3841–4681)	3776±290 (3202–4351)	0.411
	Model 3 4304±199 (3909–4699)	4127±137 (3855–4399)	4312±188 (3941–4684)	4194±266 (3667–4721)	0.830
<i>Males</i>	Model 1 3551±219 (3102–4001)	3241±159 (2932–3570)	3598±206 (3180–4016)	3218±248 (2707–3728)	0.419
	Model 2 3578±221 (3141–4016)	3266±163 (2944–3588)	3528±186 (3160–3896)	3261±219 (2828–3693)	0.546

	Normal Weight I (n=54)	Normal Weight II (n=101)	Overweight (n=67)	Obese (n=42)	p-value
Model 3	3333±212 (2913–38753)	3184±153 (2883–3486)	3655±175 (3308–4001)	3468±208 (3055–3880)	0.251

Reported as mean ± SE with 95% confidence interval

Normal weight I was classified as BMI-percentiles ranging from the 5th to the 45th percentile, and normal weight II was classified as BMI-percentiles ranging from the 45th to the 85th percentile

Model 1: Unadjusted

Model 2: Adjusted for race, age, and sex by ANCOVA

Model 3: Adjusted for race, age, sex, and brachial artery diameter by ANCOVA

* Indicates significant p-value

§ Indicates significantly different (p<0.05) compared to normal weight I as determined by Bonferroni post-hoc pairwise comparisons

Table 4

Multiple Linear Regression Examining the Associations of Age, Baseline Artery Diameter, BMI-Percentile, and %BF on EID%-peak.

	R²	β	SE	p-value
<i>Endothelium Independent Dilation – Peak Dilation (%): Model 1</i>				
	0.326			
Age (years)		0.24	0.15	0.105
Brachial Artery Diameter (mm)		−7.23	0.85	<0.001 *
Male Sex		−0.95	0.79	0.230
<i>Endothelium Independent Dilation – Peak Dilation (%): Model 2</i>				
	0.327			
Age (years)		0.23	0.15	0.136
Brachial Artery Diameter (mm)		−7.63	0.89	<0.001 *
Male Sex		−1.00	0.79	0.207
BMI-Percentile (%)		−0.004	0.01	0.761
<i>Endothelium Independent Dilation – Peak Dilation (%): Model 3</i>				
	0.330			
Age (years)		0.30	0.20	0.140
Brachial Artery Diameter (mm)		−6.46	1.31	<0.001 *
Male Sex		−1.43	1.50	0.343
%BF (%)		0.03	0.05	0.555

* Denotes significance as determined by multiple linear regression

Table 5

Multiple Linear Regression Examining the Associations of Age, Baseline Artery Diameter, BMI-Percentile, and %BF on EID%-AUC.

	R²	β	SE	p-value
<i>Endothelium Independent Dilation – Area Under the Curve (%·sec): Model 1</i>				
	0.307			
Age (years)		38.7	26.8	0.150
Brachial Artery Diameter (mm)		−1248.4	153.4	<0.001 [*]
Male Sex		−316.2	142.3	0.027 [*]
<i>Endothelium Independent Dilation – Area Under the Curve (%·sec): Model 2</i>				
	0.310			
Age (years)		45.1	27.6	0.103
Brachial Artery Diameter (mm)		−1294.3	160.3	<0.001 [*]
Male Sex		−305.4	143.5	0.034 [*]
BMI-Percentile (%)		2.7	2.6	0.302
<i>Endothelium Independent Dilation – Area Under the Curve (%·sec): Model 3</i>				
	0.296			
Age (years)		42.6	39.5	0.283
Brachial Artery Diameter (mm)		−1043.3	255.7	<0.001 [*]
Male Sex		−309.7	295.2	0.295
%BF (%)		15.3	10.5	0.148

^{*} Denotes significance as determined by multiple linear regression