Gender differences in vascular function and insulin sensitivity in young adults

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ABSTRACT

To examine influence of insulin resistance and other clinical risk factors for the MetS (metabolic syndrome) on vascular structure and function in young adults. This cross-sectional study was conducted in a cohort of young adults (mean age 22 years) and their siblings participating in a longitudinal study of cardiovascular risk (n = 370). Insulin sensitivity was determined by euglycaemic insulin clamp. EDD (endothelium-dependent dilation) was determined by flow-mediated dilation using high-resolution ultrasound imaging of the brachial artery. EID (endothelium-independent dilation) was determined by NTG (nitroglycerine)-mediated dilation. The diameter and cIMT (intima–media thickness) of the carotid artery were also measured. There was no significant difference between males and females for age or body mass index. However, males had significantly higher glucose and triacylglycerol (triglyceride) levels, while the females had significantly higher HDL-C (high-density lipoprotein-cholesterol) and insulin sensitivity (13.00 ± 0.33 compared with 10.71 ± 0.31 mg·kg·min⁻¹ of lean body mass·min⁻¹, P < 0.0001). Although peak EDD was significantly lower (6.28 ± 0.26 compared with 8.50 ± 0.28%, P < 0.0001) in males than females, this difference was largely explained by adjustment for brachial artery diameter (P = 0.15). Peak EID also was significantly lower in males than females (20.26 ± 0.44 compared with 28.64 ± 0.47%, P < 0.0001), a difference that remained significantly lower after adjustment for brachial artery diameter. Males had a significantly greater cIMT compared with females (males 0.420 ± 0.004 compared with males 0.444 ± 0.004 mm, P = 0.01), but when adjusted for carotid diameter, there was no significant difference (P = 0.163). Although there were gender differences in vascular function and structure in the young adult population examined in this study, many of the differences were eliminated simply by adjusting for artery diameter. However, the lower EID observed in males could not be explained by artery diameter. Future studies need to continue to examine influence of gender on EID and other measures of vascular function.

Key words: adolescent, cardiovascular risk, echocardiography, endothelium-derived dilation, gender, insulin sensitivity, young adulthood.

Abbreviations: BMI, body mass index; BP, blood pressure; cIMT, intima–media thickness of the carotid artery; CVD, cardiovascular disease; DBP, diastolic BP; EDD, endothelium-dependent dilation; EID, endothelium-independent dilation; GCRC, General Clinical Research Center; HDL-C, high-density lipoprotein-cholesterol; lbm, lean body mass; LDL-C, low-density lipoprotein-cholesterol; MetS, metabolic syndrome; NTG, nitroglycerine; SBP, systolic BP; WCSA, cross-sectional area of the carotid artery wall.

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INTRODUCTION

Insulin resistance is generally considered to be relevant to CVD (cardiovascular disease) risk [1]. Research studies in adults have provided support for the role of insulin resistance in the development of the MetS (metabolic syndrome) [2]. Recently, we demonstrated in a longitudinal study in adolescents that there are significant differences between males and females in the changes in cardiovascular risk factors and insulin resistance during the transition from late childhood through adolescence [3]. While males had a significant increase in insulin resistance and triacylglycerols (triglycerides) and significant decrease in HDL-C (high-density lipoprotein-cholesterol), the changes in females were in the opposite direction. BP (blood pressure) increased in both genders, but the rate of increase was significantly greater in males.

Studies by us and others have also found early changes in arterial structure and function in adolescents, with increased cIMT (carotid intima–media thickness) [4,5], significantly lower EDD (endothelium-dependent dilation) [4–8] and EID (endothelium-independent dilation) [6,9] in obese adolescents.

To date, studies of vascular structure and function in adolescents have mostly explored the link with obesity. The fact that obesity is also linked to insulin resistance suggests a role for insulin sensitivity in the development of endothelial dysfunction. The present study was conducted in a large cohort of individuals participating in a longitudinal study on the development of insulin resistance and CV risk. In this cohort, we examined whether the development of insulin resistance signals the presence of vascular dysfunction (endothelial and/or smooth muscle). In addition, we sought to examine gender differences in vascular structure and function in this population of young adults.

MATERIALS AND METHODS

The present study was approved by the Institutional Review Board Human Subjects Committee of the University of Minnesota. Consent was obtained from the children and their parents/guardians.

The initial cohort was randomly selected in 1995 after BP screening of 12,043 fifth- to eighth-grade Minneapolis, MN public school children (93% of eligible children in those grades), with stratification according to gender, race (black and non-Hispanic white) and SBP (systolic BP) percentile (half from the upper 25 percentiles and half from the lower 75 percentiles to enrich the study population with potentially higher risk children), for participation in a longitudinal study of the development of CV risk. After enrolment of the initial cohort, their siblings were enrolled [3]. Data for the present study was obtained between 2004 and 2008, the most recent examination of the cohort.

Participants were seen in a clinic dedicated to this study for anthropometric and BP measurements. Height was measured with a wall-mounted stadiometer, and weight was measured using a balance scale. BMI (body mass index) was calculated as the weight (kg) divided by the height (meters) squared. BP was measured with a random-zero sphygmomanometer on the right arm of seated subjects, and the average of two SBP and fifth-phase Korotkoff DBP (diastolic BP) measurements were analysed.

The vascular studies were conducted in the GCRC (General Clinical Research Center) of the University of Minnesota. Vascular structure and function were measured in a quiet, temperature-controlled environment. Vascular images were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions) with an 8.0-MHz linear array probe held in place by a stereotactic device. 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 ECG (end-diastolic diameter) then digitized and stored on a personal computer for later offline analysis using electronic wall-tracking software (Vascular Research Tools 5, Medical Imaging Application; LLC). All image files were averaged over a 20-s period, and peak dilation during each study was defined as the greatest percentage change from resting baseline brachial artery diameter. Digital image analysis was performed by the same trained reader blinded to group assignments.

The diameter and cIMT were measured in the common carotid segment approx. 5–11 cm proximal to the carotid bulb over a period of 10 s, while the subject rested in the supine position with the head rotated 45° from neutral. WCSA (cross-sectional area of the carotid artery wall) was calculated using the formula of Linhart et al. [10].

Assessment of flow-mediated EDD was performed by imaging the left brachial artery at the distal third of the upper arm using techniques previously described in our laboratory and by others [11,12]. After measuring resting artery diameter, a BP cuff was inflated below the elbow (distal to imaged artery segment) to a pressure of 200 mmHg maintained for 5 min to induce muscle ischaemia. Brachial artery diameter was measured continuously for a 3-min period immediately after cuff release during reactive hyperaemia to determine peak EDD (the greatest percent change from resting baseline brachial artery diameter following reactive hyperaemia during the 3-min collecting period). After a 15-min rest, 0.4 mg sublingual NTG (nitroglycerine) was administered, and the diameter of the brachial artery was continuously measured for a 15-min period post-NTG administration. Peak NTG-mediated EID was defined...
as the highest percent change from resting baseline brachial artery diameter following NTG administration during the 15-min collection period. Interindividual transducer placement matching between baseline and follow-up measurements for the carotid artery was assured through measurement between the transducer and specific external anatomical landmarks as well as the image relationship with the carotid bulb. The interindividual transducer placement matching between the baseline and follow-up measurements for the brachial artery was assured through measurement between the transducer and specific external anatomical landmarks as well as comparison of resting arterial diameters. Reproducibility of the cIMT and EDD techniques in our laboratory have shown a mean difference of 0.02 ± 0.03 % and 0.39 ± 0.65 %, respectively, for analysis separated by 1 week in healthy young adults.

Participants were seen on a separate day in the GCRC after a 10- to 12-h overnight fast for a euglycaemic insulin clamp, as previously described [13]. Briefly, insulin was infused at a rate of 1 mU/kg per min for 3 h. A variable infusion of 20 % glucose was adjusted to maintain plasma insulin infused at a rate of 1 mU/kg per min for 3 h. A variable clamp, as previously described [13]. Briefly, insulin was infused at a rate of 1 mU/kg per min for 3 h. A variable clamp, corrected for lbm (lean body mass) and expressed to maintain euglycaemia over the final 40 min of the period of the study.

TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n = 197)</th>
<th>Female (n = 173)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity (o) (white/black)</td>
<td>153/44</td>
<td>145/28</td>
<td>0.137</td>
</tr>
<tr>
<td>Age (year)</td>
<td>21.8 ± 1</td>
<td>21.7 ± 1</td>
<td>0.359</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.1 ± 0.5</td>
<td>165.8 ± 0.5</td>
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<tr>
<td>Weight (kg)</td>
<td>89.2 ± 1.2</td>
<td>86.1 ± 1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 0.5</td>
<td>26.5 ± 0.5</td>
<td>0.454</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.2 ± 1.2</td>
<td>86.1 ± 1.3</td>
<td>0.072</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.7 ± 0.8</td>
<td>37.3 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>112 ± 1</td>
<td>106 ± 1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP</td>
<td>66 ± 1</td>
<td>64 ± 1</td>
<td>0.095</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>86.1 ± 0.6</td>
<td>83.1 ± 0.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (µ units/ml)</td>
<td>9.2 ± 0.8</td>
<td>8.3 ± 0.9</td>
<td>0.499</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>163.9 ± 2.2</td>
<td>159.5 ± 2.3</td>
<td>0.169</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>43.9 ± 0.8</td>
<td>48.0 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>98.2 ± 1.9</td>
<td>94.0 ± 1.0</td>
<td>0.124</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>110.5 ± 4.3</td>
<td>87.7 ± 4.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Statistical analyses
Results are expressed as means ± S.E.M. To examine differences between males and females, a univariate general linear model using gender as a grouping variable was used (SPSS). All analyses were adjusted for age, race and BMI. EDD and EID measures were adjusted for baseline brachial artery diameter, and cIMT was adjusted for the carotid artery diameter. Pearson correlation coefficients were used to assess the relationship between measures of vascular function and body composition and metabolic variables, controlling for baseline artery diameter. To examine the possible effects of family relationship on the results, data were also analysed only for the initial participants without inclusion of siblings. Comparable results were seen in initial participants-only analysis as with the inclusion of siblings; therefore, only the results of the initial participants and their siblings are reported in this manuscript. A P value of 0.05 was used to signify statistical significance.

RESULTS

Table 1 compares the physical characteristics and laboratory studies between the male and female participants. There was no significant difference between genders for age or ethnicity. As expected, the males were heavier and taller than their female counterparts, and the females had a higher percent body fat than the males. However, there was no significant difference in BMI or waist circumference between males and females. DBP was not different between the genders, but males had a significantly higher SBP. Males
had significantly higher glucose and triacylglycerol levels, while the females had significantly higher levels of HDL-C. Fasting insulin, total cholesterol and LDL-C (low-density lipoprotein-cholesterol) levels were not significantly different. Females had a significantly higher $M_{fbm}$ ($13.00 \pm 0.33$ compared with $10.71 \pm 0.31$ mg kg$^{-1}$ h$^{-1}$, $P < 0.0001$). Given the gender differences in SBP and HDL-C, the males had a significantly higher ($0.144 \pm 0.010$ compared with $-0.200 \pm 0.041$, $P < 0.0001$) cluster score than the females (Table 1).

Results from the vascular studies are shown in Table 2. Although there was a significant difference in peak EDD between males and females, when EDD was adjusted for baseline brachial artery diameter, there was no longer a significant gender difference (Figure 1). Peak EID was also significantly lower in males than females (Table 2). However, after adjustment for baseline brachial artery diameter, the males continued to have significantly lower EID values than females (Figure 1). No risk factors were significantly related to peak EDD, and only triacylglycerols were significantly related to peak EID ($r = -0.120; P = 0.047$) (Table 3).

Males had a significantly greater cIMT in the common carotid artery compared with females (Table 2). This gender difference was attenuated, and no longer significant after adjustment for the baseline carotid diameter cIMT was related to cluster score ($r = 0.139$, $P = 0.01$) (Table 3). Although cIMT was not significantly related to peak EDD ($r = -0.029$, $P = 0.590$), it was significantly related to peak EID ($r = -0.183$, $P = 0.001$).

### DISCUSSION

This is the first study to examine gender differences in both EDD and EID in young adults. It confirms data from previous studies [16,17] in young adults showing significantly lower EDD in males compared with females that disappeared after adjustment for baseline brachial artery diameter. Thijssen et al. [18] examined the impact of artery size in different arteries (e.g. brachial artery, popliteal artery, superficial femoral artery, common femoral artery) within individuals and reported that artery size within individuals influences EDD. Providing further evidence that differences in EDD observed between males and females in the present study were a function of baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted mean</th>
<th>$P$ value</th>
<th>Adjusted mean*</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brachial artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>Male 4.04 ± 0.03</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EDD peak (%)</td>
<td>Male 6.28 ± 0.28</td>
<td>6.97 ± 0.31</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EID peak (%)</td>
<td>Male 8.50 ± 0.26</td>
<td>7.33 ± 0.34</td>
<td>0.150</td>
<td>—</td>
</tr>
<tr>
<td><strong>Carotid artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>Male 6.21 ± 0.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>cIMT (mm)</td>
<td>Male 0.444 ± 0.004</td>
<td>0.441 ± 0.004</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>WCSA (mm$^2$)</td>
<td>Male 9.30 ± 0.10</td>
<td>8.90 ± 0.08</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Female 5.66 ± 0.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Female 0.420 ± 0.004</td>
<td>0.433 ± 0.004</td>
<td>0.163</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Male 8.24 ± 0.11</td>
<td>—</td>
<td>8.70 ± 0.09</td>
<td>0.133</td>
</tr>
</tbody>
</table>
| | Female 0.33 compared with 0.33 ± 0.04 compared with 0.200 ± 0.041, $P < 0.0001$) cluster score than the females (Table 1).

Results from the vascular studies are shown in Table 2. Although there was a significant difference in peak EDD between males and females, when EDD was adjusted for baseline brachial artery diameter, there was no longer a significant gender difference (Figure 1). Peak EID was also significantly lower in males than females (Table 2). However, after adjustment for baseline brachial artery diameter, the males continued to have significantly lower EID values than females (Figure 1). No risk factors were significantly related to peak EDD, and only triacylglycerols were significantly related to peak EID ($r = -0.120; P = 0.047$) (Table 3).

Males had a significantly greater cIMT in the common carotid artery compared with females (Table 2). This gender difference was attenuated, and no longer significant after adjustment for the baseline carotid
brachial diameter. Early studies [16,17] in male and female young adults did not measure EID; however, in the present study, we observed that EID was significantly lower in young adult males than similar aged females. Thijsen et al. [18] examined the influence of artery size in different arterial bed in the same individuals on EID and reported that EID was also influenced by arterial size. Unfortunately, Thijsen et al. [18] did not examine the influence of gender on the relationship between artery size and EID. In the present study, even after correcting for the size of the artery, a significant difference in EID between males and females still existed. The significance of this finding is unclear, but it may suggest the beginning of cardiovascular changes in young adult males.

Clearly, other factors besides artery size may be involved in vascular function. The Framingham study [19] reported a heritability estimate of 0.14 for EDD, which is similar to the Northern Manhattan Family Study [20] in Caribbean Hispanic families, which reported a heritability estimate of 0.17. To our knowledge, the effects of genetics on EID have not been examined. It is possible that genetics accounts for a similar amount of the variability in EID as it does in EDD. However, interaction between genetics and gender has yet to be fully determined in either EDD or EID.

Along with the gender differences in EID, we also observed that males in the present study had lower insulin sensitivity than their female counterparts. Other studies have reported that adult men display a greater degree of insulin resistance than women [3,21,22]. Recently, it was reported that insulin resistance increased in males during the transition from late childhood (at 13 years of age) through adolescence (at 19 years of age) [3] in association with increased triacylglycerols and decreased HDL-C, despite a concurrent reduction in body fatness. The opposite occurred in females [3]. BMI was not significantly different between males and females at this age, consistent with data from this cohort through adolescence [3]. In general, BMI represents fatness and is highly correlated with other fat measurements, but there are significant differences in percentage fatness between males and females that become magnified during the adolescent years [3].

Although most research examining vascular function and CVD has focused on EDD, there is evidence that cardiovascular risk factors might impair EID as well [23–25]. In a large cohort study of asymptomatic adults, a significant impairment in EID was found in those individuals with known risk factors for atherosclerosis [23]. Similarly, impaired EID was seen in adult patients who had experienced a cardiovascular event compared with patients who had not [25]. In a cohort of healthy 35-year-old males and females, gender had the greatest influence on both EDD and EID [26].

The exact mechanism underlying the difference in EID between males and females is not understood. However, the gender-related differences in EID may be mediated via differences in HDL-C levels. It has been reported that individuals with low HDL-C concentrations have significantly impaired EID [27]. HDL-C has been shown to inhibit lipoprotein oxidation [28] and may act as an antioxidant to protect the vascular smooth muscle from the effects of oxidative stress. It is generally recognized that individuals with low HDL-C have significantly higher triacylglycerols and higher BPs, similar to what we observed in the males in the present study. The clustering of elevated BP and triacylglycerol levels with low HDL-C levels has been associated with an increased risk of CVD [29]. It may well be that combined effects of higher BP, declining sensitivity to insulin and a worsened lipid profile work together to impair smooth muscle function in young adult males.

It is also possible that the differences in EID between males and females may be related to hormonal differences between the genders [30]. Both endothelial and smooth muscle cells in vascular beds have receptors for oestrogen, progesterone and testosterone [30]. Physiological levels of 17β-oestradiol produce EID in human coronary arteries [31], and oestrogen administration caused vasodilation in de-endothelialized porcine coronary arteries that were precontracted with prostaglandin [32]. The observed gender differences in smooth muscle function may also be related to the number of sex hormone receptors. Females have higher numbers of oestrogen receptors in their arteries than males and therefore may be more sensitive to vasodilators than their male counterparts [33]. The effect of oestrogen on receptors in smooth muscle also may be age-related, with greater sensitivity in young as opposed to postmenopausal women. EID has been reported to decline with advancing age in postmenopausal women [34] and may explain why acute [34] and chronic [35] oestrogen replacement has no effect on EID in the postmenopausal population.

Males in the present study had an increased cIMT compared with females. In a similar fashion to the differences in EDD, this gender difference in cIMT was explained by differences in baseline carotid artery diameter, as reported previously [16]. An earlier longitudinal study [36] reported that cIMT was not different between boys and girls under 18 years of age, but after the age of 18 years, males had an increased cIMT compared with females. However, that study did not control for menstrual cycle by testing our female
participants during the same phase of their menstrual cycle. Given the large number of subjects tested, this would not have been possible. A number of investigators [38–42] have reported that EDD is higher during the follicular phase than either luteal or menstrual phases. The effect of the menstrual cycle on EID has only been reported in two studies [40,41], both reported that there were no significant differences in EID during the different phases of the menstrual cycle. We also did not control for oral contraceptives. However, Virdis et al. [43] studied the effect of third-generation oral contraceptives on vascular function in healthy young women and found that endothelial function remained unchanged after 6 months of oral contraceptives use.

Finally, we did not measure the postdeflation shear rate in our study. Although a few studies [44–47] have suggested that EDD be corrected for postdeflation shear rate, a recent study by Thijssen et al. [48] reported that postdeflation shear rate accounts for only 9% of the magnitude in EDD. The authors went on to suggest that although the measurement and analysis of postdeflation shear rate is important, the validity of normalizing EDD may be misleading and questionable at best. To date, no data have been published on gender differences in postdeflation shear rate. Future studies need to be done to determine the influence of gender on this variable.

In conclusion, previous reports from this cohort on the natural history of CV risk factors from childhood through adolescence to young adulthood have shown a clear pattern of change, with a significant increase in BP, triacylglycerols and insulin resistance and a significant decrease in HDL-C in males compared with females [3]. The present study adds to these earlier studies by showing significantly lower EID in males compared with females. Although a relationship between vascular function and structure and either insulin resistance or the cardiovascular risk factors might also have been expected based on the previous changes noted in this cohort, none were observed in the present study. In fact, many of the differences in vascular function and structure were eliminated simply by adjusting for baseline artery diameter. However, it should be noted that the decreased vascular smooth muscle function (EID) observed in males could not be explained by artery diameter.

**AUTHOR CONTRIBUTION**

Donald Dengel conducted all measurements of vascular structure and function, and prepared the manuscript. David Jacobs, Jr performed the statistical analysis of the data and assisted in the preparation of the manuscript. Julia Steinberger, Antoinette Moran and Alan Sinaiko recruited the subjects, performed the metabolic studies of insulin sensitivity and assisted in the preparation of the manuscript.

**FUNDING**

This work was supported by the National Institutes of Health [grant numbers HL-52851 and MO1-RR-00400 (to the General Clinical Research Centers)].

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Received 21 April 2010/5 August 2010; accepted 3 September 2010
Published as Immediate Publication 3 September 2010, doi:10.1042/CS20100223