Published in final edited form as:

Clin Sci (Lond). 2011 February ; 120(4): 153–160. doi:10.1042/CS20100223.

Gender differences in vascular function and insulin sensitivity in young adults

Donald R. DENGEL^{*,†,‡}, David R. JACOBS Jr[§], Julia STEINBERGER[‡], Antoinette M. MORAN[‡], and Alan R. SINAICO[‡]

^{*}School of Kinesiology, University of Minnesota, Minneapolis, MN 55455, U.S.A

[†]Minneapolis Veterans Affairs Medical Center, Minneapolis, MN 55417, U.S.A

[‡]Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455, U.S.A

[§]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN 55455, U.S.A

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⁻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 (females 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

Correspondence: Dr Donald R. Dengel (denge001@umn.edu).

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.

studies need to continue to examine influence of gender on EID and other measures of vascular function.

Keywords

adolescent; cardiovascular risk; echocardiography; endothelium-derived dilation; gender; insulin sensitivity; young adulthood

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 12043 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 $0.02 \pm 0.03\%$ and $0.39 \pm 0.65\%$, 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 glucose at 100 mg/dl (5.6 mmol/l). Insulin sensitivity (M) was determined from the amount of glucose required to maintain euglycaemia over the final 40 min of the clamp, corrected for lbm (lean body mass) and expressed as M_{lbm} (mg of glucose uptake·kg⁻¹ lean body mass·min⁻¹). Plasma glucose was measured immediately at the bedside with a Beckman Glucose Analyzer II (Beckman Instruments). Fasting plasma insulin levels were obtained at baseline (10 and 5 min before starting the insulin infusion) and averaged. Insulin levels were measured in the University of Minnesota Fairview Laboratory by a chemiluminescence solid phase immunometric assay (Immulite; Diagnostic Products). Immediately after inserting the intravenous lines for the clamp study, venous blood was drawn from an antecubital vein for lipid studies and analysed by the University of Minnesota Fairview Laboratory, as described previously [13].

Body composition was measured by DXA (dual energy X-ray absorptiometry) (version 6.7, Prodigy; 3M). The total body scans were performed using a fast transverse speed mode, over approximately 10–15 min. The scanner was calibrated monthly with known phantoms, and no machine drift was noted during the period of the study.

To examine the role of the combined association of the CV risk factors, a cluster score was created [14,15]. Briefly, a z-score was calculated for fasting insulin, SBP, triacylglycerols and HDL-C by determining the difference between each participant's value for the respective variable and the gender-specific mean value for that variable and then dividing the result by the corresponding S.D. The average of the z-scores for the four variables was computed (with reversed sign for HDL-C) and defined as the cluster score.

Statistical analyses

Results are expressed as means $\pm$ 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_{lbm} (13.00 ± 0.33 compared with 10.71 ± 0.31 $\text{mg}\cdot\text{kg}^{-1}\text{lbm}\cdot\text{min}^{-1}$, $P < 0.0001$). Given the gender differences in SBP and HDL-C, the males had a significantly higher (0.144 ± 0.010 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 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 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 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. Thijssen 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, Thijssen 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 correct the cIMT for carotid artery diameter, and the differences in cIMT may be explained by the larger vessel diameter observed in males. Gender differences in cIMT may also be affected by differences in sex hormones. Hormone replacement therapy has been associated with a decrease in cIMT in women [37], suggesting that the decreased cIMT in young adult women may be related to hormonal differences.

One limitation of the present study is that we 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, Viridis 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.

Acknowledgments

FUNDING

This work was supported by the National Institutes of Health [grant numbers HL-52851 and M01-RR-00400 (to the General Clinical Research Centers)].

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

References

1. National Cholesterol Education (NCEP). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III): Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002; 106:3143–3421. [PubMed: 12485966]

2. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care*. 2004; 29:2444–2449.
3. Moran A, Jacobs DR, Steinberger J, Steffen LM, Pankow JS, Hong CP, Sinaiko AR. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. *Circulation*. 2008; 117:2361–2368. [PubMed: 18427135]
4. Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. *Int J Obes Relat Metab Disord*. 2004; 28:852–857. [PubMed: 15170465]
5. Meyer AA, Kundt G, Lenschow U, Schuff-Werner P, Kienast W. Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. *J Am Coll Cardiol*. 2006; 48:1865–1870. [PubMed: 17084264]
6. 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. *Lancet*. 2001; 358:1400–1404. [PubMed: 11705484]
7. Watts K, Beye P, Siafarikas A, Davis EA, Jones TW, O'Driscoll G, Green DJ. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. *J Am Coll Cardiol*. 2004; 43:1823–1827. [PubMed: 15145107]
8. Watts K, Beye P, Siafarikas A, O'Driscoll G, Jones TW, Davis EA, Green DJ. Effects of exercise training on vascular function in obese children. *J Pediatr*. 2004; 144:620–625. [PubMed: 15126996]
9. Dengel DR, Kelly AS, Steinberger J, Sinaiko AR. Effect of oral glucose loading on endothelial function in normal and overweight children. *Clin Sci*. 2007; 112:493–498. [PubMed: 17173543]
10. Linhart A, Garipey J, Giral P, Levenson J, Simon A. Carotid artery and left ventricular structural relationship in asymptomatic men at risk for cardiovascular disease. *Atherosclerosis*. 1996; 127:103–112. [PubMed: 9006810]
11. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan D, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992; 340:1111–1115. [PubMed: 1359209]
12. Kelly AS, Kaiser DR, Dengel DR, Bank AJ. Comparison of B-mode and echo tracking methods of assessing flow-mediated dilation. *Ultrasound Med Biol*. 2004; 30:1447–1449. [PubMed: 15588955]
13. Sinaiko AR, Jacobs DR, Steinberger J, Moran A, Luepker R, Rocchini AP, Prineas RJ. Insulin resistance syndrome in childhood: associations of the euglycemic insulin clamp and fasting insulin with fatness and other risk factors. *J Pediatr*. 2001; 139:700–707. [PubMed: 11713450]
14. Sinaiko AR, Steinberger J, Moran A, Prineas RJ, Vessby B, Basu S, Tracy R, Jacobs DR. Relation of body mass index and insulin resistance to cardiovascular risk factors, inflammatory factors, and oxidative stress during adolescence. *Circulation*. 2005; 111:1985–1991. [PubMed: 15837953]
15. Sinaiko AR, Steinberger J, Moran A, Hong CP, Prineas RJ, Jacobs DR. Influence of insulin resistance and body mass index at age 13 on systolic blood pressure, triglycerides, and high-density lipoprotein cholesterol at age 19. *Hypertension*. 2006; 48:730–736. [PubMed: 16923995]
16. Juonala M, Kahonen M, Laitinen T, Hutri-Kahonen N, Jokinen E, Taittonen L, Pietikainen M, Helenius H, Viikari JSA, Raitakari OT. Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: The cardiovascular risk in Young Finns Study. *Eur Heart J*. 2008; 29:1198–1206. [PubMed: 18079136]
17. Kapuku GK, Treiber FA, Hartley B, Ludwig DA. Gender influences endothelial-dependent arterial dilation via arterial size in youth. *Am J Med Sci*. 2004; 327:305–309. [PubMed: 15201641]
18. Thijssen DHJ, Dawson EA, Black MA, Hopman MTE, Cable NT, Green DJ. Heterogeneity in conduit artery function in humans: impact of arterial size. *Am J Physiol Heart Circ Physiol*. 2008; 295:H1927–H1934. [PubMed: 18775852]
19. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, Llehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilatation in the community. *Circulation*. 2004; 109:613–619. [PubMed: 14769683]
20. Suzuki K, Juo SHH, Rundek T, Boden-Albala B, Disla N, Liu R, Park N, DiTullio MR, Sacco RL, Homma S. Genetic contribution to brachial artery flow-mediated dilation: The Northern Manhattan Family Study. *Atherosclerosis*. 2008; 197:212–216. [PubMed: 17462653]

21. Donahue RP, Prineas RJ, Donahue RD, Bean JA, Skylar JS. The female “insulin advantage” in a biracial cohort: results from the Miami Community Health Study. *Int J Obesity*. 1996; 20:76–82.
22. Sumner AE, Kushner H, Sherif KD, Tulenko TN, Falkner B, March JB. Sex differences in African-Americans regarding sensitivity to insulin’s glucoregulatory and antilipolytic actions. *Diabetes Care*. 1999; 22:71–77. [PubMed: 10333906]
23. Adams MR, Robinson J, McCredie R, Searle JP, Sorensen KE, Deanfield JE, Celermajer DS. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol*. 1998; 32:123–127. [PubMed: 9669259]
24. Bhagat K, Hingorani A, Vallance P. Flow associated or flow mediated dilatation? More than just semantics. *Heart*. 1997; 78:7–8.
25. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001; 104:2673–2678. [PubMed: 11723017]
26. Jensen-Urstad K, Johansson J, Jensen-Urstad M. Vascular function correlates with risk factors for cardiovascular disease in healthy population of 35-year-old subjects. *J Intern Med*. 1997; 241:507–513. [PubMed: 10497627]
27. Toikka JO, Ahotupa M, Viikari JSA, Niinikoski H, Taskinen MR, Irjala K, Hartiala JJ, Raitakari OT. Constantly low HDL-cholesterol concentration relates to endothelial dysfunction and increased *in vivo* LDL-oxidation in healthy young men. *Atherosclerosis*. 1999; 147:133–138. [PubMed: 10525134]
28. Parthasarathy S, Barnett J, Fong LG. High density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochem Biophys Acta*. 1990; 1044:275–283. [PubMed: 2344447]
29. Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, Heinonen OP, Frick MH. Joint effects of serum triglycerides and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study – implications for treatment. *Circulation*. 1992; 85:37–45. [PubMed: 1728471]
30. Thompson J, Khalil RA. Gender differences in regulation of vascular tone. *Clin Exp Pharmacol Physiol*. 2003; 30:1–15. [PubMed: 12542447]
31. Mugge A, Riedel M, Barton M, Kuhn M, Lichtlen PR. Endothelium independent relaxation of human coronary arteries by 17β -oestradiol *in vitro*. *Cardiovasc Res*. 1993; 27:1939–1942. [PubMed: 8287400]
32. Crews JK, Khalil RA. Antagonistic effects of 17β -estradiol, progesterone and testosterone on Ca^{2+} entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol*. 1999; 19:1034–1040. [PubMed: 10195933]
33. Collins P, Rosano GM, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, McNeill JG, Poole-Wilson PA. 17β -Estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation*. 1995; 95:24–30.
34. Sherwood A, Bower JK, McFetridge-Durdle J, Blumenthal JA, Newby LK, Hinderliter AL. Age moderates the short-term effects of transdermal 17β -estradiol on endothelium-dependent vascular function in postmenopausal women. *Arterioscler Thromb Vas Biol*. 2007; 27:1782–1787.
35. Bush DE, Jones CE, Bass KM, Walters GK, Bruza JM, Ouyang P. Estrogen replacement reverses endothelial dysfunction in postmenopausal women. *Am J Med*. 1998; 104:552–558. [PubMed: 9674718]
36. Zannad F, Sass C, Visvikis S. Environment and genetic determinants of intima-media thickness of the carotid artery. *Clin Exp Pharmacol Physiol*. 2001; 28:1007–1010. [PubMed: 11903304]
37. Westendorp ICD, In’t Veld BA, Bots ML, Akkerhuis JM, Hofman A, Grobbee DE, Witteman JCM. Hormone replacement therapy and intima media thickness of the common carotid artery. *Stroke*. 1999; 30:2562–2567. [PubMed: 10582978]
38. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation*. 1995; 92:3431–3435. [PubMed: 8521564]
39. Kawano H, Motoyama T, Kugiyama K, Hirashima O, Ohgushi M, Yoshimura M, Ogawa H, Okumura K, Yasue H. Menstrual cyclic variation of endothelium-dependent vasodilation of the

- brachial artery: possible role of estrogen and nitric oxide. *Proc Assoc Am Physicians*. 1996; 108:473–480. [PubMed: 8956371]
40. Williams MRI, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, Komesaforr PA. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab*. 1998; 86:5389–5395.
41. English JL, Jacobs LO, Green G, Andrews TC. Effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in normal young women. *Am J Cardiol*. 1998; 82:256–258. [PubMed: 9678305]
42. Adkisson EJ, Casey DP, Beck DT, Gurovich AN, Martin JS, Braith RW. Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. *Experiment Biol Med*. 2010; 235:111–118.
43. Virdis A, Pinto S, Versari D, Salvetti G, Bernini G, Fruzzetti F, Genazzin AR, Taddei S, Salvetti A. Effect of oral contraceptives on endothelial function in the peripheral microcirculation of healthy women. *J Hypertens*. 2003; 21:2275–2280. [PubMed: 14654747]
44. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF Jr, Keyes MJ, Levy D, Vasan RS, Benjamin EJ. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension*. 2004; 44:134–139. [PubMed: 15249547]
45. Pyke KE, Dwyer EM, Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J Appl Physiol*. 2004; 97:499–508. [PubMed: 15064302]
46. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol*. 2005; 568:357–369. [PubMed: 16051630]
47. Pyke KE, Tschakovsky ME. Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *J Appl Physiol*. 2007; 102:1510–1519. [PubMed: 17170205]
48. Thijssen DH, Bullens LM, van Bommel MM, Dawson EA, Hopkins N, Tinken TM, Black MA, Hopman MT, Cable NT, Green DJ. Does arterial shear explain the magnitude of flow-mediated dilation? : a comparison between young and older humans. *Am J Physiol Heart Circ Physiol*. 2009; 296:H57–H64. [PubMed: 19028795]

Figure 1. EDD and EID in males and females

Values are adjusted (age, BMI, gender, race and brachial artery diameter) means $\pm$ S.E.M.

Table 1

Physical characteristics and laboratory studies of subjects (adjusted by race and age)

Parameter	Male (<i>n</i> = 197)	Female (<i>n</i> = 173)	<i>P</i> value
Ethnicity (<i>n</i>) (white/black)	153/44	145/28	0.137
Age (year)	21.8 ± 0.1	21.7 ± 0.1	0.359
Height (cm)	180.1 ± 0.5	165.8 ± 0.5	<0.0001
Weight (kg)	89.2 ± 1.2	86.1 ± 1.3	<0.0001
BMI (kg/m ²)	26.0 ± 0.5	26.5 ± 0.5	0.454
Waist circumference (cm)	89.2 ± 1.2	86.1 ± 1.3	0.072
Body fat (%)	21.7 ± 0.8	37.3 ± 0.8	<0.0001
BP (mmHg)			
SBP	112 ± 1	106 ± 1	<0.0001
DBP	66 ± 1	64 ± 1	0.095
Glucose (mg/dl)	86.1 ± 0.6	83.1 ± 0.7	0.001
Insulin (μ-units/ml)	9.2 ± 0.8	8.3 ± 0.9	0.499
Total cholesterol (mg/dl)	163.9 ± 2.2	159.5 ± 2.3	0.169
HDL-C (mg/dl)	43.9 ± 0.8	48.0 ± 0.8	<0.0001
LDL-C (mg/dl)	98.2 ± 1.9	94.0 ± 2.0	0.124
Triacylglycerols (mg/dl)	110.5 ± 4.3	87.7 ± 4.6	<0.0001

Table 2

Mean ($\pm$ S.E.M.) and adjusted mean ($\pm$ S.E.M.) measures of vascular function (brachial artery) and structure (carotid artery)

Parameter	Unadjusted mean	P value	Adjusted mean*	P value
Brachial artery				
Diameter (mm)				
Male	4.04 $\pm$ 0.03		–	
Female	3.17 $\pm$ 0.03	<0.0001	–	–
EDD peak (%)				
Male	6.28 $\pm$ 0.28		6.97 $\pm$ 0.31	
Female	8.50 $\pm$ 0.26	<0.0001	7.73 $\pm$ 0.34	0.150
EID peak (%)				
Male	20.26 $\pm$ 0.44		22.66 $\pm$ 0.47	
Female	28.64 $\pm$ 0.47	<0.0001	25.91 $\pm$ 0.52	<0.0001
Carotid artery				
Diameter (mm)				
Male	6.21 $\pm$ 0.04			
Female	5.66 $\pm$ 0.04	<0.0001		
cIMT (mm)				
Male	0.444 $\pm$ 0.004		0.441 $\pm$ 0.004	
Female	0.420 $\pm$ 0.004	0.01	0.433 $\pm$ 0.004	0.163
WCSA (mm ²)				
Male	9.30 $\pm$ 0.10		8.90 $\pm$ 0.08	
Female	8.24 $\pm$ 0.11	<0.0001	8.70 $\pm$ 0.09	0.133

* Adjusted for brachial or carotid artery diameter.

Table 3

Correlation (r) between risk factor variables and measures of vascular structure and function for male and females combined

Variable	Correlation (r)		
	cIMT	Peak EDD	Peak EID
SBP	0.062	−0.039	−0.092
DBP	0.041	−0.027	0.068
LDL-C	−0.049	0.001	0.010
HDL-C	−0.064	−0.094	0.079
Triacylglycerols	0.085	−0.021	−0.120 *
Insulin	0.025	0.112	0.045
Glucose	−0.059	0.013	−0.100
M_{bm}	−0.020	−0.060	0.062
Cluster score	0.139 **	0.040	−0.093

cIMT was adjusted for age, BMI, gender, race and carotid artery diameter. Peak EDD and EID were adjusted for age, BMI, gender, race and brachial artery diameter.

* $P < 0.05$ and

** $P < 0.01$.