



Published in final edited form as:

Int J Obes (Lond). 2009 October ; 33(10): 1118–1125. doi:10.1038/ijo.2009.164.

Gender Differences in the Relationships Among Obesity, Adiponectin and Brachial Artery Distensibility in Adolescents and Young Adults

Elaine M. Urbina, M.D.^{a,d}, Philip Khoury, M.S.^{a,d}, Lisa J. Martin, Ph.D.^{b,d}, David D'Alessio, M.D.^e, and Lawrence M. Dolan, M.D.^{c,d}

^aCincinnati Children's Hospital Medical Center Division of Preventive Cardiology

^bCincinnati Children's Hospital Medical Center Division of Biostatistics and Epidemiology

^cCincinnati Children's Hospital Medical Center Division of Endocrinology

^dUniversity of Cincinnati, College of Medicine Department of Pediatrics

^eDepartment of Internal Medicine, Division of Endocrinology

Abstract

Background—Obesity-related CV diseases are a major cause of CV mortality. Obesity-related reduction in vascular protective adipose-derived proteins like adiponectin (APN) play a role.

Methods—We compared brachial artery distensibility (BrachD) to APN, level of adiposity and other CV risk factors (CVRFs) in 431 post-pubertal subjects (mean 17.9 years). Gender differences in average values were examined by t-tests. Correlations among BrachD, obesity and other CVRFs were examined. Regression analysis was performed to determine if APN provided independent contribution to BrachD, controlling for obesity and other CVRFs.

Results—Males had lower BrachD than females (5.72 + 1.37 vs 6.45 + 1.60 %change/mmHg, $p < 0.0001$) and lower APN (10.50 + 4.65 vs 13.20 + 6.53), (all $p < 0.04$). BrachD correlated with APN ($r = 0.25$, $p < 0.0001$). Both BrachD and APN correlated with measures of body size including height, weight, and BMI. Both correlated with higher SBP, glucose, insulin, and lower HDL-C (all $p < 0.01$). In multivariate analysis, APN, gender, APN*gender and BMI z-score predicted BrachD ($r^2 = 0.305$). By gender, only BMI z-score was significant for males ($r^2 = 0.080$). For females APN and BMI z-score contributed ($r^2 = 0.242$, all $p < 0.0001$).

Conclusions—BrachD is independently influenced by obesity in both males and females. In females, APN exerts an additional independent effect even after adjusting for BP, lipid levels and insulin. Differences in the effect of the APN — adiposity relationship on obesity-related vascular

Users may view, print, copy, download and text and data- mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

Corresponding author: Elaine Urbina, M.D. Division of Preventive Cardiology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC-7002, Cincinnati, OH 45229. Phone 513-636-8265, Fax: 513-636-0162, Elaine.Urbina@cchmc.org..

Conflict of Interest Disclosures None of the authors or persons mentioned in the acknowledgment section have other potential conflicts of interest to report.

disease may be one mechanism for gender differences in the development and progression of atherosclerosis.

Keywords

Elasticity; Pediatrics; Sex; Obesity; Risk Factors; Brachial artery

Introduction

Atherosclerosis, a major cause of cardiovascular disease (CVD)(1), is associated with vascular dysfunction.(2) Reproducible non-invasive methods for assessing atherosclerosis-related increase in arterial stiffness include the assessment of brachial artery distensibility (BrachD).(3-5) In adults, BrachD has been associated with congestive heart failure, a definitive adverse CVD outcome.(6) In addition, BrachD is reduced in adults with increased coronary artery calcium, a measure of advanced atherosclerosis.(7) In healthy young adults(3) and adolescents(8) BrachD is reduced in the presence of obesity. Despite these associations, the pathophysiologic factors that contribute to compromises in BrachD and lead to the development and progression of atherosclerosis are poorly understood.

Recent work suggests that obesity-related reduction in adiponectin (APN) level may play a role in the development of decreased vascular function(9) and atherosclerosis.(10) APN is a 244-amino acid protein secreted by adipose tissue that has potent anti-inflammatory effects. (11) APN levels are low in subjects with coronary artery disease compared to controls(12) and have been associated with multiple CV risk factors including obesity.(13) Data from animal models demonstrate a protective effect of APN on the vascular system by preservation of capacity of the endothelium to release nitric oxide in response to stress(14) and inhibition of smooth muscle cell proliferation in the arterial wall.(15) These data suggest a potential role for APN in mediating human vascular distensibility.

However, given the strong level of correlation between adiposity (i.e. BMI) and APN, a question remains whether or not adiposity and APN both independently contribute to the variability in BrachD. To address this issue, we measured BrachD in a large bi-racial population of healthy adolescents and young adults to identify associations between levels of BrachD, APN and level of adiposity. As APN levels vary by sex, we also sought to determine if the relationship differed by gender.

Methods

Study Population

The study population consisted of 431 subjects aged 14-21 years (mean age 17.9 years; 43% male, 42% non-Caucasian) who were part of the ongoing Princeton School District study(PSD). PSD was a longitudinal, population based study of the natural history of obesity, insulin resistance and diabetes in a large urban-suburban school district in Cincinnati.(16) To enter PSD, subjects had to be in the 5th through 12th grades in 2001, have no known chronic disease, and be taking no medication known to affect carbohydrate

metabolism. Pregnant females were excluded from PSD. Data were collected yearly on all participants.

The subjects included in these analyses were a sample of the cohort that participated in PSD in 2004 (the fourth year of data collection). Of the 2501 students screened, 1236 were randomly selected to undergo APN analyses. This cohort did not differ from the overall Princeton School District Study population with regard to age, sex, race, adiposity, or family history of diabetes.(17) Once BrachD testing was added to the screening procedure, it was performed on all subjects. A total of 470 subjects had both APN and BrachD. For this study, only post-pubertal subjects were included to eliminate the difference in plasma APN concentration found between pre- and post-pubertal subjects, yielding 431 subjects.(18) Pubertal stage was defined with the use of sex steroid levels and age of menarche for girls and axillary hair distribution for boys.(19) All participants with a fasting plasma glucose 100 mg/dl (5.5 mmol/L) or 2-hour post glucose load plasma glucose concentration 140 (7.8 mmol/L) were excluded.

The protocol was reviewed and approved by the Institutional Review Board at Cincinnati Children's Hospital. Written informed consent was obtained from the participant if the subject was > 18 years of age or from the parent or guardian if the participant was < 18 years of age. Written assent was obtained from all participants > 11 years of age but < 18 years of age.

Anthropometrics

After written informed consent was obtained, trained personnel obtained two measures of height using a portable stadiometer (RoadRod model; Quick Medical, North Bend, WA or Accustat, Genentech). Weight was also measured twice using a digital scale (770; SECA, Hanover, MD). The average of the two measures of height and/or weight were used in the analyses. BMI was calculated as kilograms per meter squared and BMI percentiles and z-scores were determined using the Centers for Disease Control and Prevention updated growth charts.(20)

Laboratory

Venipuncture was performed after a minimum 10 hour fast. Plasma glucose was measured using a Hitachi model 704 glucose analyzer with intra-assay and inter-assay coefficients of variation (CV) of 1.2% and 1.6%, respectively.(17) Plasma insulin was measured by radioimmunoassay using an anti-insulin serum raised in guinea pigs, 125I labeled insulin (Linco, St. Louis, MO) and a double antibody method to separate bound from free tracer. This assay has a sensitivity of 2 pmol and intra- and inter-assay CVs of 5% and 8%.(17) Hyperinsulinemia (H-I) was designated as a fasting insulin level that was >90th percentile for lean subjects in the study population. Subjects with insulin levels 90th percentile for lean subjects were classified as normal insulinemic (N-I). APN levels were determined using a radio-immunoassay kit (Linco Research, Inc., St. Charles, MO) which has a detection range from 1-200 ng/ml. It requires a ~1:500 dilution of the plasma sample, as plasma APN levels are typically 0.5-25 µg/ml.(21) The intra-assay coefficient of variation (CV) ranges from 1.8-6.2% depending on concentration, with an interassay CV from 6.9 to 9.3%.

Measured APN levels were re-converted into $\mu\text{g/ml}$ (actual plasma levels) using the procedures suggested by the kit manufacturer, to correct for dilution. An average of two measurements was used in the analysis. Appropriate negative controls and standard curve samples, were used to ensure the accuracy of the assay measurements.

Blood Pressure (BP) and Brachial Artery Distensibility (BrachD)

After 5 minutes of rest, trained personnel obtained three measures using a DynaPulse Pathway instrument (PulseMetric, Inc, San Diego, CA). Subject demographics were entered into a personal computer interfaced to the DynaPulse Pathway instrument. A BP cuff appropriate for the subject's upper arm size was applied.(22) Three automatic BP recordings of systolic, diastolic, mean arterial BP, heart rate (HR) and brachial artery pressure curves were obtained. The curves were uploaded to the on-line automated system for calculation of BrachD via the technique of pulse wave form analysis.(3) The DynaPulse Pathway instrument derives brachial artery distensibility using the technique of pulse dynamic analysis of arterial pressure signals obtained from a standard cuff sphygmomanometer.(4) The pressure waveform is calibrated and incorporated into a physical model of the cardiovascular system, assuming a straight tube brachial artery and T-tube aortic system. Validation studies of this method have been previously published.(4, 5) Correlation between compliance measurements obtained during cardiac catheterization and brachial artery compliance derived with the noninvasive method was high ($r = 0.83$). Clinical reproducibility studies demonstrated intraclass correlation coefficient for arterial compliance of 0.72 and other analyses indicated that most of the variability in measurement was due to inter-individual variation.(3) Although body size is used to estimate baseline brachial artery diameter for calculation of compliance, distensibility is equal to compliance divided by baseline brachial artery diameter. Therefore, body size is in both the numerator and the denominator of the distensibility equation. This results in calculation of a vascular measure that is independent of body size and baseline brachial artery diameter results.(3)

Statistical analyses

All analyses were performed with Statistical Analyses Software (SAS[®], version 9.1).(23) For all analyses, a p value of 0.05 was considered significant. Average values for demographic, anthropometric, laboratory and hemodynamic variables were obtained for the entire group and by gender since gender differences in BrachD were noted previously in adults(3) and children.(8) Variables were examined for extreme outliers and the shape of each variable's distribution was examined. Variance stabilizing transformations were applied as needed prior to additional analyses. T testing was performed to evaluate mean differences by gender and race. Pearson correlation coefficients were obtained between BrachD and important continuous covariates including anthropometrics, blood pressures, heart rate, fasting glucose, insulin and lipids.

Prior to performing multiple regression analysis, BrachD was adjusted for pulse pressure. Removing the influence of distending pressure on BrachD(8) allows for examination of the arterial wall properties among individuals with different baseline blood pressures. Multiple regression modeling was then performed to determine whether the key independent variable of APN provided a significant contribution to BrachD after controlling for important

covariates. Covariates in the initial model included variables which were correlated with BrachD in univariate analyses. Also, since gender(24) and obesity(25) affect plasma APN concentrations, an APN*gender interaction term and an APN*z-score of BMI interaction term were also included. Care was taken to assure all assumptions for regression were satisfied including linearity in the relationship between covariates and BrachD and homogeneity of variance. Analyses also demonstrated there were no influential outliers or collinearity among independent variables. For all models, a change in the beta slope coefficient for the key independent variable of APN of greater than 20% was considered evidence for confounding. Covariates were selected for the initial model if they were significantly correlated with BrachD in bivariate correlation analyses and were not mathematically co-linear with other terms in the model (i.e. only BMI was included as it is calculated from Height and Weight, only PP was included as it is calculated from SBP and DBP). Significance of each covariate in the initial model was assessed and non-significant terms were removed by backward elimination until all remaining covariates or their interaction effect modifier terms were significant. Regression diagnostics to determine robustness of the model fit were then performed. The final model was then examined for the significance of the key independent variable.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Gender Differences in the Cohort

Table 1 lists average demographic, anthropometric, laboratory and hemodynamic variables stratified by gender. Males were taller, heavier, and had higher BP levels, wider pulse pressure (PP) and slower heart rate (HR), but there was no BMI difference by gender. Although males were 0.6 years older ($p < 0.02$), this difference is not clinically relevant. Males differed from females (all $p = 0.04$) with lower BrachD (5.72 ± 1.37 vs 6.45 ± 1.60 %change/mmHg, $p = 0.0001$), plasma APN (10.50 ± 4.65 vs 13.20 ± 6.53 $\mu\text{g/ml}$), plasma insulin and HDL-cholesterol but higher glucose and total cholesterol. Although normal values for BrachD in youth have not been established, our means are similar to values obtained in a larger sample previously published from this cohort ($N = 582$, BrachD = 5.8 ± 1.1 to 7.1 ± 1.2 based on level of adiposity and hyperinsulinemia)(8) are also similar to data obtained in young adults from the Bogalusa Heart Study.(26)

Univariate Relationships with BrachD and APN

BrachD was associated with plasma APN (correlation coefficient = 0.25, $p = 0.0001$). There were no systematic differences in the pattern of correlations between the covariates and BrachD or APN. Both were correlated with height, weight, BMI, BMI-zscore, SBP, MAP, PP, glucose, insulin, HDL-C (all $p = 0.01$) and both BrachD and APN differed by race and gender ($p < 0.05$).

Multivariate Models

The initial multiple regression model contained the following covariates: gender, race, glucose, insulin, HDL-C, BMI z-score, APN, APN by gender and APN by BMI interaction terms. Using backward selection, the final model was: $\text{BrachD} = 0.12 - 0.0024 * \text{APN} + 0.016 * \text{gender} - 0.019 * \text{BMI z-score} + 0.0020 * \text{APN by gender interaction term}$ ($R^2 = 0.305$, p for parameter estimates < 0.02 except for gender and APN). Given the significant interaction between APN and gender, multivariate gender-specific models were also evaluated (figure 1). Interestingly, in males, only BMI z-score was significantly associated with BrachD ($\text{BrachD} = 0.13 - 0.011 * \text{BMI z-score}$ ($R^2 = 0.080$)), but in females, both BMI z-score and APN were significantly associated ($\text{BrachD} = 0.16 + 0.0013 * \text{APN} - 0.026 * \text{BMI z-score}$ ($R^2 = 0.242$, all p for parameter estimates < 0.014). The overall fit for each model was significant at $p = 0.0001$ level. Substituting HOMA-IR calculated by the method of Sinha and Caprio et al(27) for the fasting glucose and insulin in the models did not alter the result.

Discussion

While previous studies have demonstrated the importance of adiposity and APN in vascular distensibility, no study has examined the joint effects of these two factors. We have demonstrated that plasma APN concentration and adiposity are both important contributors to BrachD. However, upon further examination, we demonstrated that the relationship between APN and BrachD independent of adiposity is only present in females. To the best of our knowledge, the association of APN and BrachD has not been reported previously in healthy adolescents. In addition, males had lower BrachD than females. While BrachD correlated with traditional cardiovascular risk factors in both genders, only adiposity remained in the multivariate models suggesting the relationship between other CV risk factors and BrachD is secondary to adiposity and APN. These data suggest that the most important contributor to arterial stiffness is adiposity, with APN playing an independent role only in females. Therefore, absolute or relative hypoadiponectinemia may be one mechanism that mediates gender differences in CV disease event rates.

Reports in adults have documented the adverse impact of obesity on arterial stiffness. Even after adjusting for mean distending pressure, overweight subjects demonstrate increased carotid and aortic stiffness.(28) In pediatrics, there is a small but growing body of literature relating adiposity to decreased distensibility in the brachial(8, 29) and carotid arteries.(30) Specifically, Whincup et al, reported a strong, graded, inverse relationship between brachial distensibility and DBP, adiposity, and fasting insulin in adolescents in the United Kingdom. (29) Our data confirm that adiposity is related to increased arterial stiffness but also suggests that obesity alone does not explain gender differences in vascular function.

Other investigators have also found gender differences in arterial stiffness. In adults, central arterial stiffness (pulse wave velocity),(31) and carotid stiffness (Young's elastic pressure modulus)(32) differ significantly by gender. In addition, other studies have shown that the brachial and other arteries also have adversely lower levels of distensibility in men compared to women(3, 33) Few data are available regarding brachial artery distensibility and gender in pediatric subjects. In a previous study, we found lower BrachD in male as

compared to females adolescents. Gender remained a significant independent contributor to the variance in BrachD in multivariate models adjusted for BP and glucose.(8)

Investigators have hypothesized that gender differences in vascular function are entirely due to differences in sex hormones. This hypothesis is supported by the finding of BP-independent improvement in pulse wave velocity with estrogen replacement therapy in post-menopausal women,(34) the correlation between changes in serum estrogen compounds and progression of carotid atherosclerosis(35) and improvement in carotid stiffness with administration of estrogen.(36) In addition, data in children support the estrogen hypothesis in that gender differences in large artery stiffness occurred only after puberty was complete. (37) Our finding of gender differences in the relationship between APN and vascular function in adolescent females suggest that adipocytokines, in addition to sex hormones, may be important in explaining gender differences in vascular function and clinical outcomes.

Both animal and human research provide evidence that APN can affect vascular structure and function. Animal studies have demonstrated that APN penetrates the subendothelial space of the vascular wall after injury(11) and decreases the adhesion of monocytes to the injured vessel.(12) APN also suppresses the accumulation of lipid in monocyte-derived macrophages thus preventing their conversion to lipid-laden foam cells.(38) Conversely, hypoadiponectinemia exacerbates neointimal thickening in injured arteries by allowing the proliferation and migration of vascular smooth muscle cells into the intima of vessels.(15)

In studies of adults, hypoadiponectinemia is associated not only with the presence of advanced coronary atherosclerosis(10, 39, 40) but also traditional cardiovascular risk factors including hypercholesterolemia,(17), diabetes,(41), and hypertension.(42) Investigators have hypothesized that the reason that angiotensin II receptor blockers are more effective in treating hypertension than other classes of drugs is due to their ability to increase APN levels.(43) Hypoadiponectinemia may also have direct vascular effects by causing a decrease in endothelial function resulting in a decrease in the capacity of the endothelium to release nitric oxide in response to stress.(14) Although metabolic derangements such as carbohydrate intolerance and hyperinsulinemia are commonly found with low levels of APN, studies found a relationship between endothelial dysfunction and APN to be independent of insulin resistance(44) or diabetic status.(45) Our observation that APN is a determinant of BrachD in females despite their higher HOMA-IR values supports the conclusion that the APN-arterial stiffness relationship is independent of insulin resistance. In children, hypoadiponectinemia has also been associated with cardiovascular risk factors.(13, 17, 46) Therefore, the well-described association between endothelial dysfunction and both CV risk factors and adverse events(47) may be mediated in part by low plasma APN concentrations.

Similar to our findings, other studies have reported relationships between plasma adiponectin concentration and arterial function. Multiple adult studies have demonstrated impaired endothelial function associated with hypoadiponectinemia.(44, 45) Plasma APN level was also found to be an independent contributor to stiffness of the carotid artery (beta stiffness index) in healthy, non-diabetic subjects(48) and of the aorta (pulse wave velocity)

in patients with uncomplicated hypertension.(9) Similarly, in children, low plasma APN concentrations were associated with increased carotid intima-media thickness,(49) a condition associated with increased arterial stiffness.(50) In contrast, when Singhal et al examined the relationship among APN and endothelial function and arterial distensibility, no relationship was found.(51) However, this study was performed in a cohort that was predominantly premature making these data less generalizable to healthy adolescents born at term. Our data relating APN to vascular function in healthy adolescents independent of other CV risk factors such as fasting insulin level, HOMA-IR, and lipid levels suggest that a direct relationship between APN and arterial stiffness does exist.

Gender differences in APN are well documented. APN levels fall with increasing age due to changes in sex hormones and growth factors associated with pubertal development.(52) However, the degree of puberty-related decline differs by gender. Therefore, only post-pubertal, but not pre-pubertal girls have higher APN levels than their male counterparts.(24) These male/female contrasts may relate to the gender differences in peroxisome proliferator-activated receptor (PPARs) levels documented in animals(53, 54) that may occur early in fetal development(55) or, as seen in murine studies, estrogen may interact with PPAR alpha signaling or directly stimulate PPAR target genes.(56) PPAR gamma ligands regulate APN expression in vitro(57) and human studies demonstrate increases in APN levels in subjects with obesity or type 2 diabetes mellitus with administration of agents such as troglitazone. (58) Therefore, one potential mechanism for the gender differences in post-pubertal adolescents seen in our study may result from estrogen-related increase in PPAR activity leading to greater APN levels and higher BrachD in females.

This study has a number of limitations. First, the cross-sectional design in predominantly post-pubertal adolescents does not allow examination of time- and puberty-related changes in APN and how they relate to changes in arterial function. Furthermore, the proportion of the variability in BrachD explained by our model is low, especially in males. This suggests that other environmental factors, perhaps diet and physical activity, which were not measured in the current study, may play a role in the determination of both BrachD and APN. One such factor not measured in our study known to affect APN levels is body composition.(59) Furthermore, genetic influences were not assessed and APN levels are known to be highly heritable.(60) Finally, this is a young cohort who would be expected to have less advanced atherosclerosis hence the results may not be generalizable to an adult population with more advanced disease. Although this device has only been validated against catheterization data in adults,(4) our subjects of average age of 18 years were predominantly adult size thus reducing concern regarding any systematic differences in the applicability of the original validation results.

In conclusion, this study demonstrates that gender and adiposity are important contributors to BrachD in adolescents, with plasma APN having a strong, independent effect on BrachD in females only. Therefore, the contribution of absolute or relative hypoadiponectinemia needs to be considered as one mechanism that may be responsible for gender differences in CV disease event rates. This, in turn, may suggest new strategies for prevention of myocardial infarctions and strokes.

Acknowledgements

The authors gratefully acknowledge the work of the PSD research team and the administration, staff, teachers, students, and parents of the Princeton School District.

Funding Sources This work was supported by NIH grants DK59183, 0M01 RR 08084, NHLBI (5K23HL80447) and a Trustee Grant from Cincinnati Children's Hospital.

References

- Centers for Disease Control and Prevention. The Burden of Chronic Diseases and Their Risk Factors: National and State Perspectives 2004. 2004 <http://www.cdc.gov/nccdphp/burdenbook2004>
- Faxon DP, Creager MA, Smith SC Jr, Pasternak RC, Olin JW, Bettmann MA, et al. Atherosclerotic vascular disease conference: executive summary: atherosclerotic vascular disease conference proceeding for healthcare professionals from a special writing group of the American Heart Association. *Circulation*. 2004; 109:2595–2604. [PubMed: 15173041]
- Urbina EM, Brinton TJ, Elkasabany A, Berenson GS. Brachial artery distensibility and relation to cardiovascular risk factors in healthy young adults (The Bogalusa Heart Study). *Am J Cardiol*. 2002; 89:946–951. [PubMed: 11950433]
- Brinton TJ, Cotter B, Kailasam MT, Brown DL, Chio S-S, O'Connor DT, DeMaria AN. Development and validation of a noninvasive method to determine arterial pressure and vascular compliance. *Am J Cardiol*. 1997; 80:323–330. [PubMed: 9264426]
- Brinton TJ, Walls ED, Chio SS. Validation of Pulse Dynamic Blood pressure Measurement by Auscultation. *Blood Pressure Monitoring*. 1998; 3:121–124. [PubMed: 10212341]
- Nakamura M, Sugawara S, Arakawa N, Nagano M, Shizuka T, Shimoda Y, et al. Reduced vascular compliance is associated with impaired endothelium-dependent dilatation in the brachial artery of patients with congestive heart failure. *Journal of Cardiac Failure*. 2004; 10:36–42. [PubMed: 14966773]
- Budoff MJ, Flores F, Tsai J, Frandsen T, Yamamoto H, Takasu J. Measures of brachial artery distensibility in relation to coronary calcification. *Am J Hypertens*. 2003; 16:350–355. [PubMed: 12745195]
- Urbina EM, Bean JA, D'Alessio D, Daniels SR, Dolan LM. Overweight and Hyperinsulinemia Provide Individual Contributions to Compromises in Brachial Artery Distensibility in Healthy Adolescents and Young Adults. *JASH*. 2007; 1:200–207. [PubMed: 18431458]
- Mahmud A, Feely J. Adiponectin and arterial stiffness. *American Journal of Hypertension*. 2005; 18:1543–1548. [PubMed: 16364823]
- Maahs DM, Ogden LG, Kinney GL, Wadwa P, Snell-Bergeon JK, Dabelea D, et al. Low plasma adiponectin levels predict progression of coronary artery calcification. *Circulation*. 2005; 111:747–753. [PubMed: 15699257]
- Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Hormone & Metabolic Research*. 2000; 32:47–50. [PubMed: 10741683]
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*. 1999; 100:2473–2476. [PubMed: 10604883]
- Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *Journal of Clinical Endocrinology & Metabolism*. 2002; 87:4652–4656. [PubMed: 12364452]
- Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N, Tagawa T, et al. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *Journal of Clinical Endocrinology & Metabolism*. 2003; 88:3236–3240. [PubMed: 12843170]
- Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem*. 2002; 277:37487–37491. [PubMed: 12138120]

16. Goodman E, Adler NE, Daniels SR, Morrison JA, Slap GB, Dolan LM. Impact of objective and subjective social status on obesity in a biracial cohort of adolescents. *Obes Res.* 2003; 11:1018–1026. [PubMed: 12917508]
17. Martin LJ, Woo JG, Daniels SR, Goodman E, Dolan LM. The relationships of adiponectin with insulin and lipids are strengthened with increasing adiposity. *Journal of Clinical Endocrinology & Metabolism.* 2005; 90:4255–4259. [PubMed: 15870125]
18. Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. *J Clin Endocrinol Metab.* 2007; 92:1857–1862. [PubMed: 17284625]
19. Dolan LM, Bean J, D'Alessio D, Cohen RM, Morrison JA, Goodman E, et al. Frequency of abnormal carbohydrate metabolism and diabetes in a population-based screening of adolescents. *J Pediatr.* 2005; 146:751–758. [PubMed: 15973311]
20. Centers for Disease Control and Prevention. <http://www.cdc.gov/>
21. Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, et al. Genomic structure and mutations in adipose-specific gene, adiponectin. *Int J Obes Relat Metab Disord.* 2000; 24:861–868. [PubMed: 10918532]
22. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics.* 2004; 114:555–576. [PubMed: 15286277]
23. SAS Institute. SAS OnlineDoc. Version 9.1.3. SAS Institute; Cary, NC: 2002.
24. Tsou PL, Jiang YD, Chang CC, Wei JN, Sung FC, Lin CC, et al. Sex-related differences between adiponectin and insulin resistance in schoolchildren. *Diabetes Care.* 2004; 27:308–313. [PubMed: 14747205]
25. Sabin MA, Holly JMP, Shield JPH, Turner SJ, Grohmann MJ, Stewart CEH, et al. Mature subcutaneous and visceral adipocyte concentrations of adiponectin are highly correlated in prepubertal children and inversely related to body mass index standard deviation score. *Journal of Clinical Endocrinology & Metabolism.* 2006; 91:332–335. [PubMed: 16249291]
26. Urbina EM, Kiełtyka L, Tsai J, Srinivasan SR, Berenson GS. Impact of multiple cardiovascular risk factors on brachial artery distensibility in young adults: the Bogalusa Heart Study. *Am J Hypertens.* 2005; 18:767–771. [PubMed: 15925733]
27. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, et al. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med.* 2002; 346:802–810. [PubMed: 11893791]
28. Zebekakis PE, Nawrot T, Thijs L, Balkestein EJ, van der Heijden-Spek J, Van Bortel LM, et al. Obesity is associated with increased arterial stiffness from adolescence until old age. *J Hypertens.* 2005; 23:1839–1846. [PubMed: 16148607]
29. Whincup PH, Gilg JA, Donald AE, Katterhorn M, Oliver C, Cook DG, et al. Arterial distensibility in adolescents: the influence of adiposity, the metabolic syndrome, and classic risk factors. *Circulation.* 2005; 112:1789–1797. [PubMed: 16172286]
30. Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, et al. 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]
31. Oren A, Vos LE, Bos WJ, Safar ME, Uiterwaal CS, Gorissen WH, et al. Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? *American Journal of Hypertension.* 2003; 16:76–79. [PubMed: 12517688]
32. Urbina EM, Srinivasan SR, Kiełtyka RL, Tang R, Bond MG, Chen W, Berenson GS. Correlates of carotid artery stiffness in young adults: The Bogalusa Heart Study. *Atherosclerosis.* 2004; 176:157–164. [PubMed: 15306189]
33. van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension.* 2000; 35:637–642. [PubMed: 10679510]
34. Kawecka-Jaszcz K, Czarnańska D, Olszanańska A, Rajzer M, Jankowski P. The effect of hormone replacement therapy on arterial blood pressure and vascular compliance in postmenopausal women

- with arterial hypertension. *Journal of Human Hypertension*. 2002; 16:509–516. [PubMed: 12080436]
35. Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. *J Clin Endocrinol Metab*. 2008; 93:131–138. [PubMed: 17925335]
 36. Kallikazaros I, Tsioufis C, Zambaras P, Stefanadis C, Toutouzas P. Conjugated estrogen administration improves common carotid artery elastic properties in normotensive postmenopausal women. *Clin Cardiol*. 2002; 25:167–172. [PubMed: 12000074]
 37. Ahimastos AA, Formosa M, Dart AM, Kingwell BA. Gender differences in large artery stiffness pre- and post puberty. *J Clin Endocrinol Metab*. 2003; 88:5375–5380. [PubMed: 14602776]
 38. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*. 2001; 103:1057–1063. [PubMed: 11222466]
 39. Dzielińska Z, Januszewicz A, Wiecek A, Demkow M, Makowiecka-Ciećka M, Prejbisz A, et al. Decreased plasma concentration of a novel anti-inflammatory protein--adiponectin--in hypertensive men with coronary artery disease. *Thrombosis Research*. 2003; 110:365–369. [PubMed: 14592564]
 40. Otsuka F, Sugiyama S, Kojima S, Maruyoshi H, Funahashi T, Matsui K, et al. Plasma adiponectin levels are associated with coronary lesion complexity in men with coronary artery disease. *J Am Coll Cardiol*. 2006; 48:1155–1162. [PubMed: 16978998]
 41. Civitarese AE, Jenkinson CP, Richardson D, Bajaj M, Cusi K, Kashyap S, et al. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. *Diabetologia*. 2004; 47:816–820. [PubMed: 15105989]
 42. Mea, P Della; Lupia, M.; Bandolin, V.; Guzzon, S.; Sonino, N.; Vettor, R., et al. Adiponectin, insulin resistance, and left ventricular structure in dipper and nondipper essential hypertensive patients. *Am J Hypertens*. 2005; 18:30–35. [PubMed: 15691614]
 43. Wang ZV, Scherer PE. Adiponectin, Cardiovascular Function, and Hypertension. *Hypertension* January. 2008; 51:8–14.
 44. Fernandez-Real JM, Castro A, Vazquez G, Casamitjana R, Lopez-Bermejo A, Penarroja G, et al. Adiponectin is associated with vascular function independent of insulin sensitivity. *Diabetes Care*. 2004; 27:739–745. [PubMed: 14988295]
 45. Tan KC, Xu A, Chow WS, Lam MC, Ai VH, Tam SC, et al. Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. *Journal of Clinical Endocrinology & Metabolism*. 2004; 89:765–769. [PubMed: 14764794]
 46. Bakkaloglu SA, Buyan N, Funahashi T, Pasaoglu H, Elhan AH, Hasanoglu E, et al. Adiponectin levels and atherosclerotic risk factors in pediatric chronic peritoneal dialysis patients. *Peritoneal Dialysis International*. 2005; 25:357–361. [PubMed: 16022092]
 47. Agewall S. Is impaired flow-mediated dilatation of the brachial artery a cardiovascular risk factor? *Current Vascular Pharmacology*. 2003; 1:107–109. [PubMed: 15320837]
 48. Araki T, Emoto M, Yokoyama H, Maeno T, Hatsuda S, Mori K, et al. The association of plasma adiponectin level with carotid arterial stiffness. *Metabolism: Clinical & Experimental*. 2006; 55:587–592. [PubMed: 16631433]
 49. Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, et al. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. *J Clin Endocrinol Metab*. 2005
 50. Gullu H, Erdogan D, Caliskan M, Tok D, Yildirim E, Ulus T, et al. Interrelationship between noninvasive predictors of atherosclerosis: transthoracic coronary flow reserve, flow-mediated dilation, carotid intima-media thickness, aortic stiffness, aortic distensibility, elastic modulus, and brachial artery diameter. *Echocardiography*. 2006; 23:835–842. [PubMed: 17069601]
 51. Singhal A, Jamieson N, Fewtrell M, Deanfield J, Lucas A, Sattar N. Adiponectin Predicts Insulin Resistance but not Endothelial Function in Young Healthy Adolescents. *J Clin Endocrinol Metab*. 2005

52. Butte NF, Comuzzie AG, Cai G, Cole SA, Mehta NR, Bacino CA. Genetic and environmental factors influencing fasting serum adiponectin in Hispanic children. *J Clin Endocrinol Metab.* 2005; 90:4170–4176. [PubMed: 15827100]
53. Mattern HM, Lloyd PG, Sturek M, Hardin CD, Mattern HM, Lloyd PG, et al. Gender and genetic differences in bladder smooth muscle PPAR mRNA in a porcine model of the metabolic syndrome. *Mol Cell Biochem.* 2007; 302:43–49. [PubMed: 17318406]
54. Ciana P, Biserni A, Tatangelo L, Tiveron C, Sciarroni AF, Ottobrini L, et al. A novel peroxisome proliferator-activated receptor responsive element-luciferase reporter mouse reveals gender specificity of peroxisome proliferator-activated receptor activity in liver. *Mol Endocrinol.* 2007; 21:388–400. [PubMed: 17158222]
55. Ibabe A, Bilbao E, Cajaraville MP, Ibabe A, Bilbao E, Cajaraville MP. Expression of peroxisome proliferator-activated receptors in zebrafish (*Danio rerio*) depending on gender and developmental stage. *Histochem Cell Biol.* 2005; 123:75–87. [PubMed: 15616845]
56. Djouadi F, Weinheimer CJ, Saffitz JE, Pitchford C, Bastin J, Gonzalez FJ, et al. A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice. *J Clin Invest.* 1998; 102:1083–1091. [PubMed: 9739042]
57. Verma S, Szmítko PE, Verma S, Szmítko PE. The vascular biology of peroxisome proliferator-activated receptors: modulation of atherosclerosis. *Can J Cardiol.* 2006; 22(Suppl B):12B–17B.
58. Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes.* 2002; 51:2968–2974. [PubMed: 12351435]
59. Ryan AS, Berman DM, Nicklas BJ, Sinha M, Gingerich RL, Meneilly GS, et al. Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. *Diabetes Care.* 2003; 26:2383–2388. [PubMed: 12882866]
60. Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE, et al. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab.* 2001; 86:4321–4325. [PubMed: 11549668]

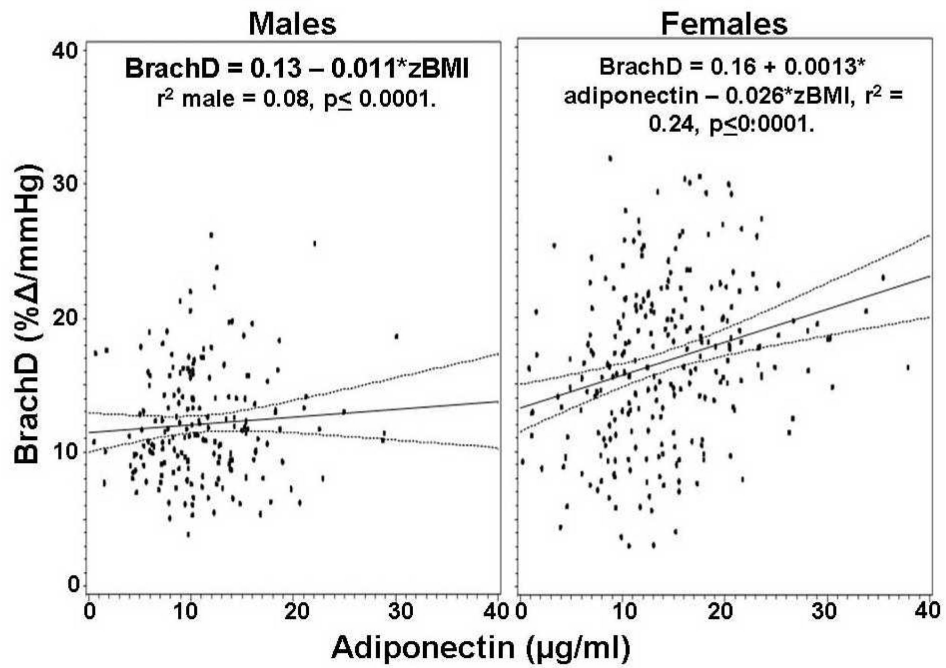


Figure 1. Scatter plot of BrachD regressed on APN by gender. Lines represent upper and lower 95th% CI for the mean. N=416. All models significant at $p < 0.0001$ and all parameter estimates at $p < 0.014$). Males: $r^2 = 0.08$; $\text{BrachD} = 0.13 - 0.011 \cdot \text{zBMI}$; Females: $r^2 = 0.24$; $\text{BrachD} = 0.16 + 0.0013 \cdot \text{APN} - 0.026 \cdot \text{zBMI}$.

Table 1

Average results overall and by gender: Means and Standard deviation (SD).

Variable	All (N = 431)	SD	Males (N = 185)	SD	Females (N = 246)	SD	P for gender difference*
Age (yrs)	18.0	1.7	18.3	1.5	17.7	1.8	0.02
Height (cm)	169.1	9.1	176.8	6.6	163.4	6.0	0.0001
Weight (kg)	72.4	19.2	80.2	18.2	66.6	17.9	0.0001
BMI (kg/m ²)	25.2	6.1	25.6	5.4	25.0	6.6	NS
BMI Z-score	0.62	1.05	0.69	1.07	0.57	1.03	NS
SBP (mmHg)	115.4	11.3	121.5	9.9	110.8	10.0	0.0001
DBP (mmHg)	69.6	7.8	70.8	7.9	68.8	7.6	0.018
MAP (mmHg)	82.9	8.1	84.6	7.9	81.6	8.1	0.0003
HR (beats/min)	73.0	11.3	67.7	10.2	76.9	10.6	0.0001
BrachD (%change/mmHg) †	6.35	1.63	5.91	1.36	6.67	1.74	0.0001
APN (microgm/ml)	12.83	6.12	11.19	5.09	14.05	6.53	0.0001
Fasting Glucose (mg/dl)	74.4	8.7	76.0	8.1	73.2	9.0	0.0018
Fasting Insulin (picomoles/L)	106.1	96.4	95.5	85.6	114.0	103.2	0.04
HOMA-IR ‡	3.31	3.07	3.01	2.70	3.55	3.32	0.04
Total Cholesterol (mg/dl)	149.1	25.6	146.0	26.7	151.4	24.5	0.025
LDL-C (mg/dl)	84.3	22.0	84.0	23.9	84.5	20.6	NS
HDL-C (mg/dl)	48.8	11.8	45.0	10.3	51.7	12.0	0.0001
Triglycerides (mg/dl)	80.4	43.8	85.6	47.8	76.6	40.2	NS

* Variance stabilizing transformations applied where needed prior to testing for group differences.

† Brachial Artery Distensibility

‡ Insulin resistance index determined by homeostatic model assessment.(27)