Published in final edited form as:

J Clin Ultrasound. 2016 January ; 44(1): 46–54. doi:10.1002/jcu.22280.

Varying Patterns of Brachial Artery Flow-Mediated Dilatation in Women with Polycystic Ovary Syndrome and Controls: An Application of the Group-Based Trajectory Modeling

Michelle L Meyer, PhD, MPH¹, Ping G Tepper, PhD², Emma Barinas-Mitchell, PhD², Mary T Korytkowski, MD³, and Evelyn O Talbott, DrPH, MPH²

¹Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

²Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania, USA

³Division of Endocrinology and Metabolism, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Abstract

Purpose—To identify changing patterns of absolute change in brachial lumen diameter (LD) after reactive hyperemia in women with polycystic ovary syndrome (PCOS) and controls, and quantify the association of PCOS status and participant factors with changing patterns.

Materials and Methods—Brachial flow-mediated dilation was measured in 128 women with PCOS and 148 controls 30–60 years old. Group-based trajectory modeling was used to investigate absolute change in LD every 30 seconds for 2 minutes after an occluding cuff deflation. Multinomial logistic regression was used to identify factors associated with trajectories.

Results—There were three patterns of absolute change in LD diameter termed non-dilators (42.2%), dilators (44.6%) and enhanced dilators (13.0%). The proportion of women with PCOS did not differ across groups. Independent of age and PCOS status, larger baseline LD (odds ratio (OR); 95% confidence interval (CI): 2.51; 1.29,4.89) and lower insulin levels (0.70; 0.52,0.93) were associated with non-dilators compared with dilators. Higher total cholesterol was associated with dilators in women with PCOS but with non-dilators in controls.

Conclusions—Trajectory modeling identified distinct patterns of change in LD and factors associated with the endothelial response. This method may be a useful tool to understand the brachial flow-mediated vasodilator response.

Keywords

Polycystic ovary syndrome; subclinical cardiovascular disease; endothelial dysfunction; endothelial function; flow-mediated dilation; group-based trajectory modeling

Corresponding Author: Michelle L Meyer, University of North Carolina at Chapel Hill, 137 E. Franklin St., Suite 306, Chapel Hill, North Carolina, USA 27514. (Fax: (919) 966-9800; Email: mlmeyer@unc.edu)

Conflict of interest: The authors have nothing to disclose.

Introduction

Women with polycystic ovary syndrome (PCOS), a common reproductive endocrine disorder, have an increase in cardiovascular disease (CVD) risk factors¹⁻⁵ and subclinical atherosclerosis compared with non-PCOS controls.⁶⁻¹⁰ Studies have also shown women with PCOS have lower flow-mediated dilation (FMD) compared with controls,^{3,8,11-13} however, results are conflicting.^{14,15} FMD is a widely used non-invasive measure of endothelial function that indicates early vascular injury.^{16,17} A lower FMD in response to an increased blood flow is a marker of endothelial dysfunction. Endothelial dysfunction has been shown in animal models of atherogenesis¹⁸ and is predictive of cardiovascular disease events.¹⁹

FMD is used in many epidemiological studies and as endpoints in randomized clinical trials, thus it is critical to have a reliable measurement. FMD is a ratio index of the change in lumen diameter (LD) divided by the baseline LD. The calculation, however, has been questioned²⁰ since it is confounded by the baseline LD.^{20,21} A smaller brachial LD would intrinsically cause higher FMD because smaller vessels are able to dilate, or compensate for an increase in blood flow and shear stress, to a greater extent than larger vessels.²² FMD has been shown to decrease with higher baseline LD^{21,23,24} whereas the absolute change in LD was unrelated to the baseline LD.^{21,25} How this potential bias in the calculation could influence the estimate and interpretation of associations with cardiovascular disease risk factors and events is unknown.

To overcome the concerns with FMD, a modified approach using group-based trajectory modeling presents an alternative approach for analyzing results from the endothelial function assessment. Semi-parametric group-based trajectory modeling, also called latent class growth mixture modeling, is commonly used to model subgroups in a population that follow similar trajectories.²⁶ This method assumes there are patterns of change in an outcome across multiple time points and identifies subgroups in the population.^{27,28} The advantage of this method is that the groups are not defined *a priori*, which differs from standard growth modeling.

This method could identify and summarize subgroups of different patterns of absolute change in LD following transient ischemia induced during the endothelial function assessment. The analysis could identify participants who have a low response and evaluate the associations between covariates and group membership. This could improve the understanding of endothelial function by removing the variability of FMD associated with calculating percent change in LD. It would also detect patterns of change, rather than individual FMD values.

The aim of this study was to use a group-based trajectory model to identify patterns of absolute change in LD after reactive hyperemia in women with PCOS and controls, and to determine if PCOS status and participant factors are associated with changing patterns.

Materials and Methods

Study Population

This analysis was based on data obtained from the Cardiovascular Health and Risk Measurement Study (CHARM) that includes women with PCOS and controls. The methodology and recruitment of the original study that occurred between 1992 and 1995 has been described previously.²⁹ Briefly, investigators identified women 19–55 years old retrospectively diagnosed with PCOS between 1970 and 1993 through medical record review in the Division of Reproductive Endocrinology at Magee-Women's Hospital (Pittsburgh, PA). Participants were also recruited from private practices and the local chapter of the Polycystic Ovarian Syndrome Association (PCOSA) support group. Investigators defined PCOS using the NIH criteria.³⁰ Investigators matched PCOS cases to controls by age (± 5 years) and race using voter's registration tapes and Cole's Cross Reference Directory of Households from 1993. The University of Pittsburgh institutional review board approved the protocols, and all participants gave consent before enrolling.

The present study includes 283 women 30–60 years old who underwent brachial artery endothelial function assessment during the second follow-up of CHARM (1997 to 1999). Investigators excluded women from endothelial tests if they had Raynaud's phenomenon, were morbidly obese, or could not carry out the test due to arm cuff limits, patient discomfort, technical problems, and patient refusal. We excluded women with a history of angina (n=4) and women taking a vasodilator medication (n=3). The final sample was 128 women with PCOS and 148 controls.

Covariates—Clinical assessments have been previously described.^{7,29,31} Briefly, investigators collected height, weight, BMI, waist circumference, blood pressure, and serum concentrations of total cholesterol, triglycerides, and fasting glucose and insulin. Participants completed a questionnaire on medical, surgical, menstrual and reproductive history, and medication use. Postmenopausal status was defined as no menstrual period in the last 12 months. Type 2 diabetes was defined as a self-reported doctor diagnosis or a glucose value of ≥ 126 mg/dL and hypertension was defined as a self-reported doctor diagnosis. For this analysis, the quantitative insulin sensitivity check index (QUICKI) was calculated ($\text{QUICKI} = 1/[\log \text{fasting insulin concentration } (\mu\text{U/mL}) + \log \text{glucose concentration } (\text{mg/dL})]$).³²

Endothelial Function Assessment—Flow-mediated dilation of the brachial artery was measured by Toshiba SSA-270A and Hewlett Packard 5500 duplex scanners during the 1997–1999 follow-up visit. Technicians placed a cuff on the right forearm and an ultrasound probe two inches above the antecubital fossa. The procedure began after the participant rested for ten minutes. The sonographer recorded baseline digital images for one minute and then inflated the cuff to 50 mmHg above the systolic blood pressure for four minutes to induce hyperemia. The sonographer recorded digital images post deflation every thirty seconds for two minutes. All images used for measurement of brachial LD were captures at end diastole. Brachial LD was measured from these digital images by one reader using the

Brachial Analysis System software (Medical Imaging Applications [MIA], University of Iowa). Three measurements of the LD for each time point were averaged.

Statistical Methods

Data preparation—To prepare the data, variables were created that represented the 30 second time variable and the corresponding absolute change in LD (for example time 1 and LD 1; time 2 and LD 2, etc.). The absolute change in LD at time zero was considered to be zero for all participants. The censored normal distribution model in SAS Proc Traj was used, which was developed by Nagin and colleagues to model group trajectories.^{26–28} The single outcome was absolute change in LD at the 30 second time points for two minutes.

Model Selection and Diagnostics—The optimal group number was determined for up to 5 groups using second order polynomial models by evaluating model fits using the BIC criteria and the estimated log Bayes factor. For these data, a three-group trajectory model was identified as the optimal number of groups. Groups accounting for no less than five percent of patients were formed. Once the three group model was determined to be the optimal model, the cubic term was added to each group. Non-significant terms were removed from each group and models were evaluated using the BIC criteria.

To check model adequacy, the average posterior probabilities of membership in each group (AvePP) and the odds of correct classification (OCC) were calculated (Figure 1).²⁶ The OCC was calculated by: $OCC_j = [(AvePP_j / (1 - AvePP_j)) / (\pi_j / (1 - \pi_j))]$; where π_j is the estimated probability of group membership. More accurate group assignments correspond to a larger OCC. Adequate models should have an AvePP of >0.70 and an OCC of >5.

Characteristics by Group—Posterior probabilities were used to classify participants into groups. Descriptive statistics were computed for categorical and continuous variables by groups. Differences in levels of characteristics across groups were evaluated using ANOVA for normally distributed variables, the Welch test for normally distributed variables with unequal variances across groups, the Kruskal-Wallis Test for non-normally distributed variables, and the Chi-square or Fisher's exact tests for categorical variables.

Group membership was modeled using multinomial logistic regression analysis to identify possible characteristics associated with trajectories. The multinomial model evaluated differences in variables between non-dilators and enhanced dilators compared to the dilators as the referent. The dilators were selected as the referent because it was the largest group and was hypothesized to represent the average endothelial response. Non-normal variables were transformed and the log of insulin and glucose were divided by the standard deviation for ease of interpreting model parameters. Total cholesterol was categorized into quartiles (Q) (Q1: 179, Q2: 180–204, Q3: 205–230, and Q4: 231mg/dL) and defined as an ordinal variable in the modeling process, as such we evaluated the linear relationship with group membership assuming effect estimates were similar across quartiles. The variables were modeled adjusting for age, PCOS status, and variables with a p-value <0.20 that were assessed using forward stepwise selection. All first order interactions with PCOS status were evaluated to investigate whether estimates differed between women with PCOS and controls.

SAS (release 9.2; SAS, Cary, NC) was used for the trajectory modeling and all other analyses were done with PASW (version 18; IBM SPSS Inc., Chicago, IL).

Results

The analysis identified three distinct patterns of absolute change in LD, which were labeled as non-dilators, dilators, and enhanced dilators (Figure 1). There were 117 (42.4%) women classified as non-dilators, 123 (44.6%) women classified as dilators and 36 (13.0%) women classified as enhanced dilators. The maximum absolute change in LD ranged from -0.19 to 0.36 mm for the non-dilators, from 0.08 to 0.40 mm for dilators, and from 0.29 to 0.70 mm for the enhanced dilators. The distribution of women with PCOS was not different across groups. The proportion of women with PCOS was 42.7% in non-dilators, 46.3% in dilators, and 58.3% in enhanced dilators, $p=0.26$ (Table 1).

When descriptive characteristics were compared across groups, insulin levels were higher across groups going from non-dilators to enhanced dilators, $p=0.03$ (Table 1). Age, BMI, fasting glucose, QUICKI, and triglyceride levels were not significantly different across groups, as well as the proportion of African Americans, hypertension, type 2 diabetes, smoking, or postmenopausal women. Significant differences across groups were seen in endothelial function parameters including baseline LD, maximum post-deflation LD, absolute change in LD, and FMD (Table 2).

In the multinomial logistic regression analysis, larger baseline LD was associated with a 2.51 greater likelihood of non-dilators compared to dilators (95% CI 1.29, 4.89; Table 3). Independent of age, PCOS status, and baseline LD, lower insulin was associated with a greater likelihood of non-dilators compared to dilators (OR 0.70, 95% CI 0.52, 0.93). There was a significant interaction term with cholesterol quartiles and PCOS status for the non-dilators compared to dilators ($p=0.02$; Table 3) after adjusting for age, baseline LD and insulin. The graph of the predicted probabilities of the non-dilators from the interaction model by PCOS status showed that as cholesterol quartiles increases, the probability of non-dilators versus dilators decreases for women with PCOS and increases for controls (Figure 2). There was no evidence of other effect modifications with PCOS status. Results did not change when stratifying by women with PCOS and controls, or excluding women with diabetes or women on medication for insulin and glucose (data not shown). There were 17 diabetics among women with PCOS and 3 diabetics among the controls ($p<0.001$), and 11 women taking diabetes medication among women with PCOS and 2 among the controls ($p=0.005$).

Discussion

Group based trajectory analysis identified three distinct patterns of absolute change in brachial artery LD following arterial occlusion. Approximately 42.4% of the women were non-dilators, 44.6% were dilators, and 13.0% were enhanced dilators. The results are surprising because it suggests 42% of the women have evidence of a low endothelial response indicative of endothelial dysfunction. Insulin and the endothelial function parameters were the only variables that differed across groups. The proportion of women

with PCOS did not differ across groups. Factors associated with non-dilators compared with dilators included higher baseline LD after adjusting for age and PCOS status, and lower insulin after adjusting for age, PCOS status, and baseline LD. Total cholesterol modified the association between PCOS status and group membership.

This analysis showed that a larger baseline LD was associated with non-dilators compared with dilators, which contradicts reports that absolute change in LD is independent of baseline LD.^{21,25} A larger baseline LD, however, has less ability to respond to increases in blood flow.²² Increases in LD are a normal process of arterial remodeling that occurs in response to changes in blood flow to preserve vascular tone and shear stress.³³ A larger baseline LD has been associated with lower endothelial function,^{34,35} coronary artery calcium,³⁶ and coronary artery disease,^{34,37} while a smaller absolute change in LD has been associated with peripheral arterial disease.³⁸

Besides baseline LD, the other determinant of non-dilators was insulin in which lower insulin levels were associated with non-dilators compared with dilators. This is similar to a report by Carmina et al. that showed insulin levels were positively related to FMD in non-PCOS women; however, no associations were seen in women with PCOS.¹⁰ Insulin acts as a vasoconstrictor³⁹ and vasodilator,⁴⁰⁻⁴⁴ and could affect important regulators of vascular function such as nitric oxide (NO) function⁴⁴ and oxidative stress. The action of insulin could be altered by states of insulin resistance that blunt the vasodilating but not the vasoconstricting signaling of insulin.³⁹ In this study, insulin resistance, as indicated by QUICKI, was not significantly associated with group membership. Similarly, using the glucose/insulin ratio as a measure of insulin resistance, Mather et al. showed no differences in FMD between insulin resistant women with PCOS compared to controls.¹⁵ Our results suggest that contribution of other factors besides insulin resistance, such as dysfunction in insulin secretion (beta cell function) may influence the endothelial response.

The interaction between cholesterol and PCOS status suggests that higher cholesterol is associated with a greater likelihood of being a dilator in women with PCOS, but being a non-dilator in controls. Total cholesterol has not been associated with FMD in women with PCOS and controls (spearman correlation $r=0.05$, $p=0.71$).¹¹ A study among women with PCOS showed that the duration of reactive hyperemia (durRH), defined as the time for the blood flow to return to baseline levels after reactive hyperemia, was shorter in women with PCOS versus controls (64 seconds versus 113 seconds, $p=0.04$).⁴⁵ The investigators showed that total cholesterol was not significantly related to the durRH, but the spearman correlations were in opposite directions between women with PCOS ($r=-0.17$, $p=0.49$) and controls ($r=0.14$, $p=0.68$). Thus, the interaction between case control status and group membership could reflect differences between women with PCOS and controls in how cholesterol affects stimulus stress or other mechanisms of the endothelial response after reactive hyperemia. Differences in the relative levels of HDL and LDL summed in total cholesterol could also explain the findings, although HDL and LDL themselves were not associated with group membership.

Guidelines for the measurement of endothelial function recommend studies to report baseline LD, absolute change in LD, FMD, area under the curve (AUC) and time to

maximum diameter.⁵⁶ A comprehensive characterization would help to interpret the results from the endothelial function assessment and reduce variability across studies. Investigators have tried to decrease the variability and increase sensitivity of the endothelial function data by using various analytic methods. Investigators have used AUC⁴⁶ as an alternative to FMD and showed it was more sensitive in detecting endothelial responses over the time course of the measurement than FMD.²¹ Other investigators have looked at the time course⁴⁷ and used it to classify responders based on the AUC of FMD.²³ These alternative methods are similar to this trajectory analysis because the participant endothelial responses were classified into groups. This analysis also allowed for the adjustment for baseline LD when analyzing the absolute change, which is not recommended when analyzing the relative change in LD because it is part of the calculation.

A limitation of this study was that the LD was measured at 30 second intervals. This might have underestimated the true maximum diameter, but may have a small impact on the results because the focus was on patterns of change rather than the maximum dilation. Another limitation was that endothelium-independent dilation was not measured so the function of the smooth muscle cells on the endothelial response could not be evaluated. Also, stimulus stress was not measured, which has been suggested to be as important as baseline LD in determining the endothelial response to shear stress.^{48,49} This limitation, however, exists in most studies because shear stress is rarely assessed and adjusting for the stimulus stress is still under debate.^{50,51} Our statistical comparisons of participant characteristics across trajectory groups were not corrected for multiple comparisons, thus there is a chance for false positives. Lastly, insulin resistance was not measured with the gold standard hyperinsulinemic-euglycemic clamp method, but QUICKI is commonly used in the literature and has been shown to be sensitive in detecting insulin resistance in women with PCOS.⁵²

Despite these limitations, this trajectory analysis identified individuals with similar patterns of absolute change in LD and factors associated with group membership. It would be important to replicate this method in different populations while taking into account the aforementioned variability in the endothelial response due to baseline LD and stimulus stress. Larger studies may be needed to increase the power of this analysis to identify factors associated with group membership.

In summary, baseline LD and insulin were identified as important factors in the endothelial response after reactive hyperemia. The proportion of women with PCOS did not differ among women grouped according to dilator status. There was evidence that higher total cholesterol was associated with dilators in women with PCOS but with non-dilators in controls, suggesting that cholesterol may have a different association with endothelial function in women with PCOS and controls. Based on the observations in this study, multiple methods could be used to analyze endothelial function data to understand the complex process of endothelial function. Applying this analytic method to past and future studies of endothelial function would allow researchers to evaluate the utility of a group-based trajectory modeling as it could be associated with less variability in how to analyze endothelial function data when compared to current methods. Trajectory modeling shows promise as an additional tool to understand endothelial function and identify individuals

with a low endothelial response that would support the use of endothelial function assessment in epidemiological and clinical studies. Further investigation is needed to investigate whether trajectory groups are clinically meaningful, but we offer a potential tool to aid in interpreting endothelial function.

Acknowledgments

Funding and acknowledgements

The Cardiovascular Health and Risk Management Study was supported by the National Institutes of Health (NHLBI RO1-44664-09-11). Dr. Snyder was supported through the National Heart, Lung, and Blood Institute predoctoral (T32 HL083825-01) and postdoctoral (T32 HL-007055) fellowships. The authors would like to thank the staff and participants of the CHARM study for their important contributions.

References

1. Glueck CJ, Morrison JA, Goldenberg N, et al. Coronary heart disease risk factors in adult premenopausal white women with polycystic ovary syndrome compared with a healthy female population. *Metabolism*. 2009; 58:714–721. [PubMed: 19375597]
2. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. *Am J Med*. 2001; 111:607–613. [PubMed: 11755503]
3. Cascella T, Palomba S, De Sio I, et al. Visceral fat is associated with cardiovascular risk in women with polycystic ovary syndrome. *Human reproduction (Oxford, England)*. 2008; 23:153–159.
4. Talbott EO, Zborowski JV, Rager JR, et al. Polycystic ovarian syndrome (PCOS): a significant contributor to the overall burden of type 2 diabetes in women. *J Womens Health (Larchmt)*. 2007; 16:191–197. [PubMed: 17388735]
5. Boulman N, Levy Y, Leiba R, et al. Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. *J Clin Endocrinol Metab*. 2004; 89:2160–2165. [PubMed: 15126536]
6. Christian RC, Dumesic DA, Behrenbeck T, et al. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2003; 88:2562–2568. [PubMed: 12788855]
7. Talbott EO, Guzick DS, Sutton-Tyrrell K, et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol*. 2000; 20:2414–2421. [PubMed: 11073846]
8. Orio F Jr, Palomba S, Cascella T, et al. Early impairment of endothelial structure and function in young normal-weight women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2004; 89:4588–4593. [PubMed: 15356067]
9. Vryonidou A, Papatheodorou A, Tavridou A, et al. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism*. 2005; 90:2740–2746. [PubMed: 15741256]
10. Carmina E, Orio F, Palomba S, et al. Endothelial dysfunction in PCOS: role of obesity and adipose hormones. *American Journal of Medicine*. 2006; 119:356.e351–356.e351. [PubMed: 16564785]
11. Tarkun I, Arslan BC, Canturk Z, et al. Endothelial dysfunction in young women with polycystic ovary syndrome: Relationship with insulin resistance and low-grade chronic inflammation. *Journal of Clinical Endocrinology and Metabolism*. 2004; 89:5592–5596. [PubMed: 15531516]
12. Lowenstein L, Damti A, Pillar G, et al. Evaluation of endothelial function in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2007; 134:208–212. [PubMed: 17374431]
13. Kravariti M, Naka KK, Kalantaridou SN, et al. Predictors of endothelial dysfunction in young women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*. 2005; 90:5088–5095. [PubMed: 15985492]

14. Arian S, Akay H, Bahceci M, et al. The evaluation of endothelial function with flow-mediated dilatation and carotid intima media thickness in young nonobese polycystic ovary syndrome patients; existence of insulin resistance alone may not represent an adequate condition for deterioration of endothelial function. *Fertility & Sterility*. 2009; 91:450–455. [PubMed: 18501898]
15. Mather KJ, Verma S, Corenblum B, et al. Normal endothelial function despite insulin resistance in healthy women with the polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism*. 2000; 85:1851–1856. [PubMed: 10843164]
16. Juonala M, Viikari JS, Laitinen T, et al. Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation*. 2004; 110:2918–2923. [PubMed: 15505080]
17. Bots ML, Westerink J, Rabelink TJ, et al. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *Eur Heart J*. 2005; 26:363–368. [PubMed: 15618057]
18. Ross R. The pathogenesis of atherosclerosis--an update. *N Engl J Med*. 1986; 314:488–500. [PubMed: 3511384]
19. Yeboah J, Crouse JR, Hsu FC, et al. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation*. 2007; 115:2390–2397. [PubMed: 17452608]
20. Atkinson G, Batterham AM. The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vascular medicine (London, England)*. 2013; 18:354–365.
21. Herrington DM, Fan L, Drum M, et al. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. *Journal of cardiovascular risk*. 2001; 8:319–328. [PubMed: 11702039]
22. Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J*. 1995; 74:247–253. [PubMed: 7547018]
23. Jarvisalo MJ, Ronnema T, Volanen I, et al. Brachial artery dilatation responses in healthy children and adolescents. *Am J Physiol Heart Circ Physiol*. 2002; 282:H87–H92. [PubMed: 11748051]
24. Juonala M, Kahonen M, Laitinen T, et al. 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]
25. Charakida M, Masi S, Luscher TF, et al. Assessment of atherosclerosis: the role of flow-mediated dilatation. *Eur Heart J*. 2010; 31:2854–2861. [PubMed: 20864485]
26. Nagin, D. Group-based modeling of development. Cambridge, Mass.: Harvard University Press; 2005.
27. Jones BL, Nagin DS. Advances in Group-Based Trajectory Modeling and an SAS Procedure for Estimating Them. *Sociological Methods & Research*. 2007; 35:542–571.
28. Jones BL, Nagin DS, Roeder K. A SAS Procedure Based on Mixture Models for Estimating Developmental Trajectories. *Sociological Methods & Research*. 2001; 29:374–393.
29. Talbott E, Guzick D, Clerici A, et al. Coronary heart disease risk factors in women with polycystic ovary syndrome. *Arterioscler Thromb Vasc Biol*. 1995; 15:821–826. [PubMed: 7600112]
30. Zawadzki, J.; Dunaif, A. Current Issues in Endocrinology and Metabolism. Bethesda, MD: 1992. Diagnostic criteria for polycystic ovary syndrome: towards a rationale approach. Consensus Conference on Polycystic Ovary Syndrome.
31. Talbott E, Clerici A, Berga SL, et al. Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. *J Clin Epidemiol*. 1998; 51:415–422. [PubMed: 9619969]
32. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*. 2000; 85:2402–2410. [PubMed: 10902785]
33. Ward MR, Pasterkamp G, Yeung AC, et al. Arterial remodeling. Mechanisms and clinical implications. *Circulation*. 2000; 102:1186–1191. [PubMed: 10973850]

34. Holubkov R, Karas RH, Pepine CJ, et al. Large brachial artery diameter is associated with angiographic coronary artery disease in women. *Am Heart J.* 2002; 143:802–807. [PubMed: 12040340]
35. Schroeder S, Enderle MD, Baumbach A, et al. Influence of vessel size, age and body mass index on the flow-mediated dilatation (FMD%) of the brachial artery. *Int J Cardiol.* 2000; 76:219–225. [PubMed: 11104877]
36. Kullo IJ, Malik AR, Bielak LF, et al. Brachial artery diameter and vasodilator response to nitroglycerine, but not flow-mediated dilatation, are associated with the presence and quantity of coronary artery calcium in asymptomatic adults. *Clin Sci (Lond).* 2007; 112:175–182. [PubMed: 16987102]
37. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet.* 1992; 340:1111–1115. [PubMed: 1359209]
38. Yataco AR, Corretti MC, Gardner AW, et al. Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *Am J Cardiol.* 1999; 83:754–758. [PubMed: 10080432]
39. Schulman IH, Zhou MS. Vascular insulin resistance: a potential link between cardiovascular and metabolic diseases. *Curr Hypertens Rep.* 2009; 11:48–55. [PubMed: 19146801]
40. Scherrer U, Randin D, Vollenweider P, et al. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest.* 1994; 94:2511–2515. [PubMed: 7989610]
41. Baron AD. Hemodynamic actions of insulin. *Am J Physiol.* 1994; 267:E187–E202. [PubMed: 8074198]
42. Lembo G, Rendina V, Iaccarino G, et al. Insulin reduces reflex forearm sympathetic vasoconstriction in healthy humans. *Hypertension.* 1993; 21:1015–1019. [PubMed: 8505085]
43. Baron AD. Insulin resistance and vascular function. *J Diabetes Complications.* 2002; 16:92–102. [PubMed: 11872375]
44. Steinberg HO, Brechtel G, Johnson A, et al. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest.* 1994; 94:1172–1179. [PubMed: 8083357]
45. Dagre A, Lekakis J, Mihas C, et al. Association of dehydroepiandrosterone-sulfate with endothelial function in young women with polycystic ovary syndrome. *Eur.* 2006; 154:883–890.
46. Dalton BS, Fassett RG, Geraghty DP, et al. No relationship between low-density lipoproteins and endothelial function in hemodialysis patients. *Int J Cardiol.* 2005; 99:307–314. [PubMed: 15749192]
47. Black MA, Cable NT, Thijssen DH, et al. Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension.* 2008; 51:203–210. [PubMed: 18086954]
48. Pyke KE, Hartnett JA, Tschakovsky ME. Are the dynamic response characteristics of brachial artery flow-mediated dilation sensitive to the magnitude of increase in shear stimulus? *J Appl Physiol.* 2008; 105:282–292. [PubMed: 18467554]
49. Padilla J, Johnson BD, Newcomer SC, et al. Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. *Cardiovasc Ultrasound.* 2008; 6:44. [PubMed: 18771594]
50. Thijssen DH, Bullens LM, van Bommel MM, et al. 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]
51. Atkinson G, Batterham AM, Black MA, et al. Is the ratio of flow-mediated dilation and shear rate a statistically sound approach to normalization in cross-sectional studies on endothelial function? *J Appl Physiol.* 2009; 107:1893–1899. [PubMed: 19833808]
52. Carmina E, Lobo RA. Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. *Fertility and sterility.* 2004; 82:661–665. [PubMed: 15374711]

Mean absolute change in lumen diameter (mm) with 95% confidence intervals by group

Group memberships and assignment accuracy

Group	Number (%)	Average posterior probabilities of group membership	Odds of correct classification
Non-dilators (ND)	117 (42.4)	0.95	25.7
Dilators (D)	123 (44.6)	0.93	17.0
Enhanced dilators (ED)	36 (13.0)	0.98	311.0

Figure 1.

Absolute change in lumen diameter over the time course of the endothelial function measurement identified as non-dilators (ND), dilators (D), and enhanced dilators (ED)

Predicted probabilities with 95% confidence intervals by PCOS status, adjusted for age, baseline lumen diameter and insulin

Figure 2.

Predicted probabilities of non-dilators versus dilators by PCOS status (PCOS in blue and controls in green) and quartiles of total cholesterol

Descriptive characteristics of women with PCOS and controls overall and by trajectory group of absolute change in lumen diameter*

Table 1

	All (n=276)	Non-Dilators (n=117)	Dilators (n=123)	Enhanced Dilators (n=36)	<i>p</i> Value
PCOS, n (%)	128 (46.4)	50 (42.7)	57 (46.3)	21 (58.3)	0.21
African American, n (%)	41 (14.9)	20 (17.1)	16 (13.0)	5 (13.9)	0.66
Age, years	42.6 ± 7.0	42.5 ± 7.2	42.7 ± 6.9	42.1 ± 7.0	0.9
BMI, kg/m ²	29.2 ± 7.2	28.5 ± 6.8	29.7 ± 7.9	29.8 ± 5.9	0.42
BMI 30 kg/m ² , n (%)	112 (40.6)	45 (38.5)	51 (41.5)	16 (44.4)	0.79
Waist circumference, cm	87.5 ± 16.5	86.4 ± 16.8	87.7 ± 17.2	89.9 ± 12.7	0.55
SBP, mm Hg	114.6 ± 14.9	115.6 ± 16.0	114.4 ± 14.7	112.6 ± 11.4	0.56
Hypertension, n (%)	34 (12.3)	18 (15.4)	14 (11.4)	2 (5.6)	0.31
Insulin, µU/mL	12.9 (9.6, 18.5)	12.0 (9.2, 16.7)	13.2 (9.7, 20.7)	15.0 (11.0, 23.1)	0.03
Glucose, mg/dL	90.1 (83.9, 97.1)	91.4 (84.8, 97.7)	89.2 (82.2, 97.1)	87.4 (82.2, 94.4)	0.25
QUICKI	0.32 ± 0.03	0.33 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.17
Type 2 diabetes, n (%)	20 (7.2)	9 (7.7)	10 (8.1)	1 (2.8)	0.65
Diabetes medication use, n (%)	13 (4.7)	4 (3.4)	9 (7.3)	0 (0)	0.13
Triglycerides, mg/dL	110.0 (75.0, 156.0)	103.5 (70.3, 141.3)	110.0 (77.0, 163.0)	137.5 (89.0, 163.0)	0.15
Total cholesterol, mg/dL	205.3 ± 35.6	204.3 ± 35.2	205.2 ± 35.6	209.2 ± 37.5	0.77
Current smoker, n (%)	50 (18.1)	18 (15.4)	22 (17.9)	10 (27.8)	0.24
Oral contraceptive use, n (%)	39 (14.1)	17 (14.5)	15 (12.2)	7 (19.4)	0.54
Hormone replacement therapy use, n (%)	37 (13.4)	16 (13.7)	18 (14.6)	3 (8.3)	0.67
Postmenopausal, n (%)	57 (20.7)	27 (23.1)	25 (20.3)	5 (13.9)	0.49

* Values are the mean ± standard deviation, median (25th, 75th percentile), or number (percent)

Endothelial function parameters of women with PCOS and controls overall and by trajectory group of absolute change in lumen diameter *

Table 2

	All (n=276)	Non-Dilators (n=117)	Dilators (n=123)	Enhanced Dilators (n=36)	p Value
Baseline lumen diameter, mm	3.0 ± 0.4	3.0 ± 0.4	2.9 ± 0.4	3.1 ± 0.5	0.05
Maximum post-deflation diameter, mm	3.2 ± 0.5	3.1 ± 0.4	3.1 ± 0.4	3.5 ± 0.5	0.0001
Absolute change in lumen diameter, mm	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	<0.0001
Flow mediated-dilation (FMD), percent	6.4 ± 4.6	2.8 ± 2.8	7.8 ± 2.4	13.5 ± 3.8	<0.0001

* Values are the mean ± standard deviation.

Table 3

Multinomial logistic regression analysis of participant characteristics associated with trajectory group of absolute change using dilators as the referent group

Characteristic	Non-Dilators		Enhanced Dilators	
	Odds Ratio (95% CI)	p Value	Odds Ratio (95% CI)	p Value
Model 1				
PCOS, vs. control	0.92 (0.54, 1.58)	0.76	1.48 (0.68, 3.23)	0.33
Age, years	0.99 (0.96, 1.03)	0.72	1.00 (0.95, 1.05)	0.93
Baseline LD, mm	2.51 (1.29, 4.89)	0.007	2.10 (0.84, 5.27)	0.11
Insulin, per SD of the log [†]	0.70 (0.52, 0.93)	0.02	1.04 (0.71, 1.51)	0.86
Model 2				
PCOS, vs. control	4.00 (1.08, 14.91)	0.04	6.79 (0.97, 47.63)	0.05
Age, years	0.99 (0.96, 1.04)	0.78	1.00 (0.94, 1.06)	0.91
Baseline LD, mm	2.70 (1.37, 5.32)	0.004	2.25 (0.89, 5.66)	0.09
Insulin, per SD of the log [†]	0.72 (0.54, 0.98)	0.04	1.07 (0.72, 1.58)	0.74
Cholesterol quartiles [*]		0.02 [‡]		0.09 [‡]
Cholesterol quartiles in PCOS	0.68 (0.33, 1.41)		0.75 (0.25, 2.27)	
Cholesterol quartiles in control	1.23 (0.90, 1.69)		1.37 (0.83, 2.27)	

PCOS: polycystic ovary syndrome; LD: lumen diameter; CI: confidence interval; SD: standard deviation

[†]The standard deviation of log insulin: 0.216

^{*}Cholesterol categorized into the following quartiles: 179, 180–204, 205–230, and 231 mg/dL

[‡]p Value corresponds to the PCOS*cholesterol quartile interaction