Published in final edited form as:
Atherosclerosis. 2011 August ; 217(2): 437–440. doi:10.1016/j.atherosclerosis.2011.04.009.

Associations of Cardiovascular Risk Factors with Two Surrogate Markers of Subclinical Atherosclerosis: Endothelial Function and Carotid Intima Media Thickness

Kathleen V. Fitch[a], **Eleni Stavrou**[a], **Sara E. Looby**[a], **Linda Hemphill**[b], **Michael R. Jaff**[c], and **Steven K. Grinspoon**[a]

[a] Program in Nutritional Metabolism and Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA., USA

[b] Massachusetts General Hospital Cardiovascular Division, Boston, MA., USA

[c] Massachusetts General Hospital Vascular Center, Boston, MA, USA

Abstract

Objective—Endothelial function and carotid intima media thickness (cIMT) were investigated in a cohort of 54 healthy adults without known cardiovascular disease.

Methods—Pulse wave amplitude was determined with peripheral arterial tonometry (PAT) to obtain the reactive hyperemia (RH)-PAT ratio. Ultrasound was used to determine cIMT.

Results—cIMT and RH-PAT were significantly associated (rho = −0.35, P = 0.02) in univariate analysis. RH-PAT was significantly associated with age, triglycerides, fasting glucose, HDL, WHR, waist circumference and VAT. cIMT was associated with age, smoking history, fasting glucose, systolic blood pressure, diastolic blood pressure and LDL. In multivariate regression analyses, triglyceride level (P = 0.04) remained a significant determinant of RH-PAT whereas systolic blood pressure (P = 0.02) and smoking pack-year history (P = 0.046) were significant determinants of cIMT.

Conclusion—Determinants of cIMT and RH-PAT were different, dominated by triglyceride and abdominal adiposity measures for RH-PAT but traditional risk factors including blood pressure, glucose, smoking and LDL for cIMT.

Keywords

Endothelial function; carotid intima media thickness; atherosclerosis

Please address correspondence and reprint requests to: Steven K. Grinspoon, MD, LON5-207; 55 Fruit St.; Boston, MA 02114, USA, sgrinspoon@partners.org Phone: 1-617-724-9109.

Disclosures: The authors have no relevant conflicts to disclose.

Clinicaltrials.gov registration: NCT00465426

Introduction

Endothelial dysfunction and carotid intima media thickness (cIMT) are both surrogate markers of atherosclerotic disease. Both have been shown to predict future CVD events [12]. However, a careful investigation of the early cardiovascular risk markers underlying these two processes, measured simultaneously in a healthy cohort of patients without known CVD, has not been performed.

Digital peripheral arterial tonometry (PAT) to assess pulse wave amplitude (PWA), is emerging as a clinical tool to measure peripheral vasodilator response [3], an indicator of endothelial function. PAT correlates with flow mediated vasodilation (FMD), a method that has been validated for evaluating endothelial function [4]. Carotid intima media thickness (cIMT), a measure of structural abnormalities, is another indicator of early atherosclerotic changes. Similar to measures of endothelial function, cIMT has also been related to traditional CVD risk factors [5]. The aim of the present study was to simultaneously evaluate determinants of endothelial dysfunction as measured by PAT and cIMT in a cohort of healthy adults.

Methods and Procedures

Fifty-four male and female subjects, ages 18–65, were recruited from July 2008 to October 2009. The subjects were healthy, asymptomatic of CVD, without a history of acute or chronic disease including current CVD and/or diabetes mellitus. The study was approved by the Massachusetts General Hospital (MGH) institutional review committee and subjects provided written informed consent. Prior data relating neck circumference to cIMT were published in this group [6], however, no data on RH-PAT or the relationship of RH-PAT to cIMT have been published.

Body Composition

Weight and anthropometric measurements were determined using standard methodologies. To assess abdominal visceral and subcutaneous adipose tissue area (VAT and SAT, respectively), a cross-sectional abdominal CT scan at the level of the L4 pedicle was performed [6].

Carotid Intima Media Thickness

Measurement of cIMT was performed as previously described [6]. The intima media thickness over the length of the left and right segment is reported as an average of the two measurements.

Endothelial Function

Digital pulse wave amplitude was measured with a PAT device placed on the tip of each index finger (Endo-PAT2000, Itamar Medical, Caesarea, Israel). Endothelial function was measured via a reactive hyperemia (RH)-PAT ratio. An RH-PAT protocol consists of a 5 minute baseline measurement, after which a blood pressure cuff on the test arm was inflated to 60 mmHg above baseline systolic blood pressure or at least 200 mmHg for 5 minutes. Occlusion of pulsatile arterial flow was confirmed by the reduction of the PAT tracing to zero. After 5 minutes, the cuff was deflated and the PAT tracing was recorded for a further 5 minutes. The ratio of the PAT signal after cuff release compared with baseline was calculated through a computer algorithm automatically normalizing for baseline signal and indexed to the contra lateral arm. The calculated ratio reflects the RH-PAT. As per prior testing protocols with the Endo-PAT device [7], subjects were not required to be fasting.

Biochemical Indices

Biochemical indices were measured using standard methodology.

Statistical Analysis

Comparison of variables was made using Student's t-test for continuous variables and the Chi-square test for noncontinuous variables. Continuous variables were tested for normality of distribution by using the Shapiro-Wilk test and examination of the histogram distribution. Because RH-PAT results were not normally distributed, Spearman correlation coefficients (rho) were assessed in univariate analyses comparing RH-PAT and cardiovascular risk parameters. Pearson correlation coefficients (r) were assessed in univariate analyses comparing cIMT and other covariates. Separate multivariate regression analyses were performed using standard least squares to determine the association of statistically significant outcomes in the univariate analyses. Gender was included in each model. Statistical significance was defined as *P* value of less than 0.05. Statistical analyses were performed using SAS JMP statistics software (SAS Institute Inc., Cary, NC).

Results

Fifty-four subjects enrolled in the study; RH-PAT data were obtained in 46 participants. Demographic, metabolic and body composition parameters are shown for all subjects in Table 1. RH-PAT and cIMT did not differ significantly among men and women. Twelve participants were active smokers during the study, but neither cIMT (0.73 ± 0.04 mm vs. 0.70 ± 0.02 mm, P = 0.45) nor RH-PAT (1.6 ± 0.2 vs. 1.9 ± 0.1, P = 0.15) differed significantly among current vs. non-smokers. Five participants (9 %) reported currently taking lipid lowering therapy and 10 participants (19 %) reported current antihypertensive use. Carotid IMT was not significantly different among the participants receiving vs. not receiving lipid lowering therapy (0.76 ± 0.07 vs. 0.70 ± 0.02 mm, P = 0.40) or antihypertensive medications (0.78 ± 0.05 vs. 0.69 ± 0.02 mm, P = 0.07). Similarly, RH-PAT was not different among the participants with respect to use of lipid lowering therapy (1.5 ± 0.28 vs. 1.9 ± 0.09, P = 0.24) or antihypertensive medications (1.9 ± 0.19 vs. 1.8 ± 0.09, P = 0.80).

Relationship of RH-PAT, cIMT and Cardiometabolic risk parameters

In univariate analysis among all subjects RH-PAT and average cIMT were significantly related (rho = −0.35, P = 0.02). RH-PAT was negatively associated with age, fasting glucose, triglycerides (Supplementary Figure 1), WHR, waist and neck circumference, as well as VAT area, and trended toward significance with BMI. RH-PAT was positively associated with HDL-cholesterol (Table 2). cIMT was also related to several cardiometabolic risk factors, however the pattern or risk factors associated with cIMT was distinct from that of RH-PAT. cIMT was positively associated with age, pack years smoking, systolic and diastolic blood pressure, fasting glucose, hemoglobin A1C, LDL-cholesterol, and neck circumference. cIMT was negatively associated with HDL-cholesterol (Table 2).

Multiple Regression Analysis of RH-PAT and Average cIMT

Multiple regression analysis was performed among the entire cohort for RH-PAT including variables that met statistical significance in univariate analysis of RH-PAT. Total R^2 for the whole model was 0.49, the parameter estimates (β) and P values from the model are shown in Table 2. Triglyceride level was found to have a significant negative association with RH-PAT in the model controlling for age, gender, HDL-cholesterol, triglycerides, fasting glucose, average cIMT, VAT, WHR, waist circumference and neck circumference.

Triglyceride level was the only factor that remained significantly associated with RH-PAT in the model, accounting simultaneously for other risk factors.

Multiple regression analysis was performed among the entire cohort for cIMT including variables that met statistical significance in univariate analysis of cIMT. Total R^2 was 0.52, the parameter estimates (β) and P values from the model are shown in Table 2. In this analysis, pack-year smoking history and systolic blood pressure were significant predictors of cIMT controlling for age, gender, diastolic blood pressure, hemoglobin A1C, HDL- and LDL-cholesterol, fasting glucose, and neck circumference.

Discussion

In this study we simultaneously examined associations between cardiometabolic risk factors and two markers of subclinical atherosclerosis, endothelial function and cIMT. We investigated several known risk factors for heart disease and identified significant factors that are independently associated with cIMT and endothelial dysfunction as measured by RH-PAT. Pulse wave amplitude to assess endothelial function is a relatively new, noninvasive technique to measure this marker of CVD. Few studies have explored associations of CVD risk factors with RH-PAT [4, 8, 9].

In the present study, in multivariate regression analysis controlling for several cardiovascular risk factors, only triglyceride level was independently associated with RH-PAT. In the Framingham Cohort Study, triglyceride level was correlated with RH-PAT in univariate analysis. However, this relationship was not present in multivariate analysis controlling for traditional CVD risk factors [8]. Several studies have evaluated triglycerides, exploring the effect of chronic or transient elevations of triglyceride levels on the endothelium and have found that both states are associated with endothelial dysfunction [10, 11], although results are not consistent [12]. Triglycerides or triglyceride-rich proteins may activate the expression of pro-inflammatory molecules such as NF-κB and CREB [13]. In addition, hypertriglyceridemia has been linked with higher plasma levels of asymmetric dimethylarginine (ADMA), which decreases nitric oxide production and has been found to be related to decreased endothelial function [10].

In contrast to RH-PAT, systolic blood pressure and pack-year smoking history remained significant predictors of cIMT in multivariate regression modeling in the present study. Large studies have evaluated the association of cardiovascular risk factors with cIMT [14]. Hypertension and smoking have been associated with cIMT and atherosclerosis [15], but risk factors have not been simultaneously compared to RH-PAT and cIMT in prior studies.

Strengths of our study include the simultaneous assessment of two markers of subclinical atherosclerosis and their association with traditional cardiovascular risk factors in a healthy cohort without known cardiovascular disease. We demonstrated that although cIMT and RH-PAT are correlated with each other to a modest degree, very distinct patterns of risk association were seen comparing the two indices. This observation has implications for the pathophysiology of atherosclerotic disease, suggesting that hypertriglyceridemia may relate most strongly to functional vasodilatory properties assessed in a specific and increasingly commonly used test of endothelial function, whereas smoking and blood pressure more strongly influence the development of atherosclerotic plaque. In addition, we demonstrate strong relationships of measures of central adiposity to RH-PAT. However, these associations were no longer statistically significant in multivariate regression testing, suggesting that the relationship demonstrated in univariate analysis was a function of hypertriglyceridemia associated with abdominal fat accumulation. These results also have implications for the choice of noninvasive tests to investigate early subclinical

cardiovascular disease. Our data demonstrate that in such patients, cIMT will reflect more traditional risk factors, but will be insensitive to triglyceride-related changes in endothelial function. Conversely, RH-PAT, as assessed by the Endo-PAT method is more modestly associated with traditional risk factors that contribute to development of atherosclerotic plaque, but highly associated with hypertriglyceridemia. The cross-sectional design of this study is a limitation, however, and causality cannot be determined from this design.

Conclusion

To our knowledge, this is the first study to simultaneously assess cIMT and RH-PAT in a cohort of asymptomatic adults. We found a relationship between cIMT and RH-PAT, two surrogate markers of subclinical atherosclerosis. Both of these markers were significantly associated with several cardiovascular risk factors although in unique patterns. These findings suggest that abnormalities of endothelial function and cIMT are likely two distinct processes in the development of atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank the subjects who participated in this study and the nursing and bionutrition staff of the MGH Clinical Research Center for their excellent patient care. The authors have no relevant conflicts of interest to disclose.

Funding: Funding was provided by NIH R01 DK049302 and K24 DK064545-08 to S.K.G. and by NIH M01-RR-01066 and 1 UL1 RR025758-01, Harvard Clinical and Translational Science Center, from the National Center for Research Resources. NIH funding also provided through K23 NR011833-01A1 to S.E.L. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

References

1. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000; 101:1899–1906. [PubMed: 10779454]
2. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997; 96:1432–1437. [PubMed: 9315528]
3. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, Karas RH, Udelson JE. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J. 2003; 146:168–174. [PubMed: 12851627]
4. Kuvin JT, Ramet ME, Patel AR, Pandian NG, Mendelsohn ME, Karas RH. A novel mechanism for the beneficial vascular effects of high-density lipoprotein cholesterol: enhanced vasorelaxation and increased endothelial nitric oxide synthase expression. Am Heart J. 2002; 144:165–172. [PubMed: 12094204]
5. Urbina EM, Srinivasan SR, Tang R, Bond MG, Kieltyka L, Berenson GS. Impact of multiple coronary risk factors on the intima-media thickness of different segments of carotid artery in healthy young adults (The Bogalusa Heart Study). Am J Cardiol. 2002; 90:953–958. [PubMed: 12398961]
6. Fitch K, Stanley T, Looby S, Rope A, Grinspoon S. Relationship between neck circumference and cardiometabolic parameters in HIV-infected and non-HIV-infected adults. Diabetes Care. 2011 Epub ahead of print.
7. Kuvin JT, Mammen A, Mooney P, Alsheikh-Ali AA, Karas RH. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vasc Med. 2007; 12:13–16. [PubMed: 17451088]

8. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, Mitchell GF, Sheffy J, Vita JA, Benjamin EJ. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation. 2008; 117:2467–2474. [PubMed: 18458169]

9. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, Pumper GM, Lerman LO, Lerman A. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J. 2010; 31:1142–1148. [PubMed: 20181680]

10. Lundman P, Eriksson MJ, Stuhlinger M, Cooke JP, Hamsten A, Tornvall P. Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. J Am Coll Cardiol. 2001; 38:111–116. [PubMed: 11451259]

11. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol. 1997; 79:350–354. [PubMed: 9036757]

12. Raitakari OT, Lai N, Griffiths K, McCredie R, Sullivan D, Celermajer DS. Enhanced peripheral vasodilation in humans after a fatty meal. J Am Coll Cardiol. 2000; 36:417–422. [PubMed: 10933351]

13. Norata GD, Grigore L, Raselli S, Redaelli L, Hamsten A, Maggi F, Eriksson P, Catapano AL. Post-prandial endothelial dysfunction in hypertriglyceridemic subjects: molecular mechanisms and gene expression studies. Atherosclerosis. 2007; 193:321–327. [PubMed: 17055512]

14. Polak JF, Backlund JY, Cleary PA, Harrington AP, O'Leary DH, Lachin JM, Nathan DM. Progression of Carotid Artery Intima-Media Thickness During 12 Years in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. Diabetes. 2011; 60:607–613. [PubMed: 21270271]

15. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, Jarvisalo MJ, Uhari M, Jokinen E, Ronnemaa T, Akerblom HK, Viikari JS. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA. 2003; 290:2277–2283. [PubMed: 14600186]

Table 1

Characteristics of the study subjects

	Mean ± SEM (n = 54)	Range
Demographics		
Age (years)	49 ± 1	25–63
Gender [n (%)]		
Male	26 (48)	
Female	28 (52)	
Race [n (%)]		
White	27 (50)	
Black	27 (50)	
American Indian/Alaska Native	0 (0)	
More than one race	0 (0)	
Lifetime smoking (pack-years)	8.7 ± 2.7	0–103.5
Metabolic Parameters		
Systolic Blood Pressure (mmHg)	119 ± 2	95–143
Diastolic Blood Pressure (mmHg)	76 ± 1	42–100
Fasting Glucose (mg/dl)	86 ± 1	68–112
2-h Glucose (mg/dl)	109 ± 5	43–234
Fasting Insulin (μU/ml)	3.4 ± 0.4	0.1–15.4
Hgb A1C (%)	5.6 ± 0.07	4.7–6.7
Total Cholesterol (mg/dl)	174 ± 4	120–260
HDL-Cholesterol (mg/dl)	53 ± 2	25–90
LDL-Cholesterol (mg/dl)	105 ± 4	40–173
Triglycerides (mg/dl)	82 ± 5	28–186
C-reactive protein (mg/L)	3.40 ± 0.81	0.04–27.7
Endothelial function (RH-PAT)	1.8 ± 0.08	1.1–3.4
cIMT	0.70 ± 0.02	0.45–1.10
Body Composition Parameters		
BMI (kg/m^2)	28.2 ± 0.7	19.5–42.5
WHR	0.91 ± 0.01	0.72–1.07
SAT area (cm^2)	285 ± 20	59–711
VAT area (cm^2)	111 ± 10	9–294
Iliac waist (cm)	97 ± 1.9	69–131
Neck circumference (cm)	38.1 ± 0.5	29.9–44.8

Data reported as mean ± standard error of the mean (SEM) or percentage. HDL, high-density lipoprotein; LDL, low-density lipoprotein; cIMT, carotid intima media thickness; BMI, body mass index; WHR, waist to hip ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; RH-PAT, reactive hyperemia-peripheral arterial tonometry.

Table 2

Univariate & Multivariate Associations Between Endothelial Function, cIMT and Cardiometabolic Parameters

	Endothelial Function				cIMT			
	Univariate Analysis		Multivariate Analysis†		Univariate Analysis		Multivariate Analysis¥	
	rho§	P	β	P	r*	P	β	P
Demographics								
Age (yrs)	−0.32	0.03	0.001	0.94	0.51	0.0001	0.004	0.08
Smoking history (pack years)	−0.28	0.06			0.43	0.001	**0.002**	**0.046**
Gender	N/A	0.10	−0.03	0.85	N/A	0.33	−0.02	0.40
Metabolic and Cardiovascular Parameters								
Systolic blood pressure (mmHg)	−0.14	0.35			0.40	0.003	**0.006**	**0.02**
Diastolic blood pressure (mmHg)	0.08	0.61			0.30	0.03	−0.003	0.23
Fasting glucose (mg/dl)	−0.32	0.04	0.003	0.79	0.47	0.0005	0.001	0.72
2-hr glucose (mg/dl)	−0.19	0.24			0.19	0.18		
Fasting insulin (μU/ml)	0.05	0.75			0.22	0.12		
Hemoglobin A1c (%)	−0.11	0.50			0.36	0.008	0.04	0.28
Total cholesterol (mg/dl)	−0.11	0.45			0.26	0.06		
HDL-cholesterol (mg/dl)	0.47	0.001	0.01	0.09	−0.31	0.02	−0.002	0.80
Triglycerides (mg/dl)	−0.52	0.0002	**−0.006**	**0.04**	0.21	0.12		
LDL-cholesterol (mg/dl)	−0.15	0.33			0.35	0.01	−0.0002	0.80
C-reactive protein (mg/L)	−0.22	0.15			−0.21	0.13		
cIMT (mm)	−0.35	0.02	−1.07	0.14	N/A	N/A		
Body Composition Parameters								
BMI (cm/kg^2)	−0.29	0.054			0.10	0.49		
WHR	−0.37	0.01	−1.09	0.44	0.25	0.08		
Iliac waist circumference (cm)	−0.38	0.01	−0.005	0.64	0.22	0.11		
SAT area (cm^2)	−0.23	0.13			0.11	0.46		
VAT area (cm^2)	−0.34	0.03	0.002	0.24	0.23	0.09		
Neck Circumference (cm)	−0.34	0.02	0.004	0.94	0.29	0.03	0.006	0.43

rho§ is Spearman correlation coefficient; r* is Pearson correlation coefficient.

† $R^2 = 0.49$, P = 0.008

¥ $R^2 = 0.52$, P = 0.0004

LDL, low density lipoprotein; HDL, high density lipoprotein; WHR, waist-to-hip ratio; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; cIMT, carotid intima media thickness