Published in final edited form as:

Atherosclerosis. 2009 November ; 207(1): 277–283. doi:10.1016/j.atherosclerosis.2009.04.024.

Tissue factor pathway inhibitor, vascular risk factors and subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis

CT Mitchell^{*}, A Kamineni[†], W Palmas[‡], and M Cushman^{*}

^{*} Department of Medicine, University of Vermont, Burlington, VT, USA

[†] Department of Biostatistics, University of Washington, Seattle, WA, USA

[‡] Department of Medicine, Columbia University, New York, NY, USA

Abstract

Background—Tissue factor pathway inhibitor (TFPI) is an endothelial membrane-associated anticoagulant protein. Higher circulating levels might reflect endothelial damage.

Objective—We hypothesized an association of higher total TFPI with subclinical atherosclerosis.

Patients/Methods—Total TFPI was measured in 1000 participants of the Multi-Ethnic Study of Atherosclerosis, a cohort of 6814 men and women without clinical vascular disease, aged 45–84, from 4 ethnic groups. Subclinical atherosclerosis measures were coronary artery calcium (CAC), carotid intima-media thickness (IMT) and ankle-brachial index (ABI).

Results—TFPI was higher with age, male gender, higher LDL-cholesterol, smoking and diabetes, but not ethnicity. Adjusting for risk factors, TFPI in the 4th versus 1st quartile was associated with a 1.2-fold increased risk of detectable CAC (95% CI 1.0–1.4), a 2.1-fold increased risk of CAC >400 Agatston units (95% CI 1.1–4.0) and a 1.6-fold (95% CI 1.1–2.5) increased risk of internal carotid IMT above the 80th percentile, but not with external carotid IMT or low ABI. Findings were consistent across ethnic groups.

Conclusions—In this diverse population, higher total TFPI was associated with prevalent CAC (limited to levels >400 units), and elevated internal carotid IMT, independent of other factors. Higher TFPI may indicate endothelial dysfunction. Further study is needed of TFPI and progression of atherosclerosis.

Keywords

atherosclerosis; coronary heart disease; tissue factor pathway inhibitor; risk factor

Tissue factor pathway inhibitor (TFPI) is an endothelial membrane-associated protein¹. TFPI co-localizes with tissue factor and macrophages in atherosclerotic plaques, where it may reduce thrombotic and inflammatory potential^{2–4}. In vascular injury models, TFPI infusion or local gene delivery had favorable effects on thrombosis and restenosis^{5–7}, while TFPI deficiency

Corresponding Author: Mary Cushman, MD, MSc, Department of Medicine, University of Vermont. 208 South park Drive, Suite 2, Colchester, VT, USA, 05446. fax: 802-656-8965; phone: 802-656-8968; mary.cushman@uvm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

in atherosclerosis-prone mice was associated with more atherosclerosis, more plaque tissue factor activity and decreased time to occlusive thrombosis with vascular injury⁸.

TFPI circulates primarily bound to lipoproteins with a smaller free component; both can be measured with commercial assays^{9, 10}. Both free and total TFPI concentration may be higher with acute or chronic vascular disease^{11, 12}. Not all studies agree, however^{13–15}. Epidemiologic studies have reported conflicting findings concerning correlation of TFPI with vascular risk factors.^{10, 16}

Only a few studies assessed associations of TFPI with subclinical atherosclerosis measures.^{12, 16, 17}. Given the few available studies of TFPI epidemiology, and lack of information on distributions by ethnicity, we evaluated associations of plasma total TFPI with cardiovascular risk factors, subclinical atherosclerosis and biomarkers of endothelial function in four ethnic groups in the Multiethnic Study of Atherosclerosis (MESA).

Methods

Subjects

The MESA cohort of 6814 subjects aged 45–84 was recruited and examined in 2000–2002 at six U.S. field centers. All participants were free of clinical vascular diagnoses. Women were 53% of the cohort and there were four ethnic groups; 38% Caucasian, 28% African-American, 22% Hispanic, and 12% Chinese. A detailed description of recruitment methods, study design, and examination components has been published¹⁸. All participants provided informed consent for the study using procedures approved by institutional human subjects committees.

The baseline examination included assessment of medical history and demographic information. Blood pressure measurement, electrocardiography, carotid ultrasound, ankle-brachial blood pressure index (ABI), and cardiac computed tomography for presence of coronary artery calcium (CAC) were performed. Medication use (statins, aspirin, and postmenopausal hormone therapy) was ascertained by self-report and review of prescription bottles.

After enrollment of 5030 participants, a random sample of 1000 was selected for measurement of biomarkers of vascular disease risk, including total TFPI. Eligibility included completion of coronary computed tomography and consent for use of DNA samples for research.

Laboratory Methods

Fasting morning blood samples were collected and promptly centrifuged for =30,000 g-minutes, at –4 °C. Plasma and serum were shipped overnight on dry ice to a central laboratory and stored at –80 °C. Total TFPI was measured using a sandwich ELISA using polyclonal antibodies for capture and a monoclonal detection antibody specific for the Kunitz domain 1 of TFPI (Imubind Total TFPI, American Diagnostica, Inc, Stamford, CT). Intra-assay and inter-assay coefficients of variation (CVs) ranged from 6.2–7.1% and 5.5–7.3%. von Willebrand factor was measured by immunoturbidometric assay on the Sta-R analyzer (Liatest vWF, Diagnostica Stago, Parsippany, NJ), with intra-assay and inter-assay CVs of 3.7% and 4.5%, respectively. Intercellular adhesion molecule-1 (ICAM-1) was measured by ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN) with CVs of 5.0%.

Definitions of Risk Factors

Smoking status was categorized as never, former, or current use. Diabetes status was defined as normal, impaired fasting glucose (IFG; glucose 100–125 mg/dl), or diabetes (glucose ≥126 mg/dl or use of diabetes medications). Hypertension was defined as self-reported history with

use of anti-hypertensive medications, or systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg. Chronic kidney disease was defined as estimated glomerular filtration rate < 60 ml/min/1.73m² based on the Modification of Diet in Renal Disease equation.

Assessment of Subclinical Atherosclerosis

The ABI was calculated using bilateral brachial artery pressure and dorsalis pedis or posterior tibial artery pressure (whichever was larger) measured by Doppler probe¹⁸. Values < 0.9 were considered abnormal. The CAC score was determined using chest computed tomography with each participant scanned twice and the average Agatston score calculated. Scores were adjusted with a standard calcium phantom scanned simultaneously with the participant, and images read centrally¹⁹. CAC was expressed as zero versus non-zero values and categorized into four groups; zero, 0–100, 101–400, and > 400 Agatston units²⁰. Internal and common carotid IMT were measured by ultrasound and standardized methods using a GE scanner, with videotaped scans read centrally²¹. Values of maximum IMT for each artery above the gender-specific 80th percentile were classified as abnormal. A composite subclinical disease variable was created classifying any subject with either ABI < 0.9 , CAC > 0 Agatston units, common or internal IMT $> 80^{\text{th}}$ percentile or presence of major ECG abnormalities.

Statistical Analysis

Analyses were performed using Stata 9.2 (College Station, Texas). Risk factor levels by gender and ethnic group were compared using t-tests or chi-squared tests. Distributional characteristics of TFPI were examined by gender and ethnic group. Linear regression was used to analyze associations of TFPI with risk factors. All risk factors significantly associated with TFPI in univariate analyses were included in a multivariable model to assess independent correlates of TFPI. Effect modification by gender or ethnicity was tested creating interaction terms for variables showing an independent association with TFPI. A p-value < 0.10 for the interaction was defined as significant. Next, we evaluated the association of TFPI categorized in sex and race-specific quartiles of the distribution, with subclinical atherosclerosis. Relative risk regression models were used with each subclinical disease measure as the dependent variable and TFPI as the main predictor variable, with and without adjustment for risk factors related to TFPI in previous analysis. Those variables that did not change the TFPI coefficient (expressed as a continuous variable) by $> 10\%$ were not included in the final model. Finally, to qualitatively compare associations of TFPI and LDL-cholesterol with atherosclerosis, we constructed similar models assessing the relative risk of each subclinical disease by quartiles of LDL-cholesterol, adjusted for lipid lowering therapy and TFPI.

Results

Participant Characteristics

There were 995 participants with TFPI results. Characteristics by gender are shown in Table 1. The mean age was 59.4 years and 57.1% were women. More women than men were hypertensive or obese. Men were more likely to have a glucose disorder, smoking history and subclinical disease and had lower HDL than women. CAC was present in 57.9% of men and 34.3% of women. Men were more likely to have CAC scores greater than 400 Agatston units than women.

TFPI concentrations ranged between 15.0 – 100.0 ng/ml with a mean (SD) of 48.6 (14.3) ng/ml. Figure 1 shows the distribution by gender and ethnic group. Men had higher values than women (table 1). Among ethnic groups mean TFPI was similar, except Chinese subjects had lower values, 42.9 (14.2) ng/ml compared to 49.4 (14.1) ng/ml for the other three groups.

Associations of TFPI and Risk Factors

Table 2 shows associations of TFPI with demographic characteristics and cardiovascular risk factors. For each continuous variable, the difference in TFPI concentration per SD higher value of that continuous variable is shown. For categorical variables the difference in TFPI among groups is shown. In models including age, gender, race and other vascular risk factors related univariately with TFPI, TFPI was significantly higher with age, male gender, higher LDL, current smoking, and diabetes. Levels remained lower among Chinese compared to Caucasians, but this was only statistically significant among men (p for ethnicity*gender 0.076). TFPI levels were similar among African-Americans, Hispanics and Caucasians. Bivariate associations of TFPI with blood pressure, kidney disease and body size measures were not significant after adjustment for other risk factors correlated with TFPI. In gender-specific models, the associations of TFPI with LDL and diabetes were larger among women than men (p for interaction 0.009 and 0.076, respectively). Women with kidney disease had higher TFPI, but not men. Interpretation of results on kidney function was not altered when estimated glomerular filtration rate was considered as a continuous variable. Among women, current use of hormone replacement therapy was strongly associated with lower TFPI, with levels 9.4 ng/ml lower than non-users. Women using estrogen plus progesterone had higher TFPI than women taking estrogen alone, but this difference was not statistically significant (data not shown).

Supplemental Table 1 shows the associations between TFPI and risk factors stratified by ethnicity. LDL cholesterol was the only risk factor significantly associated with higher TFPI in all four ethnic groups, an association that did not differ by ethnicity ($p=0.85$). The association of TFPI with older age also did not differ by ethnicity ($p=0.43$). The association of TFPI with male gender was most apparent among Caucasians and least apparent among African-Americans and Hispanics ($p=0.068$ for ethnic group differences). Current smokers had higher TFPI among Caucasians and African-Americans but not other ethnic groups ($p=0.063$ for ethnic group differences). The association of diabetes with higher TFPI appeared larger among Caucasians and Hispanics, but this difference by ethnicity was not statistically significant ($p=1.0$).

The unadjusted increment difference in TFPI for each standard deviation higher von Willebrand factor and ICAM-1 were +2.5 ng/ml and +1.6 ng/ml, respectively ($p<0.001$). Adjustment for age, sex and race had little impact on these associations; increment differences +2.1 and +1.3 ng/ml, respectively ($p<0.001$ and <0.01).

Associations between TFPI and Subclinical Disease Measures

Figure 2 shows the unadjusted mean (SD) TFPI by subclinical disease measures. TFPI was higher in all groups with compared to without subclinical disease, except for major ECG abnormalities (all other $p<0.004$). It was also higher across the four increasing CAC categories. Associations were similar in gender and race-stratified analyses (data not shown).

Table 3 shows the crude and adjusted associations of TFPI quartiles with each subclinical disease measure. In unadjusted analysis, TFPI was associated with every subclinical disease measure except major ECG abnormalities. Those with TFPI in the fourth compared to the first quartile had a 3-fold higher risk of ABI <0.9 , a 1.4-fold higher risk of any CAC, a 4.1-fold higher risk of CAC >400 Agatston units, a 2.5-fold higher risk of an internal carotid IMT above the 80th percentile, and a 1.7-fold increased risk of elevated common carotid IMT. Adjusting for age, gender, and LDL cholesterol, the only influential risk factors in the models, the associations of TFPI in the 4th quartile with low ABI and higher common carotid IMT were no longer apparent. There was a 1.2-fold increased risk of CAC >0 Agatston units, and this was mainly driven by an association with CAC >400 units, where the adjusted odds ratio was

2.1. There was a 1.6-fold increased risk of elevated internal carotid IMT for TFPI in the 4th quartile. Adjustment for lipid lowering therapy did not alter interpretation of any of these models. In analyses excluding 186 women using postmenopausal hormones, associations of TFPI with subclinical disease were similar (data not shown). In analyses including only participants with detectable CAC, adjusting for age, gender and LDL, each 1 unit higher log CAC was associated with 0.57 ng/ml higher TFPI (95% CI -0.13–1.27 ng/ml; $p = 0.11$). Inclusion of other risk factors correlated with TFPI (from table 2) as covariates in all of these relative risk regression models did not change the results substantially.

Discussion

The major findings of this study of a multi-ethnic cohort were associations of higher total TFPI concentration with CAC and internal carotid artery IMT, independent of cardiovascular risk factors including LDL cholesterol, which was correlated with TFPI. In unadjusted models, higher TFPI was also associated with lower ABI and higher common carotid IMT, but these associations were explained by age and LDL in adjusted models. The association of TFPI with CAC was not graded, and was limited to those with CAC >400 Agatston units.

Our findings in nearly 1000 participants add to previous smaller studies of TFPI and carotid IMT^{12, 16, 17} by demonstrating clear associations with higher internal, but not common carotid IMT, and a newly described association with CAC, independent of other risk factors. Similar to our findings for TFPI antigen, the Cardiovascular Health Study reported higher total TFPI activity with carotid artery stenosis and higher internal carotid IMT, independent of other risk factors, in 400 elderly participants without clinical cardiovascular disease¹⁶. In the Suita Study of 245 men and 277 women in Japan, higher free TFPI was associated with higher internal carotid IMT in men only, however the association was not independent of vascular risk factors¹⁷. A study of 20 healthy patients with carotid plaque showed higher total TFPI antigen and TFPI activity with plaque, but there was no adjustment for vascular risk factors¹².

TFPI has been evaluated in three prospective studies assessing risk of vascular events; all measured the free form with conflicting results. In the AtheroGene Study, among 523 patients with unstable angina or myocardial infarction, higher free TFPI antigen was associated with baseline disease severity, but not with 2-year risk of cardiovascular death¹⁵. The Prospective Epidemiological Study of Myocardial Infarction demonstrated lower, but not higher, free TFPI antigen as a risk factor for future coronary events in healthy men¹⁴, presumably reflecting impaired anticoagulant function²². Conversely, in a small study that did not include adjustment for potential confounders, higher free TFPI antigen was associated with future coronary events in patients with unstable angina¹¹.

LDL-cholesterol and total TFPI were closely related in all four ethnic groups and both genders because over 80% of circulating TFPI is bound to lipoprotein complexes, with the remainder as free TFPI²³. Our findings of associations of total TFPI with subclinical disease, independent of LDL-cholesterol suggest the possibility that measurement of total TFPI may reflect the free component if LDL is accounted for. Further studies are needed to assess the role of TFPI independent of LDL, and the association of free TFPI with the progression of atherosclerosis.

Previous studies do not all agree, but variably reported that TFPI is higher with older age, male gender, increased LDL, smoking, diabetes, and markedly decreased in women taking exogenous female hormones^{10, 16}. Our study yielded similar results, clarifying prior discrepant findings on associations of TFPI with diabetes and smoking. We extend prior findings by our large sample size and by evaluating gender and ethnicity differences in associations. Unlike some other coagulation or endothelial function biomarkers²⁴, TFPI levels were similar in Caucasians, Hispanics and African-Americans. Lower TFPI in Chinese men is similar to

findings for other coagulation and endothelial markers, except plasminogen activator inhibitor-1, which is higher in this ethnic group²⁴. As this finding was independent of other participant characteristics, genetic differences might play a role. Although findings were not statistically significant the weaker association of total TFPI with diabetes in men seen here was similar to one prior report that free TFPI was more weakly associated with diabetes in men¹⁰. Although two ethnic-specific findings were seen; a larger association of TFPI with gender in Caucasians and no association with smoking among Chinese, there were no large differences in other TFPI correlates among the four ethnicities studied here. The ethnicity-smoking interaction may relate to the rarity of smoking and the smaller-sized Chinese sample.

We chose to measure total TFPI antigen and not free TFPI or TFPI activity, or other related factors such as tissue factor. Given the current literature on correlations of different TFPI measurements with clinical and subclinical vascular disease and with risk factors, it is likely that free TFPI better reflects the anticoagulant function of TFPI while total TFPI is a marker of endothelial damage. Our findings that TFPI was correlated with von Willebrand factor and ICAM-1, which are known markers of endothelial function, support this hypothesis and some previous data¹³. Future studies should measure both forms for fuller analysis.

Strengths of this study include selection of a multiethnic cohort free of cardiovascular disease at enrollment, who will be followed over time for progression of subclinical disease and vascular events. We are not aware of other studies addressing differences in TFPI in multiple ethnic groups. Standardized methods for data collection were used, including a central laboratory. Some limitations deserve discussion. The population was volunteers selected from six centers, and may not represent the overall population. TFPI was measured in 995 participants, so power to observe some associations in subgroups was not optimal. For example, there were only 99 Chinese participants. Finally, observational studies do not allow inferences about causality, and our cross-sectional design cannot examine temporal relationships between TFPI and subclinical CVD. It is possible that atherosclerosis causes higher total TFPI due to endothelial damage and/or that higher TFPI contributes to the atherosclerotic process²⁻⁹.

In summary, associations of total TFPI with cardiovascular risk factors were clarified in this multiethnic sample. Total TFPI concentration was associated with higher internal carotid artery IMT and CAC, after adjusting for cardiovascular risk factors. Results were consistent in men and women and among four ethnic groups. Higher TFPI may be caused by, or contribute to, atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the other investigators, the staff, and the participants of the MESA for their valuable contributions. A full list of participating MESA investigators and institutions can be found at <http://www.mesa-nhlbi.org>. The research was supported by contracts N01-HC-95159 through N01-HC-95166 from the National Heart, Lung, and Blood Institute (NHLBI) and NHLBI research training grant T32 HL076132-01.

References

1. Kato H. Regulation of functions of vascular wall cells by tissue factor pathway inhibitor: basic and clinical aspects. *Arterioscler Thromb Vasc Biol* 2002;22:539–548. [PubMed: 11950688]
2. Drew AF, Davenport P, Apostolopoulos J, Tipping PG. Tissue factor pathway inhibitor expression in atherosclerosis. *Lab Invest* 1997;77:291–298. [PubMed: 9354763]

3. Caplice NM, Mueske CS, Kleppe LS, Simari RD. Presence of tissue factor pathway inhibitor in human atherosclerotic plaques is associated with reduced tissue factor activity. *Circulation* 1998;98:1051–1057. [PubMed: 9736590]
4. Crawley J, Lupu F, Westmuckett AD, Severs NJ, Kakkar VV, Lupu C. Expression, localization, and activity of tissue factor pathway inhibitor in normal and atherosclerotic human vessels. *Arterioscler Thromb Vasc Biol* 2000;20:1362–1373. [PubMed: 10807755]
5. Jang Y, Guzman LA, Lincoff AM, Gottsauner-Wolf M, Forudi F, Hart CE, Courtman DW, Ezban M, Ellis SG, Topol EJ. Influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. *Circulation* 1995;92:3041–3050. [PubMed: 7586275]
6. Asada Y, Hara S, Tsuneyoshi A, Hatakeyama K, Kisanuki A, Marutsuka K, Sato Y, Kamikubo Y, Sumiyoshi A. Fibrin-rich and platelet-rich thrombus formation on neointima: recombinant tissue factor pathway inhibitor prevents fibrin formation and neointimal development following repeated balloon injury of rabbit aorta. *Thromb Haemost* 1998;80:506–511. [PubMed: 9759635]
7. Yin X, Yutani C, Ikeda Y, Enjyoji K, Ishibashi-Ueda H, Yasuda S, Tsukamoto Y, Nonogi H, Kaneda Y, Kato H. Tissue factor pathway inhibitor gene delivery using HVJ-AVE liposomes markedly reduces restenosis in atherosclerotic arteries. *Cardiovasc Res* 2002;56:454–463. [PubMed: 12445886]
8. Westrick RJ, Bodary PF, Xu Z, Shen YC, Broze GJ, Eitzman DT. Deficiency of tissue factor pathway inhibitor promotes atherosclerosis and thrombosis in mice. *Circulation* 2001;103:3044–3046. [PubMed: 11425765]
9. Kokawa T, Enjyoji K, Kumeda K, Kamikubo Y, Harada-Shiba M, Koh H, Tsushima M, Yamamoto A, Kato H. Measurement of the free form of TFPI antigen in hyperlipidemia. Relationship between free and endothelial cell-associated forms of TFPI. *Arterioscler Thromb Vasc Biol* 1996;16:802–808. [PubMed: 8640408]
10. Morange PE, Renucci JF, Charles MA, Aillaud MF, Giraud F, Grimaux M, Juhan-Vague I. Plasma levels of free and total TFPI, relationship with cardiovascular risk factors and endothelial cell markers. *Thromb Haemost* 2001;85:999–1003. [PubMed: 11434709]
11. Soejima H, Ogawa H, Yasue H, Kaikita K, Nishiyama K, Misumi K, Takazoe K, Miyao Y, Yoshimura M, Kugiyama K, Nakamura S, Tsuji I, Kumeda K. Heightened tissue factor associated with tissue factor pathway inhibitor and prognosis in patients with unstable angina. *Circulation* 1999;99:2908–2913. [PubMed: 10359735]
12. Novo G, Caplice N, Tantillo R, Bonura F, Simari R, Novo S. TFPI antigen and activity levels in patients with asymptomatic atherosclerosis and target organ acute and chronic complications. *Int Angiol* 2005;24:366–371. [PubMed: 16355095]
13. Blann AD, Amiral J, McCollum CN, Lip GY. Differences in free and total tissue factor pathway inhibitor, and tissue factor in peripheral artery disease compared to healthy controls. *Atherosclerosis* 2000;152:29–34. [PubMed: 10996336]
14. Morange PE, Simon C, Alessi MC, Luc G, Arveiler D, Ferrieres J, Amouyel P, Evans A, Ducimetiere P, Juhan-Vague I. Endothelial cell markers and the risk of coronary heart disease: the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. *Circulation* 2004;109:1343–1348. [PubMed: 15023872]
15. Morange PE, Blankenberg S, Alessi MC, Bickel C, Rupprecht HJ, Schnabel R, Lubos E, Munzel T, Peetz D, Nicaud V, Juhan-Vague I, Tiret L. Prognostic value of plasma tissue factor and tissue factor pathway inhibitor for cardiovascular death in patients with coronary artery disease: the AtheroGene study. *J Thromb Haemost* 2007;5:475–482. [PubMed: 17204132]
16. Sakkinen PA, Cushman M, Psaty BM, Kuller LH, Bajaj SP, Sabharwal AK, Boineau R, Macy E, Tracy RP. Correlates of antithrombin, protein C, protein S, and TFPI in a healthy elderly cohort. *Thromb Haemost* 1998;80:134–139. [PubMed: 9684799]
17. Sakata T, Mannami T, Baba S, Kokubo Y, Kario K, Okamoto A, Kumeda K, Ohkura N, Katayama Y, Miyata T, Tomoike H, Kato H. Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries. *Atherosclerosis* 2004;176:355–360. [PubMed: 15380459]
18. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacobs DR Jr, Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic

study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002;156:871–881. [PubMed: 12397006]

19. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology* 2005;234:35–43. [PubMed: 15618373]
20. Greenland P, Bonow RO, Brundage BH, Budoff MJ, Eisenberg MJ, Grundy SM, Lauer MS, Post WS, Raggi P, Redberg RF, Rodgers GP, Shaw LJ, Taylor AJ, Weintraub WS, Harrington RA, Abrams J, Anderson JL, Bates ER, Grines CL, Hlatky MA, Lichtenberg RC, Lindner JR, Pohost GM, Schofield RS, Shubrooks SJ Jr, Stein JH, Tracy CM, Vogel RA, Wesley DJ. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography). *Circulation* 2007;115:402–426. [PubMed: 17220398]
21. Sharrett AR, Ding J, Criqui MH, Saad MF, Liu K, Polak JF, Folsom AR, Tsai MY, Burke GL, Szklo M. Smoking, diabetes, and blood cholesterol differ in their associations with subclinical atherosclerosis: the Multiethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 2006;186:441–447. [PubMed: 16154575]
22. Dahm AE, Andersen TO, Rosendaal F, Sandset PM. A novel anticoagulant activity assay of tissue factor pathway inhibitor I (TFPI). *J Thromb Haemost* 2005;3:651–658. [PubMed: 15842349]
23. Lwaleed BA, Bass PS. Tissue factor pathway inhibitor: structure, biology and involvement in disease. *J Pathol* 2006;208:327–339. [PubMed: 16261634]
24. Lutsey PL, Cushman M, Steffen LM, Green D, Barr RG, Herrington D, Ouyang P, Folsom AR. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. *J Thromb Haemost* 2006;4:2629–2635. [PubMed: 17002663]

Figure 1.
TFPI Distribution by Gender and Ethnicity

Figure 2. TFPI by Subclinical Disease Status. Bars represent the mean and error bars the standard error of the mean.

Table 1

Characteristics of Study Population by Gender.*

	Female (n=568)	Male (n=427)	p Value [†]
Age (years)	59.2 ± 9.6	59.5 ± 9.9	0.63
BMI (kg/m ²)			
25–29.9	33.8%	45.4%	
≥30	35.4%	29.0%	0.001
Waist circumference (cm)	96.2 (16.1)	99.9 (12.4)	<0.001
Cholesterol (mmol/L)	5.15 (0.91)	4.90 (0.88)	<0.001
HDL-cholesterol (mmol/L)	1.45 (0.39)	1.16 (0.31)	<0.001
LDL-cholesterol (mmol/L)	3.03 (0.80)	3.06 (0.80)	0.44
Triglyceride (mmol/L)	1.29 (0.91–1.78)	1.28 (0.95–1.84)	0.30
Smoking			
Former	27.2%	44.4%	
Current	14.5%	16.2%	<0.001
Diabetes			
Impaired fasting glucose	16.9%	28.6%	
Diabetes	12.0%	13.2%	<0.001
Hypertension	43.3%	37.7%	0.08
Regular aspirin use (≥3 d/wk)	15.7%	22.2%	0.01
Statin use	10.8%	13.1%	0.25
Current HRT	33.2%		
Estrogen	59.2%		
Estrogen + progestin	40.8%		
Chronic Kidney Disease	10.6%	6.3%	0.02
Ankle-brachial index <0.9	2.7%	2.6%	0.94
Ankle-brachial index	1.1 (0.1)	1.2 (0.10)	<0.001
Common carotid IMT (mm)	0.8 (0.2)	0.9 (0.2)	<0.001
Internal carotid IMT (mm)	0.9 (0.5)	1.1 (0.6)	<0.001
CAC score (Agatston units)			
nonzero	34.3%	57.9%	<0.001
0	65.7%	42.2%	
1–100	22.5%	32.1%	
101–400	7.2%	14.1%	
>400	4.6%	11.7%	<0.001
Major ECG abnormality	8.7%	17.2%	<0.001
Any subclinical disease	50.7%	67.9%	<0.001
TFPI (ng/ml)	46.6 (15.0)	51.2 (12.8)	<0.001

* Data are mean (standard deviation) or proportions except triglyceride which is median (25th–75th percentile)

[†] p value represents difference in means or proportions between women and men

Table 2

Associations Between TFPI and Risk Factors; All Persons and Stratified by Gender.*

	Crude Model (n=995)	All (n=975)	Multivariable Models Women (n=554)	Men (n=414)
Age (per 10 years)	2.2 [§]	2.2 [§]	2.5 [§]	1.7 [‡]
Male gender (vs. female)	4.7 [§]	3.6 [§]		
Ethnicity (vs. Caucasian)				
Chinese	-5.9 [§]	-2.4	-1.8	-5.7 [‡]
Black	.7	0.8	1.4	-1.9
Hispanic	1.1	1.8	1.6	0.4
Systolic pressure (per 21 mmHg)	1.8 [§]			
Diastolic pressure (per 10 mmHg)	1.9 [§]	0.8	0.7	0.6
HDL-cholesterol (per 0.37 mmol/L)	-0.9 [‡]			
LDL-cholesterol (per 0.80 mmol/L)	6.2 [§]	6.2 [§]	6.3 [§]	4.9 [§]
Triglyceride (per 1.04 mmol/L) [¶]	0.4			
Waist circumference (per 14.8 cm)	2.0 [§]	0.6	0.9	-0.7
BMI (vs. <25 kg/m ²)				
≥25–<30 kg/m ²	2.7 [‡]			
≥30 kg/m ²	3.1 [‡]			
Smoking (vs. never)				
Former	3.4 [‡]	1.7	2.1	1.0
Current	3.8 [‡]	4.1 [§]	4.3 [‡]	3.9 [‡]
Kidney Disease (vs. none)	3.9 [‡]		4.3 [‡]	-1.3
Diabetes (vs. none)				
Impaired fasting glucose	2.7 [‡]	-0.2	-2.3	1.1
Diabetes	4.1 [‡]	3.9 [‡]	4.0 [‡]	3.0
Statin use (vs. none)	0.4			
Current HRT use (vs. none)	NA	NA	-8.1 [§]	NA

* Data shown are increment difference in TFPI per SD higher value of each continuous risk factor, or compared to the referent group for categorical risk factors. Samples sizes do not add across the table due to missing data for covariates in multivariable models (7 women missing hormone use status). The model R² for the multivariable model (column 2) was 0.27.

[‡] P<.05

[‡] P<.01

[§] P<.001.

[¶] Exponential function of the standard deviation of log-transformed triglyceride.

Table 3

Relative Risk of Subclinical Disease by TFPI Quartiles

	N	RR (95% CI) by TFPI Quartiles (ng/ml)				C-statistic
		1	2	3	4	
Ankle-brachial index < 9						
crude	990	1.0				
adjusted *	973	1.0	0.8 (0.2–3.4)	2.1 (0.6–6.9)	3.0 (1.0–9.3)	
			0.5 (0.0–6.8)	1.2 (0.1–15.3)	1.0 (0.2–6.0)	0.743
CAC > 0 (vs. 0) Agatston units						
crude	995	1.0	1.1 (0.9–1.3)	1.4 (1.1–1.7)	1.4 (1.2–1.8)	
adjusted *	978	1.0	1.0 (0.9–1.2)	1.2 (1.0–1.4)	1.2 (1.0–1.4)	0.781
CAC 1–100 (vs. 0) Agatston units						
crude	818	1.0	0.9 (0.7–1.2)	1.3 (1.0–1.7)	1.4 (1.1–1.8)	
adjusted *	805	1.0	1.0 (0.7–1.3)	1.2 (0.9–1.6)	1.2 (0.9–1.6)	0.729
CAC 101–400 (vs. 0) Agatston units						
crude	654	1.0	1.3 (0.7–2.2)	1.8 (1.1–3.0)	1.5 (0.9–2.6)	
adjusted *	639	1.0	1.5 (0.8–2.9)	1.6 (0.8–3.0)	1.3 (0.7–2.5)	0.830
CAC > 400 (vs. 0) Agatston units						
crude	629	1.0	2.2 (1.0–4.7)	2.3 (1.1–5.1)	4.1 (2.0–8.3)	
adjusted *	616	1.0	1.7 (0.9–3.2)	2.0 (1.1–3.8)	2.1 (1.1–4.0)	0.899
Elevated common carotid IMT						
crude	987	1.0	1.3 (0.9–1.9)	1.3 (0.9–1.9)	1.7 (1.2–2.4)	
adjusted *	970	1.0	1.1 (0.8–1.6)	1.0 (0.7–1.5)	1.1 (0.8–1.5)	0.800
Elevated internal carotid IMT						
crude	977	1.0	1.3 (0.9–2.0)	1.6 (1.1–2.5)	2.5 (1.7–3.6)	
adjusted *	960	1.0	1.1 (0.7–1.7)	1.3 (0.9–2.0)	1.6 (1.1–2.5)	0.772
Any subclinical disease						
crude	995	1.0	1.0	1.1 (0.9–1.3)	1.2 (1.0–1.4)	
adjusted *	978	1.0	1.0	1.0 (0.9–1.2)	1.1 (0.9–1.2)	0.787

* Adjusted for age, gender, and LDL-cholesterol