Metabolic Syndrome, Inflammation, and Risk of Symptomatic Peripheral Artery Disease in Women
A Prospective Study

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Background—The metabolic syndrome (MetS) is associated with incident myocardial infarction and stroke and is linked with subclinical inflammation; however, prospective data pertaining to MetS and future peripheral artery disease (PAD) are sparse, with few studies examining the role of inflammation. We therefore evaluated the relationship between MetS, inflammation, and incident PAD.

Methods and Results—We conducted a prospective cohort study among 27,111 women free of baseline cardiovascular disease who were participating in the Women’s Health Study. Subjects were followed for incident symptomatic PAD (n = 1,110; median cohort follow-up 13.3 years). We used Cox proportional hazards models to compare PAD risk among women with and without MetS. We also evaluated relationships between MetS and subclinical inflammation as measured by high-sensitivity C-reactive protein and soluble intercellular adhesion molecule-1 and adjusted for these biomarkers in multivariable models. Women with MetS had a 62% increased risk of future PAD (hazard ratio 1.62, 95% confidence interval 1.10 to 2.38). After multivariable adjustment, MetS remained significantly associated with PAD (adjusted hazard ratio 1.48, 95% confidence interval 1.01 to 2.18), with a 21% risk increase per additional MetS-defining trait (adjusted hazard ratio 1.21, 95% confidence interval 1.06 to 1.39). In women with and without MetS, respectively, median levels of high-sensitivity C-reactive protein were 4.0 versus 1.5 mg/L (P < 0.0001), and median levels of soluble intercellular adhesion molecule-1 were 374 versus 333 ng/mL (P < 0.0001). When high-sensitivity C-reactive protein and soluble intercellular adhesion molecule-1 were added to multivariable models, risk associated with MetS was substantially attenuated and no longer significant (hazard ratio 1.14, 95% confidence interval 0.75 to 1.73).

Conclusions—MetS is associated with an increased risk of future symptomatic PAD in women. This risk appears to be mediated largely by the effects of inflammation and endothelial activation.

Key Words: peripheral vascular disease ■ metabolic syndrome X ■ inflammation ■ endothelium ■ women

The metabolic syndrome (MetS), a cluster of cardiovascular risk factors associated with insulin resistance and obesity, has been consistently associated with a moderate increase in the incidence of coronary heart disease, stroke, and cardiovascular mortality.1–6 Much less information is available on the relationship between MetS and incident lower-extremity peripheral artery disease (PAD), with most data deriving from cross-sectional reports.7–10 One of 2 previously published prospective evaluations suggested that MetS is a predictor of end-stage PAD (lower-extremity amputation or revascularization) but that this risk increase is largely attributable to the impact of diabetes alone.11 Another recent analysis did not find a significant association of MetS with incident PAD, although a strong and independent relationship between MetS and cerebrovascular disease was noted.12 This potential difference in the relationship between MetS and coronary heart disease and stroke on one hand and PAD on the other may be due to the absence of large prospective studies for incident PAD or to differences in the atherogenic mechanisms that affect these distinct vascular beds.13

Clinical Perspective on p 1047

Systemic inflammation, assessed through plasma levels of high-sensitivity C-reactive protein (hsCRP), is an important predictor of cardiovascular morbidity and mortality14–17 and may be an important mediator of the association between MetS and cardiovascular disease.3,5 Furthermore, both in-
flammation and endothelial injury or activation (as measured by soluble cellular adhesion molecule-1 [sICAM-1]) have recently been shown to be strongly and independently associated with incident PAD. These factors may therefore explain potential associations between MetS and PAD; however, few prospective data have been available. In an attempt to clarify these issues, we assessed the relationships between MetS, inflammation, and future symptomatic PAD, defined as intermittent claudication or lower-extremity artery revascularization, in a large cohort of initially healthy women.

Methods

Participants
All study subjects were participants of the Women’s Health Study, a completed randomized trial evaluating the risks and benefits of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer. Details of the study design have been described previously. Briefly, beginning in 1993, 39,876 female health professionals in the United States who were 45 years or older and free of cardiovascular disease, cancer, or other major illnesses were randomized to receive 100 mg of aspirin every other day, 600 IU of vitamin E every other day, both agents, or placebo. The trial initially had a betacarotene arm that was terminated early. Information on baseline variables was collected by use of mailed questionnaires. Follow-up questionnaires asking participants about study outcomes and other information were sent every 6 months during the first year and every 12 months thereafter. For the purpose of the present study, we excluded 11,935 participants because they did not provide a baseline blood sample, 7 participants with confirmed per randomization PAD, and 823 participants with missing information on body mass index (BMI), blood pressure, or history of hypertension. Thus, the final study population for the present analysis consisted of 27,111 women. Written informed consent was obtained from all participants. The study was approved by the institutional review board of Brigham and Women’s Hospital, Boston, and was monitored by an external data and safety monitoring board.

Laboratory Analyses
All blood analyses were performed in a core laboratory certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were ascertained with direct measurement assays (Roche Diagnostics, Indianapolis, Ind). Triglyceride levels were measured enzymatically, with correction for endogenous glycerol, by use of a Hitachi 917 analyzer and reagents and calibrators from Roche Diagnostics. Plasma hsCRP was measured by a validated highsensitivity immunoturbidimetric method (Denka Seiken, Niigata, Japan). The interassay coefficients of variation with 2 levels of control materials ranged from 1.07% to 5.20%. sICAM-1 was measured by quantitative sandwich ELISA (R&D Systems, Minneapolis, Minn) with a reproducibility of 8.89% and 6.39% at concentrations of 171.8 and 289.1 ng/mL, respectively.

Definition of MetS
According to the Adult Treatment Panel III guidelines, women with 3 or more of the following traits are defined as having MetS: (1) Waist circumference ≥88 cm; (2) triglycerides ≥150 mg/dL; (3) HDL-C <50 mg/dL; (4) blood pressure ≥130/85 mm Hg; and (5) abnormal glucose metabolism as identified by a fasting blood glucose level ≥100 mg/dL. For the present study, we used a modified Adult Treatment Panel III definition of MetS previously validated in this cohort. Because waist circumference was not available at baseline, a BMI cut point for obesity of ≥26.7 kg/m^2 was used as a surrogate. When waist circumference was collected at the 6-year follow-up, this value corresponded to the same percentile for BMI as did a waist circumference of 88 cm. In addition, prior data in the WHS have demonstrated that BMI is equivalent to waist circumference in predicting major cardiovascular events, and a recent meta-analysis of MetS and vascular risk found no heterogeneity of effects whether waist circumference, waist-to-hip ratio, or BMI was used.

Outcome Ascertainment
Participants are surveyed annually for multiple health outcomes, including symptomatic PAD events, defined as intermittent claudication and/or peripheral artery surgery, inclusive of catheter-based interventions. Case confirmation occurred by telephone interview, during which the presence of vascular claudication was established by use of the Edinburgh Claudication Questionnaire. This instrument is a modified version of the World Health Organization/Rose Claudication Questionnaire that has been validated in a community outpatient setting with 92% sensitivity and 99% specificity for physician-diagnosed intermittent claudication. In addition, medical records were obtained to assess the concordance of reported symptoms with diagnostic testing when available. Reports of peripheral arterial surgery were confirmed after review of operative notes or procedural reports in the case of peripheral angioplasty or stenting. Of 456 self-reported PAD events that had occurred as of November 23, 2007, 114 were confirmed with these methods. Among dis confirmed events, venous disease, lower-extremity arthritis, lumbar disc disease, and peripheral neuropathy were the main causes of nonischemic leg pain. There were 73 unrefuted events in women who were unreachable for telephone interview, deceased, or unwilling to participate. Only confirmed events were considered in the analysis. Among women with confirmed PAD, there were 111 cases of intermittent claudication, and 56 of these women underwent revascularization. In addition, there were 3 cases of peripheral artery revascularization without antecedent claudication.

Statistical Analyses
Baseline characteristics were compared according to the presence or absence of MetS with Wilcoxon rank sum tests for continuous variables and χ^2 tests for categorical variables. Age-adjusted incidence rates were calculated by internal standardization.

The divergence of PAD incidence over time between groups with 0, 1 to 2, and ≥3 MetS traits was estimated with Kaplan-Meier survival curves, and the log-rank test was computed to compare curves. To account for potential confounders, we constructed multivariable Cox proportional hazards models to estimate the adjusted hazard ratio (HR) of PAD for women with 1 to 2 and ≥3 MetS traits by creating indicator variables for these 2 categories and adding terms for potential confounders. Cox models were also used to compare the risk according to presence versus absence of MetS. The HR per increasing number of MetS-defining traits was estimated using the number of traits (ranging from 0 to 5) as an ordinal variable in the model. For each woman, person-years of follow-up were calculated from the date of return of the baseline questionnaire to the date of incident PAD, death, or November 23, 2007, whichever came first. All risk estimates are presented as HRs with the 95% confidence interval (CI).

Crude models were first adjusted for age and smoking status and then additionally adjusted for total cholesterol and exercise. We also assessed the effect of hormone replacement therapy on incident PAD; because it was neither a significant predictor nor a confounder for the association between MetS and PAD, hormone replacement therapies were not included in the analyses.
therapy was not included in the final models. To assess whether the increased risk associated with MetS is mediated by inflammation and/or endothelial activation, we included hsCRP and sICAM-1 in the model-building process. Both hsCRP and sICAM-1 were log-transformed to normalize the variable distribution and better meet the assumption of linearity in risk across increasing levels of these biomarkers. We fitted separate models that adjusted for each biomarker in a first step and subsequently added both markers to the same model. Finally, to evaluate whether the risk associated with MetS is independent of baseline diabetes mellitus, we repeated the main analyses among women without established diabetes on entry into the study.

Effect modification was assessed with multiplicative interaction terms. The proportional hazards assumption was examined for all models by the inclusion of a MetS-by–logarithm-of-time interaction variable into the model.28 No violation of this assumption was detected. All analyses were performed with SAS version 9 (SAS Institute Inc, Cary, NC). A 2-tailed probability value \( <0.05 \) was considered to indicate statistical significance.

Results

Baseline characteristics of the study population according to MetS status are shown in Table 1. Overall, 6290 (25.5%) participants had MetS. Compared with women without MetS, those with MetS were significantly older \( (P<0.0001) \), more likely to smoke \( (P=0.009) \), and less likely to exercise on a regular basis \( (P<0.0001) \). Among participants with MetS, an HDL-C level \( <50 \text{ mg/dL} \) was the most prevalent individual MetS-defining trait (88.0%), followed by elevated BMI (81.2%), elevated blood pressure (77.8%), elevated triglycerides (77.7%), and dysglycemia as identified by baseline or incident diabetes mellitus (28.2%).

During a median (interquartile range) follow-up of 13.3 (12.5 to 13.8) years, 114 symptomatic PAD events occurred. There were 44 and 70 events among women with and without MetS, respectively. Age-adjusted incidence rates in these 2 groups were 0.43 and 0.30 events per 1000 person-years of follow-up, respectively. Figure 1 provides the cumulative incidence curves for PAD after subjects were classified according to the presence of 0, 1 to 2, or \( \geq 3 \) MetS traits and shows the increasing risk of PAD with a higher number of traits. Multivariable regression analysis confirmed these relationships (Figure 2). Compared with women without any MetS traits, those who had 1 or 2 criteria had a 2.5-fold increased PAD risk, and women with established MetS had a 3-fold increased risk independent of age, smoking, total cholesterol, and physical activity.

When examined according to presence or absence of MetS or per the number of individual traits (Table 2), we found similar results. The hazard ratio for symptomatic PAD among...
women with MetS versus those without MetS was 1.62 (95% CI 1.10 to 2.38) on univariable analysis and 1.48 (95% CI 1.01 to 2.18) after adjustment for age and smoking status. Additional adjustment for total cholesterol and exercise had virtually no effect on these risk estimates. Adjustment for LDL cholesterol instead of total cholesterol did not change the results (data not shown). When assessed as an ordinal risk factor, after multivariable adjustment, there was a 21% (95% CI 6% to 38%, \( P = 0.004 \)) greater hazard of PAD per additional MetS-defining trait.

Both hsCRP and sICAM-1 were strongly associated with MetS and with an increasing number of MetS traits. Median levels of hsCRP were 4.0 and 1.5 mg/L (\( P < 0.0001 \)) among women with and without MetS, respectively. The corresponding levels of sICAM-1 were 374 and 333 ng/mL (\( P < 0.0001 \)). Among participants with 0 to 5 MetS-defining traits, median hsCRP levels gradually increased from 1.0 to 5.9 mg/L (\( P < 0.0001 \)), and median sICAM-1 levels increased from 321 to 413 ng/mL (\( P < 0.0001 \)).

To address whether elevated hsCRP or sICAM-1 levels might account for the relationship of MetS and incident PAD, we sequentially added these variables to multivariable models (Table 2). After the inclusion of hsCRP, the adjusted HR for MetS was reduced to 1.23 (95% CI 0.82 to 1.85). A similar effect was observed after the inclusion of sICAM-1; the adjusted HR for MetS in this model was 1.30 (95% CI 0.87 to 1.95). When both biomarkers were added to the same model, the association was markedly attenuated (adjusted HR 1.14, 95% CI 0.75 to 1.73). Findings were similar when assessed according to the number of MetS-defining traits (Table 2). In analyses stratified by age, smoking status, or approximate tertiles of hsCRP and sICAM-1, we found consistent results (data not shown). None of the probability values for effect modification by these factors were statistically significant (\( P > 0.14 \) for all interactions tested).

Finally, we evaluated the risk associated with the individual MetS-defining traits. The HRs (95% CIs) for PAD associated with elevated BMI, elevated triglycerides, hypertension, low HDL-C, and dysglycemia were 0.98 (0.65 to 1.46), 1.39 (0.96 to 2.01), 1.50 (1.02 to 2.19), 1.60 (1.10 to 1.33), and 2.05 (1.26 to 3.36), respectively. By comparison, the HR associated with current smoking was 12.7 (7.6 to 21.2). Whether MetS had an effect independent of established diabetes was evaluated among 26 364 women free of diagnosed diabetes at baseline. Among this subgroup, 105 symptomatic PAD events occurred. Similar to the main analyses,
the multivariable adjusted hazard ratio for MetS was 1.43 (95% CI 0.95 to 2.16, P=0.09), and this risk estimate was reduced to 1.15 (95% CI 0.73 to 1.78) after additional adjustment for hsCRP and sICAM-1.

Discussion

In this prospective study of initially healthy, middle-aged women, similar to previously published data for coronary and cerebrovascular disease, we found that MetS is associated with a moderate increase in risk of future symptomatic PAD. This finding persisted after adjustment for age, smoking status, total cholesterol, and physical activity (adjusted HR 1.48, 95% CI 1.01 to 2.18). Furthermore, the magnitude of effect was comparable in women who were nondiabetic at baseline. Risk relationships were largely attenuated after control for hsCRP and sICAM-1, which suggests that in this generally low-risk population of women, the excess risk associated with MetS may be mediated through heightened inflammation and/or endothelial activation.

Most prior cross-sectional reports have demonstrated a positive association between MetS and prevalent PAD. Among participants in the National Health and Nutrition Examination Survey (NHANES), MetS was linked to a high likelihood of prevalent PAD (OR 4.8, 95% CI 2.2 to 34.0), and the presence of PAD increased with increasing levels of C-reactive protein. In another study from NHANES, insulin resistance as measured by the homeostasis model was also associated with prevalent PAD (OR 2.1 for highest versus lowest quartile of insulin resistance, 95% CI 1.1 to 4.0) independent of traditional risk factors, including other components of MetS. However, in this population with a high prevalence of cardiovascular risk factors and established cardiovascular disease, adjustment for C-reactive protein did not attenuate this relationship. In a third cross-sectional study of patients with diagnosed vascular disease, MetS was more common in those who had PAD (57%) than in those with atherosclerosis that affected other vascular beds (40%, 43%, and 45% for coronary disease, cerebrovascular disease, and aortic aneurysm, respectively).

Despite such evidence from cross-sectional reports, few prospective comparisons of MetS and incident PAD have been available. A small prospective study in a Finnish cohort demonstrated a 2-fold increased hazard of end-stage PAD (n=57 amputations or revascularization procedures) among those with MetS; however, this relationship appeared to be due primarily to prevalent diabetes. After analyses that adjusted for diabetes or excluded diabetic subjects, the authors concluded that MetS does not predict PAD beyond the risk associated with established diabetes. However, in this evaluation of end-stage PAD, diabetes-related chronic conditions such as neuropathic ulcers and concurrent infection rather than ischemia per se may have been major contributors, because nearly 50% of events were amputations. A dominant effect of preexisting diabetes was not confirmed in the present analysis, which included milder cases of PAD (intermittent claudication and limb ischemia).

The Edinburgh Artery Study did not find a significant association between MetS and incident PAD (HR 0.89, 95% CI 0.57 to 1.28). Compared with the present study population, the Edinburgh cohort was older (mean age 65 years), of mixed gender, and had a high prevalence of baseline smoking (25.3%) and asymptomatic disease (17% with enrollment ankle-brachial index <0.9). Nonetheless, these null findings from a higher-risk group in contrast to the current positive results among low-risk women invoke the intriguing possibility that MetS has a greater impact on atheroma initiation than on its progression in individuals with extant subclinical disease. This hypothesis requires corroboration in other cohorts but is supported by results of a recent meta-analysis of MetS and incident cardiovascular events in which MetS had a larger effect (HR 1.96 versus 1.43, P=0.04) in studies of individuals at lower baseline cardiovascular risk (<10%).

As demonstrated in prior reports, the present data also confirm that markers of inflammation and endothelial activation are strongly associated with MetS. We found an increase in plasma levels of hsCRP and sICAM-1 per additional MetS-defining trait such that women with MetS had substantially higher plasma levels than those without MetS. Furthermore, the addition of either hsCRP or sICAM-1 individually to multivariable models substantially attenuated the effect of MetS on subsequent PAD, whereas inclusion of both markers virtually abolished this association. The present findings thus suggest that in this relatively healthy population of women, inflammation and endothelial activation may be potential mediators of the heightened PAD risk conferred by this risk factor cluster.

The present data also emphasize the importance of smoking in the pathogenesis of PAD. Women who smoked had a 12.7-fold increased risk of developing PAD compared with nonsmokers, and therefore, smoking was by far the strongest risk factor in this population. Although prior risk estimates for the association between smoking and PAD are somewhat
weaker than in the present study, our finding may reflect a impact of smoking in women that requires further investigation. The relative magnitude of smoking as a risk factor compared not only with MetS but also with diabetes in the present study underscores the importance of abstinence from smoking for the prevention of PAD.

The present results should be interpreted in the context of several potential limitations. First, our study included women who were of predominantly Caucasian origin, and our findings may not be generalizable to other groups. Second, the use of symptomatic PAD as our primary a priori end point by definition excluded subclinical disease, which otherwise may have been detected through abnormal pulse examination or ankle-brachial index. However, we believe our data to be not only relevant from a mechanistic perspective but also of clinical importance for the following reasons. First, claudication and limb ischemia requiring revascularization are the principal clinical manifestations of PAD. Second, all events evaluated in the present analysis were confirmed by a validated claudication questionnaire and medical record review. Third, women enrolled in the present study are health professionals and are therefore less likely to encounter barriers to medical care, which may otherwise have led to underdiagnosis. Although potential misclassification resulting from atypical or occult disease may have occurred, this, if anything, would have biased our results toward the null. On the other hand, a low ankle-brachial index has been shown to strongly and independently predict the occurrence of cardiovascular events independent of claudication symptoms, which highlights the importance of this end point. Nevertheless, we believe that the use of confirmed symptomatic PAD is not only valid but also represents an important clinical end point for this disease. Finally, a modified version of the official Adult Treatment Panel III MetS definition was used in the present analysis. The criteria for MetS have been variable and continue to evolve over time. Our use of baseline or incident type 2 diabetes mellitus instead of fasting glucose levels as a MetS-defining trait likely underestimated the number of women who satisfied the MetS criteria. Again, this could have biased our results toward the null. The definition used in the present report has been previously associated with incident cardiovascular disease in this cohort. Furthermore, the present study was undertaken to describe a potential etiologic role for this risk factor cluster in the development of PAD rather than to validate the utility of the syndrome for risk prediction.

In conclusion, similar to the previously noted relationship between MetS and incident coronary heart disease and stroke, the present study demonstrated a modest positive association with future PAD in a population of otherwise low-risk women. Substantially increased plasma levels of hsCRP and sICAM-1 were evident in subjects with MetS, and a strong influence of these factors on the relationship between MetS and PAD was noted, suggesting a possible pathophysiological role. Prospective data from other cohorts are greatly needed not only to corroborate our results but also to further elucidate mechanistic links between risk factor clustering and onset of this disease.

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