Soluble P-Selectin and the Risk of Future Cardiovascular Events

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Background—P-selectin, a cell-surface adhesion molecule involved in leukocyte rolling and attachment, has been hypothesized to play a role in the initiation of atherosclerosis. However, little clinical data are available evaluating the role of soluble P-selectin in determining vascular risk.

Methods and Results—In a large-scale prospective study of apparently healthy women, we measured baseline plasma concentration of soluble P-selectin among 115 participants who subsequently developed cardiovascular events and among 230 age- and smoking-matched participants who remained free of disease during 3.5 years of follow-up. Overall, mean levels of soluble P-selectin were significantly higher at baseline among women who subsequently experienced cardiovascular events compared with those who did not (83.2 versus 69.3 ng/mL; \(P=0.003\)). The risk of future cardiovascular events increased with increasing quartiles of soluble P-selectin (\(P=0.02\)), such that women in the highest quartile at study entry had an age- and smoking-matched relative risk 2.2 times higher than those in the lowest quartile (95% confidence interval, 1.2 to 4.2; \(P=0.01\)). This effect was independent of traditional risk factors. For each quartile increase in soluble P-selectin, the risk of future cardiovascular events increased 28% (\(P=0.03\)) after additional adjustment for obesity, hypertension, hyperlipidemia, diabetes, and exercise frequency. The highest risks were observed among women with the very highest levels of P-selectin (>137.3 ng/mL, the 95th percentile cut point of the control distribution).

Conclusions—Soluble P-selectin levels are elevated among apparently healthy women at risk for future vascular events. (Circulation. 2001;103:491-495.)

Key Words: inflammation • selectins • atherosclerosis • risk factors • cell adhesion molecules • myocardial infarction

Inflammation plays a major role in atherosclerosis,\(^1\) and plasma markers of inflammation such as high-sensitivity C-reactive protein may have clinical utility in the detection of atherothrombotic risk.\(^2,3\) Among the earliest inflammatory steps leading to atherogenesis is the slowing, tethering, and subsequent attachment of circulating leukocytes to the vascular endothelium, a process mediated partly by the selectin family of cell adhesion molecules.\(^4,5\) In particular, P-selectin is an adhesion receptor expressed on activated endothelial cells that mediates initial leukocyte “rolling.”\(^6\) Evidence supporting a role for P-selectin in atherogenesis includes the observation that P-selectin is preferentially expressed in the endothelium overlying atherosclerotic plaques,\(^7\) that P-selectin-deficient mice develop reduced fatty streaks,\(^8-10\) and that anti-P-selectin antibodies inhibit monocyte rolling and attachment across carotid endothelium.\(^11\) In addition, atherogenic factors, including oxidized LDL,\(^12,13\) induce P-selectin expression.

Levels of soluble P-selectin can be measured in plasma and derive from both platelet and endothelial sources. However, clinical data relating soluble P-selectin to atherogenesis are sparse and limited to cross-sectional or retrospective studies describing elevated levels of soluble P-selectin among hypertensive,\(^14\) hyperlipidemic,\(^15\) and atherosclerotic patients.\(^16-19\) To date, no prospective data are available describing the role of soluble P-selectin among apparently healthy individuals being followed for incident myocardial infarction or stroke. Thus, whether plasma levels of soluble P-selectin are a marker for a high risk of future vascular events is uncertain.

Methods

We evaluated the role of soluble P-selectin as a potential marker for cardiovascular risk among initially healthy participants in the Women’s Health Study, an ongoing trial of aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer.\(^20\) Details of the Women’s Health Study have been provided elsewhere; in brief, 28,263 women free of reported cardiovascular disease provided baseline plasma samples, which were collected in EDTA and stored in liquid nitrogen until the time of analysis. All study participants were then prospectively followed over an average period of 3.5 years for the occurrence of first-ever cardiovascular events (myocardial infarction).
infarction, stroke, coronary revascularization, or cardiovascular death). The end point of myocardial infarction was confirmed if symptoms of ischemia were present and if the event was associated with diagnostic changes in cardiac enzyme levels or if there were diagnostic electrocardiographic changes. Reports of coronary revascularization procedures were confirmed by record review. The diagnosis of stroke was confirmed if the patient had a new neurological deficit of >24-hour duration; computed tomography or magnetic resonance scanning was available for the majority of cases. Deaths from coronary heart disease were confirmed by record review, death certificates, autopsy reports, and information provided by family members.

For this analysis, baseline plasma samples were obtained from 115 study participants who subsequently developed a confirmed cardiovascular end point during follow-up (cases). For each of these women, baseline plasma samples were also obtained from 2 control women, who were selected from the pool of remaining study participants who did not develop cardiovascular events during follow-up. Two controls were matched to each case on the basis of age (±1 year) and smoking status (former, current, never). Thus, a total of 345 women (115 cases and 230 controls) were included in this prospective, nested, case-control analysis.

Baseline plasma samples from each case and control participant were thawed and assayed for soluble P-selectin by enzyme-linked immunosassay (R&D Systems). Matched plasma specimens were analyzed in blinded triplets, with the position of the case specimen varied at random to reduce systematic bias and minimize interassay variability. In pilot data performed for this study, intra-assay and interassay coefficients of variation were <8% across expected ranges of soluble P-selectin. Lipid profiles and other markers of inflammation were measured in these women using techniques described elsewhere.3,21,22

Means and proportions for baseline risk factors were computed for case and control participants and compared using the Student’s t test or χ2 analysis. Tests for trend were used to evaluate evidence of association between increasing levels of soluble P-selectin and subsequent vascular risk after dividing the study population into quartiles on the basis of the distribution of control values. Adjusted risk estimates were obtained with the use of logistic regression models that, in addition to accounting for the matching variables of age and smoking status, also adjusted for potential confounding variables. Finally, to evaluate threshold effects, we repeated these analyses using a prespecified series of cut points for soluble P-selectin based on the 50th, 75th, 90th, and 95th percentile cut points of the control distribution. All probability values are 2-tailed, and confidence intervals were computed at the 95% level.

### Results

Table 1 shows the baseline clinical characteristics of the study participants. As expected, initially healthy women who subsequently developed cardiovascular events (cases) were more likely at baseline to have a history of hypertension, obesity, diabetes, or hyperlipidemia when compared with women who remained free of reported disease (controls). Because of matching, age and smoking status were similar in the 2 study groups.

The distribution of soluble P-selectin levels observed in our study is consistent with that expected in studies of unselected healthy individuals (R&D Systems; Figure 1). However, as shown in Table 1 and Figure 2, both mean (83.2 versus 69.3 ng/mL; P=0.003) and median (68.6 versus 62.7 ng/mL; P=0.02) levels of soluble P-selectin were significantly higher at baseline among study participants who subsequently developed a first-ever cardiovascular event (cases) than in those who did not (controls).

In these data, increasing levels of soluble P-selectin at baseline were associated with increased risks of future cardiovasular events over the 3.5-year follow-up period. As shown in Table 2, the age- and smoking-matched relative risk of future cardiovascular events increased 27% (95% confidence interval, 4% to 56%) for each quartile increase in baseline level of soluble P-selectin (P=0.02). These effects persisted after controlling for other baseline clinical characteristics, such that the adjusted relative risks of future cardiovascular events for women with the lowest (referent) to

![Figure 1. Frequency distribution and cumulative frequency of baseline soluble P-selectin levels among apparently healthy women.](image-url)
highest baseline quartiles of soluble P-selectin were 1.0, 1.3, 1.3, and 2.2 (P-trend=0.03) after additional control for body mass index (kg/m²), hypertension, hyperlipidemia, family history of coronary disease, diabetes, and exercise frequency (Table 2).

Cigarette consumption was associated with elevated levels of soluble P-selectin, such that nonsmokers had significantly lower levels of P-selectin (67.9 ng/mL) than did former (76.8 ng/mL) or current smokers (80.6 ng/mL; P=0.01; Figure 3). However, because our study design matched cases and controls on smoking status, the potential for confounding on this basis is minimal. To assess for effect modification, analyses limited to nonsmokers were performed; they had almost identical overall results.

To evaluate potential threshold effects, we repeated these analyses using a series of prespecified cut points for soluble P-selectin. As shown in Table 3, the relative risks associated with baseline soluble P-selectin concentrations exceeding the 50th, 75th, 90th, and 95th percentiles of the control distribution were 1.4 (P=0.1), 1.9 (P=0.01), 2.0 (P=0.03), and 2.3 (P=0.04), respectively. Thus, much of the excess risk associated with baseline soluble P-selectin was due to an excess of high values among the case subjects. Similar effects were observed in analyses stratified by the presence or absence of hypertension, obesity, diabetes, and hyperlipidemia.

In prior work from this cohort, the inflammatory markers soluble intercellular adhesion molecule 1 (sICAM-1), high-sensitivity C-reactive protein, and interleukin-6 were also predictive of future cardiovascular events. In these data,
were obtained several years before the onset of first-ever vascular occlusion. By contrast, in our study, plasma samples elevations of soluble P-selectin are a cause or a result of been impossible from these prior studies to discern whether P-selectin levels may increase with acute ischemia, it has thus processes leading to atherosclerotic vascular occlusion. confirmation of an important role for P-selectin in the hypothesis that P-selectin may play an important role in the initiation of atherosclerosis.

In this prospective epidemiological evaluation of apparently healthy women, elevated baseline levels of soluble P-selectin were associated with increasing risks of future myocardial infarction, stroke, coronary revascularization, and cardiovascular death. This association was independent of age and smoking status and persisted after additional control for several lipid and nonlipid cardiovascular risk factors. In these data, the strongest correlates of P-selectin were smoking status and sICAM-1. However, the effects of P-selectin on risk were only minimally attenuated in analyses further controlling for these factors and for other traditional markers of cardiovascular risk. These data thus provide strong clinical confirmation of an important role for P-selectin in the processes leading to atherosclerotic vascular occlusion.

Prior clinical data relating soluble P-selectin to coronary artery disease are limited and have been derived primarily from small cross-sectional or retrospective studies of patients with acute coronary syndromes. Because soluble P-selectin levels may increase with acute ischemia, it has thus been impossible from these prior studies to discern whether elevations of soluble P-selectin are a cause or a result of vascular occlusion. By contrast, in our study, plasma samples were obtained several years before the onset of first-ever vascular events, eliminating the possibility that acute ischemia was the underlying cause of soluble P-selectin release.

In these data, cigarette consumption was directly associated with increased levels of soluble P-selectin. These data are thus in accord with prior work demonstrating potential adverse effects of smoking on endothelial function and on the expression of other adhesion molecules, including ICAM-1 and vascular cell adhesion molecule-1. However, because we matched case and control subjects on smoking status, it is highly unlikely that this effect explains our result. Further, we observed almost identical findings in a post hoc subgroup analysis limited to nonsmokers.

Potential limitations of our study include the fact that we relied on a single, frozen baseline plasma sample and that we evaluated only postmenopausal women. However, observed soluble P-selectin levels in our study are very similar to those expected in fresh plasma (Figure 1); these data suggest that our collection and storage procedures, which include freezing at −170°C are sufficient to preserve the integrity of our samples. However, sex-specific findings for soluble P-selectin are possible; thus, care must be taken when generalizing these data to men. Finally, because soluble P-selectin derives from both platelets and endothelial cells, the present measurements cannot distinguish between these 2 potential sources of circulating soluble P-selectin. Furthermore, the plasma levels of soluble P-selectin could increase due to increased expression on the platelet or endothelial surface or be due to excessive shedding by as-yet poorly understood mechanisms.

In summary, the current report, together with prior data for other adhesion molecules, provides strong epidemiological support for the hypothesis that the very early inflammatory processes of leukocyte rolling, tethering, attachment, and transmigration across the vascular endothelium are critical initiating events in atherothrombosis. These data also provide a human extension of basic work demonstrating that P-selectin–deficient mice have delayed fatty streak formation and that anti–P-selectin antibodies inhibit cell adhesion in early atherogenesis, at least in apoE-deficient mice. Thus, accumulating data support the potential for adhesion molecules to serve as novel targets for the treatment and prevention of cardiovascular disorders.

**Acknowledgments**

Supported by grants HL-58755 and HL-63293 from the National Heart, Lung, and Blood Institute. Dr Ridker is additionally supported by an Established Investigator Award from the American Heart Institute and by grants HL-63293.
Association and by a Distinguished Clinical Scientist Award from the Doris Duke Charitable Foundation. The authors thank Dr Peter Libby for reviewing this manuscript.

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Circulation. 2001;103:491-495
doi: 10.1161/01.CIR.103.4.491

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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