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Relation of C-Reactive Protein to Endothelial Fibrinolytic Function in Healthy Adults

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Abstract

Elevated plasma concentrations of C-reactive protein (CRP) independently predict future atherothrombotic events in healthy, asymptomatic adults. CRP may promote atherothrombosis by altering fibrinolytic balance; however, the influence of elevated plasma CRP concentrations on endothelial fibrinolysis in healthy adults is unclear. We tested the hypothesis that endothelial release of tissue-type plasminogen activator (t-PA) is impaired in adults with elevated plasma CRP concentrations independent of other cardiometabolic risk factors. Fifty-four healthy adults were studied: 24 with CRP < 1.0 mg/L (low CRP; 18M/6F; age: 55±2 yr); 18 with CRP 1.0-3.0 mg/L (moderate CRP; 8M/10F; 58±2 yr); and 12 with CRP > 3.0 mg/L (high CRP; 7M/5F; 56±2 yr). Net endothelial release of t-PA was determined, *in vivo*, in response to intrabrachial infusions of bradykinin (125-500 ng/min) and sodium nitroprusside (2.0-8.0 µg/min). Capacity of the endothelium to release t-PA was significantly lower (~30%) in the high (from 0.32±0.5 to 38.9±6.0 ng·100 ml tissue⁻¹·min⁻¹) and moderate (-0.05±0.4 to 39.3±5.2 ng·100 ml tissue⁻¹·min⁻¹) compared with the low (0.42±0.9 to 61.8±5.2 ng·100 ml tissue⁻¹·min⁻¹) CRP groups. There was no significant difference in t-PA release between the high and moderate CRP groups. Plasma CRP concentrations were inversely related to t-PA release (r=-0.38, P<0.05). In conclusion, these results indicate that the capacity of the endothelium to release t-PA is diminished in adults with plasma CRP ≥ 1.0 mg/L. Endothelial fibrinolytic dysfunction may underlie the increased atherothrombotic risk associated with elevations in plasma CRP concentrations in otherwise healthy adults.

Keywords

endothelium; tissue-type plasminogen activator; fibrinolysis; inflammation

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CONFLICT OF INTEREST None declared.

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INTRODUCTION

We tested the hypothesis that the capacity of the endothelium to acutely release t-PA is impaired in adults with elevated plasma CRP concentrations independent of other cardiometabolic risk factors. To address this hypothesis, we employed an isolated forearm model to assess endothelial t-PA release *in vivo* in adults with a range of plasma CRP concentrations.

METHODS

Fifty-four sedentary adults (32 males/21 females) were studied: 24 with CRP < 1.0 mg/L (low CRP; 18M/6F; age: 55±2 yr), 18 with CRP 1.0-3.0 mg/L (moderate CRP; 8M/10F; 58±2 yr), and 12 with CRP > 3.0 mg/L (high CRP; 7M/5F; 56±2 yr). CRP classification tertiles were based on published guidelines regarding plasma CRP concentrations and associated cardiovascular risk^{1,2}. All subjects were normotensive (arterial blood pressure ≤140/90 mmHg) and free of overt cardiovascular and metabolic disease as assessed by medical history, physical examination, fasting blood chemistries, and electrocardiograms and blood pressure at rest and during incremental exercise performed to exhaustion. None of the subjects smoked, were taking medications (including vitamins), or performed regular physical exercise for at least 1 year before the start of the study. Prior to participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder. This study was approved by the Institutional Review Board of the University of Colorado at Boulder and all experiments conformed with the principles outlined in the *Declaration of Helsinki*.

Body mass was measured to the nearest 0.1 kg. Percent body fat was determined by dual energy x-ray absorptiometry (Lunar Radiation Corporation, Madison, WI). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines³. Fasting plasma lipid, lipoprotein, glucose and insulin concentrations were determined using standard techniques by the clinical laboratory affiliated with the Clinical Translational Research Center at the University of Colorado at Boulder.

Blood samples for the determination of circulating levels of CRP were collected in chilled EDTA tubes with minimal venostasis, after a 12 hour overnight fast. Plasma concentrations of high sensitivity CRP were determined in duplicate by enzyme immunoassay (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation were <7% and <8%, respectively.

All measurements of endothelial fibrinolytic function were performed in a temperature-controlled room between 7 and 10 AM after an overnight fast as previously described by our laboratory^{4,5}. Briefly, an intravenous catheter was placed in a deep antecubital vein of the non-dominant arm. Thereafter, a 5-cm, 20-gauge catheter was introduced into the brachial artery of the same arm under local anesthesia (1% lidocaine). Forearm blood flow (FBF) was measured using strain-gauge venous occlusion plethysmography and presented as mL/100 mL forearm volume/min. Following the measurement of resting blood flow for 5 minutes, bradykinin was infused intra-arterially at rates of 12.5, 25, 50 ng·100 ml tissue⁻¹·min⁻¹ and sodium nitroprusside at 1.0, 2.0, 4.0 µg·100 ml tissue⁻¹·min⁻¹ for 5 min at each dose. To avoid an order effect, the sequence of drug administration was randomized.

Net endothelial release of t-PA antigen and PAI-1 antigen in response to bradykinin and sodium nitroprusside was calculated according to Jern et al.⁶ using the following equation:

$$\text{Net Release} = (C_V C_A) \times (\text{FBF} \times [101 \text{ hematocrit} / 100])$$

where C_V and C_A represent the concentration in the vein and artery, respectively. For both t-PA and PAI-1, a positive difference indicated a net release and a negative difference, net uptake. Arterial and venous blood samples were collected simultaneously at baseline and the end of each drug dose. Enzyme immunoassay was used to determine t-PA and PAI-1 antigen concentrations. Hematocrit was measured in triplicate using the standard microhematocrit technique and corrected for trapped plasma volume within the erythrocytes⁷. The total amount of t-PA antigen released across the forearm in response to bradykinin was calculated as the incremental area under each curve using a trapezoidal model. In order to avoid confounding effects from potential infection or acute inflammation on fibrinolytic function, all subjects were free of recent infection/inflammation (> 2 weeks) as determined by questionnaire⁸.

Differences in subject baseline characteristics and area under the curve data were determined by between-groups analysis of variance (ANOVA). Group differences in FBF and endothelial t-PA and PAI-1 antigen release in response to bradykinin and sodium nitroprusside were determined by repeated-measures ANOVA. When indicated by a significant F value, a *post hoc* test using the Newman-Keuls method was performed to identify differences between the groups. Relations between variables of interest were assessed by means of Pearson's correlation coefficient and linear regression analysis. There were no significant gender interactions in any of the primary outcome variables; therefore, the data were pooled and presented together. Because of the skewed distribution of plasma CRP concentrations, the data were log transformed to satisfy basic assumptions for parametric testing. However, per joint American College of Cardiology and Centers for Disease Control recommendations¹, the absolute values for CRP are presented to facilitate clinical interpretation. All data are expressed as mean $\pm$ SEM. Statistical significance was set *a priori* at $P < 0.05$.

RESULTS

Selected subject characteristics are presented in the table. By design, plasma CRP concentrations were significantly different between the groups. Plasma CRP concentrations were ~300% higher ($P < 0.05$) in the moderate than the low CRP group, and ~400% and ~1200% higher (both $P < 0.05$) in the high compared with the moderate and low CRP groups, respectively. There were no differences in any anthropometric, hemodynamic, or metabolic variables between the groups.

Forearm blood flow responses to bradykinin were not different between the low (4.7 ± 0.3 to 16.1 ± 0.8 ml·100 ml tissue⁻¹·min⁻¹), moderate (3.7 ± 0.2 to 14.5 ± 0.8 ml·100 ml tissue⁻¹·min⁻¹), and high (4.1 ± 0.3 to 14.1 ± 1.1 ml·100 ml tissue⁻¹·min⁻¹) CRP groups (Figure 1). Similarly, there were no group differences in the FBF responses to SNP (Figure 1). Forearm blood flow in the non-infused arm remained constant throughout the infusion protocols and did not differ significantly between groups.

Basal endothelial t-PA antigen release was not significantly different among the groups. However, compared with the low CRP group, the capacity of the endothelium to release t-PA in response to bradykinin was significantly blunted in both the moderate and high CRP groups. Net release of t-PA antigen was ~35% lower ($P < 0.05$) in the moderate (from -0.1 ± 0.4 to 39.3 ± 5.1 ng·100 ml tissue⁻¹·min⁻¹) and high (from 0.1 ± 0.5 to 38.9 ± 6.0 ng·100 ml tissue⁻¹·min⁻¹) compared with the low (from 0.4 ± 0.9 to 61.8 ± 5.2 ng·100 ml

tissue⁻¹·min⁻¹) CRP group (Figure 2). As a result, the total amount of t-PA antigen released (area under the bradykinin curve) was markedly lower (~30%; $P < 0.05$) in the moderate (209 ± 30 ng/100 ml tissue) and high (216 ± 30 ng/100 ml tissue) compared with the low (318 ± 33 ng/100 ml tissue) CRP group (Figure 2). There was a significant inverse relation between CRP and peak t-PA release to bradykinin ($r = -0.38$) in the overall study population (Figure 3). Sodium nitroprusside did not stimulate significant changes in t-PA release in either the low (from -0.2 ± 0.4 to 1.2 ± 2.2 ng·100 ml tissue⁻¹·min⁻¹), moderate (from 1.8 ± 1.4 to 3.9 ± 2.0 ng·100 ml tissue⁻¹·min⁻¹) or high (from -0.4 ± 1.0 to 4.0 ± 3.5 ng·100 ml tissue⁻¹·min⁻¹) CRP groups. Neither bradykinin nor sodium nitroprusside elicited significant changes in PAI-1 antigen release in any group (data not shown).

DISCUSSION

The novel finding of the present study is that adults with elevations in plasma CRP concentrations (≥ 1.0 mg/L) demonstrate diminished endothelial capacity to release t-PA compared with adults with low plasma CRP concentrations. To our knowledge, this is the first study to examine the association between plasma CRP concentrations and *in vivo* endothelial t-PA release in healthy adult humans.

Plasma CRP concentrations were first identified as a strong, independent predictor of future cardiovascular events in healthy men by Ridker and colleagues⁹ with data from the Physicians Health Study in 1997. Since that initial report, the ability of CRP to predict myocardial infarction, ischemic stroke, and cardiovascular mortality has been confirmed in more than 20 diverse population cohorts¹⁰. CRP may promote the initiation and progression of atherosclerosis by directly altering endothelial cell function. In addition to *in vitro* models, several clinical studies have shown an inverse relation between plasma CRP concentrations and endothelium-dependent vasodilation¹¹⁻¹³, and acute intravenous infusion of recombinant CRP impairs endothelial vasoreactivity in hypercholesterolemic patients¹⁴. The results of the present study significantly extend these findings by demonstrating that plasma CRP concentrations greater than 1.0 mg/L, a range considered to confer an elevated risk of cardiovascular disease, are associated with endothelial fibrinolytic dysfunction.

The ability of endothelial cells to release t-PA, the primary activator of plasminogen on the surface of a developing clot, is a critical endogenous defense mechanism against thrombosis^{15,16}. Consequently, reduced endogenous fibrinolytic activity is considered to be a major contributor to the development, progression, and severity of atherothrombotic vascular disease¹⁷⁻²⁰. For example, in humans with coronary artery disease, impaired endothelial t-PA release predicts the future risk of atherothrombotic events²⁰. The present results indicate that elevations in CRP above 1.0 mg/L are associated with diminished capacity of the endothelium to release t-PA, independent of other cardiovascular risk factors. Interestingly, the degree of impairment in endothelial t-PA release was similar in the moderate and high CRP groups, indicating that even modest elevations (1.0 – 3.0 mg/L) in plasma CRP are associated with impaired endothelial fibrinolytic function. These findings significantly extend those of Speidl and colleagues²¹ demonstrating a relation between CRP concentrations ≥ 3.0 mg/L and impaired t-PA responses to venous occlusion in patients with coronary artery disease. Indeed, data presented herein provide the first evidence of a link between elevations in plasma CRP levels and endothelial fibrinolytic dysfunction in healthy adults. Furthermore, our results complement epidemiological data indicating that CRP concentrations independently predict future atherothrombotic events in apparently healthy, asymptomatic adults^{9,10} by providing a possible mechanism for this elevation in thrombotic risk.

The mechanisms underlying the link between elevations in CRP and endothelial fibrinolytic dysfunction are unclear. One possibility is that CRP acts directly on the endothelium to diminish t-PA production and/or release. This notion is supported by *in vitro* data demonstrating that CRP binds to FC γ receptors expressed by human aortic endothelial cells and decreases t-PA antigen release, activity, and intracellular protein levels²². Alternatively, it is plausible that elevations in CRP are a surrogate marker of underlying inflammatory processes that diminish the capacity of the endothelium to release t-PA. For example, several inflammatory cytokines that promote CRP release from the liver, such as interleukin-1 β and tumor necrosis factor- α ²³, also downregulate t-PA release from the endothelium^{22,24}. Moreover, reactive oxygen species that are produced under inflammatory conditions have been shown to reduce t-PA synthesis and release in cultured human endothelial cells^{25,26}. These potential mechanisms are not mutually exclusive; indeed, CRP is intimately involved in the genesis of a pro-inflammatory and oxidative environment that together may damage the endothelium, ultimately impairing t-PA release. Given our study design, we are unable to discern the factors underlying the apparent strong relation between elevations in CRP concentration and endothelial fibrinolytic dysfunction in asymptomatic adults.

There are 3 experimental considerations of the present study that should be mentioned. Firstly, given the cross-sectional approach employed in the present study, we cannot rule out the possibility that genetic and/or lifestyle factors might have influenced the results. To minimize the influence of lifestyle behaviors, we studied sedentary, non-smoking middle-aged and older adults who were free of overt cardiometabolic disease and not currently taking medication that could influence endothelial fibrinolytic function. Secondly, we relied on a single blood sample for the measurement of CRP and therefore cannot account for changes in plasma CRP concentrations that may occur over time. However, all subjects in the present study were free of recent inflammatory/infectious events and known chronic inflammatory conditions, thereby minimizing the potential influence of acute inflammation on plasma CRP levels. Finally, our study population included relatively few women, particularly in the low CRP group. Although we observed no gender interactions, the limited number of women in the study does not permit us to be conclusive regarding possible gender differences regarding the influence of elevated CRP concentrations on endothelial t-PA release.

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Figure 1. Forearm blood flow responses to bradykinin (A) and sodium nitroprusside (B) in the low, moderate, and high CRP groups. Values are mean $\pm$ SEM.

Figure 2. Net release (A) and total amount (area under the curve; B) of tissue-type plasminogen activator (t-PA) antigen released across the forearm in response to bradykinin in the low, moderate, and high CRP groups. Values are mean $\pm$ SEM; *P < 0.05 vs. low CRP.

Figure 3. Correlation between peak t-PA release to bradykinin and plasma CRP concentration (logarithmic transformation).

Table

Selected Subject Characteristics.

Variable	C-Reactive Protein Group		
	Low (< 1.0 mg/L) (n=24)	Moderate (1.0 – 3.0 mg/L) (n=18)	High (> 3.0 mg/L) (n=12)
Male/Female	18 / 6	8 / 10	7 / 5
C-reactive protein (mg/L)	0.53 ± 0.04	1.61 ± 0.13 [*]	6.08 ± 0.58 ^{*†}
Age (years)	55±2	58 ± 2	56 ± 2
Body mass (kg)	81.8 ± 3.2	82.8 ± 3.1	86.0 ± 3.6
Body mass index (kg/m ²)	27.1 ± 0.8	28.2 ± 0.8	28.4 ± 0.8
Body fat (%)	30.2 ± 2.0	34.8 ± 1.6	34.2 ± 2.0
Waist circumference (cm)	96.0 ± 2.6	97.8 ± 2.3	97.0 ± 3.6
Systolic blood pressure (mmHg)	123 ± 2	117 ± 2	117 ± 3
Diastolic blood pressure (mmHg)	78 ± 2	76 ± 2	75 ± 2
Total cholesterol:			
mmol/L	4.9 ± 0.2	4.7 ± 0.2	4.8 ± 0.2
mg/dL	187.9 ± 6.9	182.7 ± 8.9	185.0 ± 8.3
Low-density lipoprotein cholesterol:			
mmol/L	3.1 ± 0.1	2.9 ± 0.2	2.9 ± 0.2
mg/dL	118.4 ± 5.7	112.3 ± 8.0	113.1 ± 7.4
High-density lipoprotein cholesterol:			
mmol/L	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
mg/dL	49.4 ± 2.6	51.7 ± 2.8	45.3 ± 3.6
Triglycerides:			
mmol/L	1.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.2
mg/dL	101.0 ± 10.9	93.4 ± 7.6	133.1 ± 17.8
Glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1	5.4 ± 0.2
Insulin (pmol/L)	35.4 ± 3.1	41.2 ± 4.0	44.0 ± 5.7

Values are mean±SEM.

^{*} P < 0.05 vs. Low CRP.[†] P < 0.05 vs. Moderate CRP