C-Reactive Protein Is Associated With Arterial Stiffness in Apparently Healthy Individuals

Yasmin, Carmel M. McEniery, Sharon Wallace, Isla S. Mackenzie, John R. Cockcroft, Ian B. Wilkinson

Objective—C-reactive protein (CRP) levels predict outcome in healthy individuals and patients with atherosclerosis. Arterial stiffness also independently predicts all-cause and cardiovascular mortality and may be involved in the process of atherosclerosis. The aim of this study was to investigate the relationship between stiffness and inflammation in a cohort of healthy individuals.

Methods and Results—Pulse wave velocity (PWV) and blood pressure were assessed in 427 individuals. Subjects with cardiovascular disease, diabetes, hypercholesterolemia and those using medication were excluded. CRP correlated with age, mean arterial pressure (MAP), brachial and aortic PWV, and pulse pressures. In multiple regression models, aortic PWV correlated independently with age, CRP, male gender, and MAP ($R^2=0.593; P<0.001$). CRP was also independently associated with brachial PWV. Aortic augmentation index correlated with age, gender, MAP, and inversely with heart rate and height, but not with CRP ($R^2=0.794; P<0.001$). Aortic, carotid, and brachial pulse pressures were also independently associated with CRP levels.

Conclusion—Aortic and brachial PWV, and pulse pressure, relate to levels of inflammation in healthy individuals, suggesting that inflammation may be involved in arterial stiffening. Anti-inflammatory strategies may, therefore, be of benefit in reducing arterial stiffness and thus cardiovascular risk, especially in patients with premature arterial stiffening.

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Key Words: C-reactive protein ■ pulse wave analysis ■ augmentation index ■ pulse wave velocity ■ inflammation

The pathogenesis of atherosclerosis remains incompletely understood, but inflammation is thought to play an important role. Several studies have demonstrated that serum levels of the acute phase protein, C-reactive protein (CRP), independently predict outcome in patients with cardiovascular disease, and in apparently healthy individuals. Levels of CRP also correlate with endothelial function, an independent predictor of cardiovascular risk, in patients with coronary artery disease. Moreover, CRP has direct pro-inflammatory effects on human endothelial cells in vitro and can induce endothelial dysfunction.

Aortic pulse wave velocity (PWV), a measure of aortic distensibility, predicts mortality in patients with end-stage renal failure, hypertension, diabetes, and older otherwise healthy individuals, independently of known confounding factors. Interestingly, recent evidence suggests that brachial pulse pressure, a surrogate measure of arterial stiffness, is correlated with CRP and interleukin-6 (IL-6) levels in apparently healthy men. Moreover, an association between CRP and aortic PWV has been reported in subjects with end-stage renal failure. However, whether there is any relationship between inflammation and central pulse pressure or more direct indices of arterial stiffness such as aortic PWV in healthy individuals is unclear. We hypothesized that CRP levels would be correlated with aortic PWV and central pulse pressure in healthy individuals without manifest cardiovascular disease. The aim of the present study was to test this hypothesis in an unselected cohort of individuals across a range of blood pressures and ages.

Methods

Subjects
A total of 427 healthy subjects, across a wide age range, were studied as part of an ongoing investigation into the factors influencing arterial stiffness. Individuals were selected at random from local General Practice lists. The overall response rate to the initial invitation was 85%. Subjects with diabetes mellitus, a serum cholesterol ≥6.5 mmol/L, renal disease (defined as a clinical history, creatinine ≥150 μmol/L or an active urinary sediment), or cardiovascular disease (defined as a clinical history or evidence on examination) were excluded from the study, as were subjects receiving any medication. Untreated hypertensive subjects (blood pressure ≥140/90 mm Hg) were included. Approval for all studies was obtained from the Local Research Ethics Committee, and written informed consent obtained from each participant.

Hemodynamics
Blood pressure was recorded in the dominant arm using a validated oscillometric technique (HEM-705CP; Omron Corporation, Japan).
Radial artery waveforms were recorded with a high-fidelity micromanometer (SPC-301; Millar Instruments, Tex) from the wrist of the dominant arm. Pulse wave analysis (SphygmoCor; AtCor Medical, Sydney, Australia) was then used to generate a corresponding central (ascending aortic) waveform using a transfer function, as described previously. This transfer function has been prospectively validated for the assessment of ascending aortic blood pressure, and the system shows good repeatability of measurements. Aortic augmentation index (AIx) and heart rate were determined using the integral software. Augmentation index, a measure of systemic arterial stiffness, was calculated as the difference between the second and first systolic peaks, expressed as a percentage of the pulse pressure. The aortic PWV was measured using the same device by sequentially recording ECG-gated carotid and femoral artery waveforms, as previously described in detail. The brachial PWV was determined by sequential recording of pressure waveforms at the carotid and radial arteries. In addition, carotid pulse wave pressures were recorded directly, using applanation tonometry and the SphygmoCor apparatus. These waves were then scaled to the brachial mean and diastolic pressures using the SphygmoCor software, and carotid AIx and systolic pressure calculated using the SphygmoCor software. This approach provided central hemodynamic data without the use of any transfer function, in a similar manner to that described previously. All measurements were made in duplicate, and the mean values were used in the subsequent analysis.

Protocol
All studies were conducted in a quiet temperature-controlled room (22 ± 2°C). Height and weight were recorded. After 20 minutes of supine rest, blood pressure and radial artery waveforms were recorded, and then the aortic and brachial PWV measured. All arterial stiffness measurements were made by trained investigators (Y., C.M.M., S.W., I.S.M.), and the within- and between-observer measurement reproducibility values were in agreement with our previously published data. Ten milliliters of blood were then drawn from the antecubital fossa into plain tubes. The samples were then centrifuged at 4°C (4000 rpm for 20 minutes), and the serum separated and stored at −80°C for subsequent analysis. A highly sensitive latex-based immunoassay (Dade Behring, Milton Keynes, UK) was used to determine levels of CRP. Cholesterol (total, and LDL and HDL fractions), triglycerides, glucose, and creatinine were determined using standard methodology in an accredited laboratory.

Data Analysis
Data were analyzed using SPSS software (version 11.0) and linear regression analysis. Multiple regression analysis was conducted using the “enter method.” Values of CRP and body mass index (BMI) were significantly skewed and, therefore, data were log-normalized before inclusion in regression analyses. ANOVA with Bonferroni post hoc test was used to assess differences between groups based on category of CRP. All values represent means ± SD, and P < 0.05 was considered significant. Variables for the stepwise linear regression model were chosen based on simple correlation analyses and those variables known or thought to be associated with arterial stiffness, from published observations. R² change indicates percent change for each parameter.

Results
The baseline characteristics of the subjects studied are shown in Table 1. The mean age of the study group was 47 years (range 16 to 83); 68 subjects were younger than 20 years, 66 between 20 to 29 years, 42 between 30 to 39, 46 between 40 to 49, 58 between 50 to 59, 87 between 60 to 69, and 60 were 70 years or older. There was an equal sex distribution across age ranges.

Of the 427 subjects studied, 115 had hypertension (blood pressure ≥ 140/90 mm Hg); 106 subjects had a CRP < 1 mg/L, 170 a value of 1 to 3 mg/L, and 151 had a value ≥ 3 mg/L. When subjects were categorized according to CRP levels that relate to future cardiovascular risk (≤ 0.9, 1 to 3, and ≥ 3 mg/L), aortic PWV (7.0 ± 2.4; 7.6 ± 2.5; 8.2 ± 2.8 m/sec), central pulse pressure (37 ± 12; 39 ± 11; 43 ± 14 mm Hg), and brachial pulse pressure (52 ± 13; 53 ± 12; 56 ± 13 mm Hg) were significantly related to CRP category (P < 0.001; P < 0.001; P = 0.02, respectively). The mean age differed between the CRP categories (42, 46, and 50 years, respectively; P = 0.005), as did mean arterial pressure (92, 93, 96 mm Hg, respectively; P = 0.02), but there was no difference in the gender distribution between groups (P = 0.6). Categorizing CRP into tertiles did not meaningfully alter any of the associations.

Values of CRP were significantly skewed (median value 1.9 mg/L); therefore, log-normalized data were used for subsequent analyses. With simple linear regression models, serum CRP levels were significantly correlated with age, mean arterial pressure, brachial and aortic PWV, and with central, carotid, and peripheral pulse pressures. However, in a multiple regression model including all the aforementioned parameters, only mean pressure remained significantly associated with CRP (P = 0.027).

The aortic PWV was significantly related to age, BMI, mean pressure, LDL cholesterol, triglycerides, and CRP. When these parameters were entered into a stepwise linear regression model, together with gender, glucose, smoking,
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and heart rate, the aortic PWV was positively associated with age, CRP, male gender, and mean arterial pressure (Table 2). Similarly, brachial PWV was positively associated with age, mean arterial pressure, CRP, male gender, triglycerides, HDL cholesterol, and smoking (Table 2).

In a further multivariate model, AIx was positively correlated with age, female gender, and mean arterial pressure, and inversely correlated with heart rate and height, but there was no correlation with CRP ($R^2=0.794$; $P<0.001$). Use of carotid AIx rather than aortic values improved the predictive value of the model ($R^2=0.837$; $P<0.001$) but did not meaningfully alter any of the associations (acceptable waveforms were available for 400 subjects). Indeed, there was a high degree of correlation between derived aortic AIx and carotid AIx ($r=0.85$; $P<0.001$), and the values for both indices are in agreement with those reported previously. Substitution of mean arterial pressure with peripheral diastolic pressure did not significantly alter any of the models. Similarly, PWV was still independently associated with CRP when separate models were constructed for men and women, and for hypertensive (blood pressure $\geq 140$ and/or $90$ mm Hg) and normotensive (blood pressure $<140$ and $90$ mm Hg) individuals.

Two further stepwise linear regression models were constructed to investigate the factors related to pulse pressure. Peripheral pulse pressure was related to age, CRP, male gender, and smoking, and central pulse pressure was associated with age, heart rate, BMI, and CRP (Table 3). The predictive value of the models was improved when subjects were categorized according to age. However, the associations between variables were not significantly altered. Moreover, use of carotid, rather than derived aortic, pulse pressure did not meaningfully alter the regression models. Indeed, there was a high degree of correlation between carotid and derived aortic pulse pressure values ($r=0.82$; $P<0.001$).

**Table 2. Stepwise Regression Analyses for Aortic and Brachial PWV**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>Beta</th>
<th>Significance</th>
<th>$R^2$ Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic PWV*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.079</td>
<td>0.007</td>
<td>0.659</td>
<td>$&lt;0.001$</td>
<td>54</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>0.030</td>
<td>0.011</td>
<td>0.152</td>
<td>0.008</td>
<td>22</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.719</td>
<td>0.227</td>
<td>0.142</td>
<td>0.002</td>
<td>16</td>
</tr>
<tr>
<td>LnCRP</td>
<td>0.309</td>
<td>0.106</td>
<td>0.127</td>
<td>0.004</td>
<td>15</td>
</tr>
<tr>
<td>Brachial PWV†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.018</td>
<td>0.005</td>
<td>0.275</td>
<td>$&lt;0.001$</td>
<td>23</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>0.024</td>
<td>0.009</td>
<td>0.208</td>
<td>0.006</td>
<td>35</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.668</td>
<td>0.263</td>
<td>-0.144</td>
<td>0.012</td>
<td>20</td>
</tr>
<tr>
<td>LnCRP</td>
<td>0.165</td>
<td>0.071</td>
<td>0.131</td>
<td>0.021</td>
<td>14</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.686</td>
<td>0.206</td>
<td>0.213</td>
<td>$&lt;0.001$</td>
<td>14</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.429</td>
<td>0.171</td>
<td>0.153</td>
<td>0.013</td>
<td>17</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.184</td>
<td>0.091</td>
<td>0.128</td>
<td>0.045</td>
<td>12</td>
</tr>
</tbody>
</table>

Variables excluded in the aortic PWV model were triglycerides, HDL and LDL cholesterol, smoking, glucose, body mass index, and heart rate. Variables excluded from the brachial PWV model were LDL cholesterol, glucose, body mass index, and heart rate.

Stepwise linear regression analysis using all 427 subjects. The regression co-efficient provides the slope of the regression line relating each parameter to the PWV, and beta provides a measure of the relative strength of the association independent of the measurement units. Replacing LDL cholesterol with total cholesterol did not significantly alter either model.

**Discussion**

The main novel findings from the present study are that, even after controlling for other confounding factors, aortic and brachial PWV are both associated with the inflammatory marker CRP in healthy individuals. In addition, we have extended previous observations by demonstrating that serum CRP is independently correlated with both peripheral and central pulse pressure. Interestingly, there was no correlation between AIx and CRP. These data suggest that arterial stiffness is related to the level of systemic inflammation, and that inflammation may play a role in the process of arterial stiffening.

Arterial stiffness is an independent predictor of all-cause and cardiovascular mortality in selected populations, and may play a more direct role in the process of atherosclerosis itself. Serum levels of CRP, an acute phase reactant, also predict cardiovascular risk, and inflammation is believed to be a key process in atheroma formation. London et al found a correlation between aortic PWV and CRP in patients with end-stage renal failure and also demonstrated that there was an inverse correlation between CRP level and the efficacy of antihypertensive medication. C-reactive protein also correlates with large artery elasticity in patients with rheumatoid arthritis and in patients with acute antineutrophil cytoplasmic antibody–associated vasculitis. However, to our knowledge, only 1 previous study, reported after the present study was completed, has explored the relationship between inflammation and arterial stiffness in healthy individuals. Although the authors demonstrated an associ-
ation between CRP and peripheral pulse pressure, there was no association with aortic stiffness or with atherosclerotic load, and the influence of CRP on central pulse pressure was not explored. Moreover, 40% of the study population were hypertensive and a number were receiving vasoactive or anti-inflammatory medication, which may have confounded the observations relating to aortic stiffness.

When CRP was grouped using previously published categories, which relate to cardiovascular risk (<1, 1 to 3, and >3 mg/L), aortic PWV was related to CRP in a stepwise manner. The difference in aortic PWV between the highest and lowest CRP categories was equivalent to an additional age difference of almost 12 years. Although the PWV provides a robust measure of arterial distensibility, it is influenced by a number of factors including age and mean pressure. Therefore, to explore more fully the relationship between aortic PWV and inflammation, a multiple regression model was constructed with known or likely determinants of arterial stiffness. As expected, age, mean arterial pressure, and male gender were correlated with aortic PWV, but AIx did not. Augmentation index provides a composite measure of arterial stiffness and, as such, depends on the site and degree of wave reflection, and the speed of wave travel (the PWV). Therefore, the current data suggest that inflammation increases large and muscular artery stiffness rather than wave reflection from sites of impedance mismatch in the periphery. Although normally related, drugs and various physiological maneuvers can affect AIx and PWV differently.

Peripheral pulse pressure, frequently considered as a surrogate measure of large artery stiffness, correlates with CRP and IL-6 levels, in healthy middle-aged men. In the present study, we have confirmed the relationship between peripheral pulse pressure and CRP, and between pulse pressure and age. However, peripheral pulse pressure does not always provide reliable information concerning aortic stiffness or central pulse pressure. This is potentially important because pulse pressure differs throughout the arterial tree, and central pulse pressure more closely relates to carotid intima-media thickness, and outcome than peripheral pulse pressure. Importantly, in the present study there was also a positive, independent correlation between central pulse pressure and CRP levels, even after adjustment for age and heart rate. The lack of association between CRP and AIx suggests that an increase in aortic stiffness, rather than enhanced wave reflection, mediates the increase in central pulse pressure with increasing inflammatory load.

In the present study, we did not observe any independent relationship between LDL cholesterol or glucose and arterial stiffness, although HDL cholesterol was positively associated with brachial PWV. These findings are perhaps surprising in light of our previous published work and that of others but may reflect the a priori exclusion of patients with diabetes mellitus and hypercholesterolemia. Moreover, CRP is likely to be a better predictor of cardiovascular risk than serum cholesterol. Another potential criticism is the use of a transfer function to derive central parameters from the radial artery waveform. However, the transfer function has previously been validated for the measurement of central pressure and substitution of carotid indices, which were recorded directly from the carotid artery and therefore not subject to any mathematical transfer function, did not meaningfully alter any of our observations.

The cross-sectional nature of the present study also limits our ability to infer a causal relationship between inflammation and arterial stiffness; therefore, further studies are likely to be required to determine whether inflammation per se leads to arterial stiffening. Although we did not explore the potential mechanisms by which inflammation may increase arterial stiffness, we and others have recently demonstrated...
strated that endothelial-derived nitric oxide is important in the functional regulation of large artery stiffness in vivo, and both acute and chronic inflammation are known to impair endothelial function. Therefore, endothelial dysfunction may provide one possible mechanism linking inflammation and stiffness. Alternatively, inflammation may induce structural changes in the arterial wall, by altering the balance between elastin breakdown and synthesis. Indeed, several elastolytic enzymes are known to be upregulated by inflammatory cytokines, including matrix metalloproteinase-9. However, further studies are required to address these hypotheses.

Summary

Large artery stiffness and central pulse pressure are important determinants of cardiovascular risk. The data presented in the current study indicate that both indices are related to the level of inflammation in apparently healthy individuals across a wide age range. These data suggest that an inflammatory process may be involved in large artery stiffening. Therefore, strategies that reduce inflammation, such as HMGCoA reductase inhibitors (statins), which have recently been shown to reduce stiffness in a small cohort of patients with isolated systolic hypertension, or anti-TNFα therapy, may potentially be of benefit in reducing arterial stiffness in individuals with exaggerated or premature arterial stiffening, and thus potentially in reducing cardiovascular risk.

Acknowledgments

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References

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