C-Reactive Protein Concentrations Are Very High and More Stable over Time Than the Traditional Vascular Risk Factors Total Cholesterol and Systolic Blood Pressure in an Australian Aboriginal Cohort

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BACKGROUND: Stability of circulating high-sensitivity C-reactive protein (hsCRP) concentrations has implications for its utility in assessing cardiovascular disease (CVD) risk. We sought to determine hsCRP reproducibility in an indigenous Australian cohort with a view to use hsCRP as a marker of future CVD in community-based risk-factor screenings.

METHODS: Seventy people living in a community on the northern coast of Australia participated in 2 risk-factor screenings over a median (interquartile range) follow-up time of 829 (814–1001) days. hsCRP was measured by high-sensitivity nephelometry.

RESULTS: Geometric mean hsCRP concentrations at baseline and follow-up were 4.5 and 5.1 mg/L, respectively \((P = 0.220)\), and Pearson product-moment correlation was 0.775. The proportion of people at high CVD risk \((\text{hsCRP} > 3.0 \text{ mg/L})\) at baseline was 67.1% and remained consistently high \((68.6\%)\) at follow-up. Linear regression analysis for follow-up hsCRP as a function of baseline hsCRP, sex, and differences in total and regional body fatness showed that baseline hsCRP was the single predictor in the model, accounting for 63.9% of the total variance in follow-up hsCRP \((P_{\text{model}} < 0.001)\). Prevalence agreement (95% CI) between baseline and follow-up for the hsCRP \(\geq 3.0 \text{ mg/L}\) category was 84% \((73\%–92\%)\) \((P_{\text{McNemar}} = \text{not significant})\), and \(\kappa\) coefficient was fair \((0.64, \text{compared with} 0.31\) for systolic blood pressure \(\geq 140 \text{ mmHg}\) and 0.43 for total cholesterol \(\geq 5.5 \text{ mmol/L}\)).

CONCLUSIONS: hsCRP concentrations remained consistently reproducible over time across a wide concentration range in an Aboriginal cohort. Correlations between concentrations over time were better than for other traditional CVD risk factors. hsCRP concentration has potential as a marker of future CVD risk.

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The pentraxin C-reactive protein (CRP) is an acute-phase reactant that is mainly produced in the liver in response to infection or injury. Recently, low concentrations of C-reactive proteins (measured using highly sensitive assays and thus abbreviated to hsCRP) have emerged as a potentially useful marker of cardiovascular disease (CVD) risk (1). The American Heart Association (AHA) has advocated for its use as a predictor of CVD in clinical practice (2). High premature CVD mortality is an important contributor to the 20-year shorter median life expectancy of indigenous Australians relative to nonindigenous Australians (3). We have previously reported high hsCRP concentrations in Australian Aboriginal populations (4), and we hypothesized that chronic inflammation may be an important contributor to the excess CVD risk (5). The aim of this study was to determine the degree of reproducibility of hsCRP concentrations over time in an Aboriginal cohort, to determine if subclinical elevation of hsCRP is a chronic condition in this population, with a view to using hsCRP as a marker of CVD risk in community-based risk-factor screenings.

Research Design and Methods

The research design and methods are reported in detail elsewhere (4). Briefly, we screened 70 participants living in an isolated coastal Aboriginal community in the northeast of Arnhem Land in 2 risk-factor screening programs between 2001 and 2004. The median time...
The interquartile range between the first and second assessment of hsCRP concentrations in this cohort was 829 (814–1001) days. Venous blood samples were collected and kept cold until centrifuged, and serum was stored under liquid nitrogen until transferred to storage at −80 °C. We measured serum hsCRP using a commercial, high-sensitivity nephelometric assay (BN-II Nephelometer; Dade Behring Diagnostics), with a lower limit of detection of 0.175 mg/L. In-house intra- and interassay CVs for hsCRP at 2.4 mg/L were 1.9% and 4.3%, respectively. We measured cholesterol in lithium heparin plasma, glucose in fluoridated plasma, and hemoglobin A1c in EDTA-treated whole blood. Lipid and glucose analyses were performed by routine methods in a diagnostic laboratory accredited by the National Association of Testing Authorities (SouthPath Flinders Medical Centre, SA Australia). We have not reported estimates of LDL cholesterol concentration because the Friedewald equation used to calculate LDL cholesterol has not been validated for use in Aboriginal populations, which are characterized by very low HDL cholesterol concentrations, increased triglycerides, and small, dense LDL particles (6). The Friedewald equation is based on assumptions about the distribution of triglycerides across lipoprotein fractions that may not hold in this context (7).

Statistical analyses were performed using SPSS (ver. 15.0). We used paired t-test to compare baseline and follow-up measurements and a multivariate linear regression model to determine follow-up hsCRP as a function of baseline hsCRP, sex, and change in total and regional adiposity. We used Pearson correlation and Bland–Altman (the relationship of the difference between the 2 measurements to the mean of the 2 measurements (8)) scatterplots to visually illustrate the relationship between follow-up and baseline hsCRP measurements. For categorical analyses of hsCRP and traditional CVD risk factors, we assessed agreement between paired results using the κ statistic. κ Coefficient <0.40 was considered poor agreement, 0.40–0.75 fair agreement, and >0.75 excellent agreement (9). We used the McNemar test to assess the level of agreement in prevalence of categories at baseline and follow-up.

### Table 1. Characteristics of participants in baseline and follow-up screenings (n = 70).

<table>
<thead>
<tr>
<th>Variable</th>
<th>R&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean hsCRP, mg/L (95% CI)</td>
<td>0.775</td>
<td>4.6 (3.5–5.8)</td>
<td>5.1 (3.9–6.7)</td>
<td>0.220</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>0.731</td>
<td>4.7 (1.0)</td>
<td>4.8 (0.9)</td>
<td>0.148</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.539</td>
<td>2.08 (1.32)</td>
<td>2.30 (1.34)</td>
<td>0.176</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.825</td>
<td>0.95 (0.27)</td>
<td>0.96 (0.25)</td>
<td>0.521</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>0.815</td>
<td>6.1 (1.6)</td>
<td>6.5 (1.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>0.541</td>
<td>6.5 (3.3)</td>
<td>6.8 (4.0)</td>
<td>0.443</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.964</td>
<td>26.1 (5.5)</td>
<td>26.1 (5.4)</td>
<td>0.942</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0.722</td>
<td>96 (13)</td>
<td>96 (18)</td>
<td>0.998</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.969</td>
<td>74 (16)</td>
<td>75 (16)</td>
<td>0.201</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>0.582</td>
<td>119 (20)</td>
<td>119 (22)</td>
<td>0.902</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>0.610</td>
<td>71 (10)</td>
<td>71 (12)</td>
<td>0.987</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>0.556</td>
<td>48 (14)</td>
<td>49 (14)</td>
<td>0.846</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are mean (SD) unless noted otherwise.  
<sup>b</sup> Pearson product-moment correlation, baseline vs follow-up.  
<sup>c</sup> Paired t-test.
follow-up screenings. Statistical significance was accepted at $P < 0.05$.

**Results**

Two discrepant values (>3 SDs of the mean difference between baseline and follow-up hsCRP) were excluded from analyses of hsCRP as a continuous variable but not from categorical analyses. Fig. 1 shows the distribution of hsCRP in the population at baseline and follow-up screenings: no bimodality was detected for hsCRP distribution in the population. Table 1 shows clinical characteristics of the participants: there were trends toward increases in all biochemical and anthropometric measurements, but only the increases in hemoglobin A1c and hsCRP were statistically significant. Importantly, hsCRP concentrations were very high in the cohort in both baseline and follow-up screenings (the median hsCRP concentration for adults in the Busselton Health Survey, which is representative of the general Australian population, was about 1.4 mg/L; Hung et al. (10)). The correlation between baseline and follow-up hsCRP concentrations was greater than those for glucose, blood pressure, and cholesterol (Table 1), but not greater than for HDL cholesterol, hemoglobin A1c, or body mass index. Fig. 2 shows the relationship between baseline and follow-up hsCRP measurements using Pearson correlation and Bland–Altman scatterplots: Pearson product-moment correlation for baseline and follow-up screenings for (log-transformed) hsCRP was 0.775 (Fig. 2A). Bland–Altman analysis showed a modest increase in hsCRP concentrations over time in the population (Fig. 2B). Difference between baseline and follow-up concentrations was not associated with mean CRP (regression coefficient $= 0.188$, $P = 0.876$). Linear regression analysis for follow-up hsCRP as a function of baseline hsCRP, sex, and differences in total and regional body fatness showed that baseline hsCRP was the single significant predictor in the model [$P < 0.001$, accounting for 63.9% ($P_{\text{model}} < 0.001$) of the total variance in hsCRP at follow-up].

Finally, we tested the prevalence agreement between baseline and follow-up hsCRP measurements across AHA hsCRP risk categories for CVD and hsCRP >10.0 mg/L, shown in Table 2: 67% and 69% of the cohort were at high risk (hsCRP >3.0 mg/L) of CVD at baseline and follow-up, respectively. Additionally, there were more cases with hsCRP >10.0 mg/L during follow-up ($n_{\text{baseline}} = 17$ vs $n_{\text{follow-up}} = 21$), but this increase was not statistically significant ($P_{\text{McNemar}} = 0.424$). The proportions of all hsCRP-risk categories did not change significantly over time (all $P_{\text{McNemar}} = \text{not significant}$), and agreement for placing individuals within categories was fair at hsCRP >10.0 mg/L ($\kappa = 0.50$, $P < 0.001$) and higher for hsCRP >3.0 mg/L ($\kappa = 0.64$, $P < 0.001$). For comparison, agreement ($\kappa$ statistics) for the conventional CVD risk factors total cholesterol ($\geq 5.5$ mmol/L) and systolic blood pressure ($\geq 140$ mmHg) in this population were lower than for hsCRP >3.0 mg/L (Table 2). $\kappa$ Statistics for low HDL cholesterol, overweight, and hyperglycemia were greater than for hsCRP >3.0 mg/L.

**Discussion**

Circulating hsCRP concentrations were highly reproducible in this cohort at the population level, even after...
The median interval of >2 years between the 2 measurements. Reproducibility of hsCRP concentrations in this population indicates that the first measurement of hsCRP reflects a chronic inflammatory condition. The proportion of the cohort in different hsCRP-risk categories did not change significantly over the median period of 829 days, indicating that the CVD risk profile remained consistent at the population level.

As expected, within-person hsCRP concentrations varied somewhat more across all risk categories as indicated by \( \kappa \) statistics. However, there was good agreement with respect to individuals having hsCRP >3.0 mg/L, that is, falling within the AHA high-risk category on both occasions. The observed within-person variability was actually lower when compared with that of the conventional traditional CVD risk factors total cholesterol and blood pressure, whether considered as continuous (Table 1) or binary (Table 2) variables. In support, Ockene et al. (11) reported that >60% of hsCRP concentrations in apparently healthy adults in the US (mean hsCRP 2.8 mg/L) remained in the same quartile in a repeat measurement after 12 months. Furthermore, Ridker et al. (12) reported strong correlation \( (r = 0.60, P < 0.001) \) of circulating hsCRP concentrations after 60 months of follow-up in post–myocardial infarction patients. In the British Regional Heart Study, underestimation of CVD risk due to time-related variability in risk factors was similar for CRP and systolic blood pressure (but slightly lower than for total cholesterol) (13). Thus hsCRP measurements at the population and individual levels in this Aboriginal population are stable and comparable with other traditional risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline, % (n)</th>
<th>Follow-up, % (n)</th>
<th>Agreement, % (95% CI)*</th>
<th>( \kappa ) (P)</th>
<th>McNemar ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.0</td>
<td>7.1 (5)</td>
<td>8.6 (6)</td>
<td>87 (76–94)</td>
<td>0.11 (0.343)</td>
<td>1.000</td>
</tr>
<tr>
<td>1.0–3.0</td>
<td>25.7 (18)</td>
<td>22.9 (16)</td>
<td>93 (83–97)</td>
<td>0.38 (0.001)</td>
<td>0.804</td>
</tr>
<tr>
<td>3.0–10.0</td>
<td>42.9 (30)</td>
<td>38.6 (27)</td>
<td>67 (55–78)</td>
<td>0.32 (0.007)</td>
<td>0.678</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>24.3 (17)</td>
<td>30.0 (21)</td>
<td>80 (68–88)</td>
<td>0.50 (&lt;0.001)</td>
<td>0.424</td>
</tr>
<tr>
<td>&gt;3.0</td>
<td>67.1 (47)</td>
<td>68.6 (48)</td>
<td>84 (73–92)</td>
<td>0.64 (&lt;0.001)</td>
<td>1.000</td>
</tr>
<tr>
<td>Systolic blood pressure ≥140 mmHg</td>
<td>18.6 (13)</td>
<td>10.1 (7)</td>
<td>83 (71–90)</td>
<td>0.31 (0.006)</td>
<td>0.146</td>
</tr>
<tr>
<td>Total cholesterol ≥5.5 mmol/L</td>
<td>17.1 (12)</td>
<td>16.2 (11)</td>
<td>84 (72–91)</td>
<td>0.43 (&lt;0.001)</td>
<td>1.000</td>
</tr>
<tr>
<td>HDL cholesterol &lt;1.03 (male)/&lt;1.29 (female) mmol/L</td>
<td>81.4 (57)</td>
<td>76.5 (52)</td>
<td>93 (83–97)</td>
<td>0.78 (&lt;0.001)</td>
<td>0.375</td>
</tr>
<tr>
<td>Body mass index ≥25.0 kg/m²</td>
<td>55.7 (39)</td>
<td>55.7 (39)</td>
<td>94 (89–99)</td>
<td>0.88 (&lt;0.001)</td>
<td>1.000</td>
</tr>
<tr>
<td>Fasting plasma glucose ≥6.1 mmol/L</td>
<td>22.9 (16)</td>
<td>34.3 (24)</td>
<td>86 (75–93)</td>
<td>0.66 (&lt;0.001)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

* Proportion of the total sample falling within the same category on both occasions.

The AHA has suggested repeating measures of hsCRP >10.0 mg/L within 2 weeks to exclude an acute inflammatory condition at the time of testing (2). The exclusion of samples with hsCRP >10.0 mg/L has been widely accepted in cross-sectional studies, including studies on indigenous populations (14, 15). In the present study, no bimodality for hsCRP distribution was evident in either baseline or follow-up. These findings are in agreement with other large-scale studies, such as the Women’s Health Study (16), where the risk of the first CVD event increased linearly across increasing deciles of hsCRP, even after stratifying by the Framingham risk score and controlling for diabetes, with no evidence of any threshold effect. Moreover, the authors also proposed that people with hsCRP >10.0 mg/L should be regarded at very high risk of CVD, recommending another hsCRP-risk category for CVD. Taken together, there was no firm evidence for a threshold effect at hsCRP 10.0 mg/L, prompting a revision of its use in clinical settings. Other groups with increased circulating hsCRP in association with chronic inflammatory conditions (rheumatoid arthritis and systemic lupus erythematosus) also experience greatly increased CVD risk and shorter life expectancy (17, 18).

That hsCRP concentrations were highly reproducible over time is consistent with other published data on inflammation, diet, infections, and abnormal metabolism. In the Reykjavik prospective study, the correlation of hsCRP concentrations at a 12-year interval was 0.59, similar to blood pressure and total cholesterol in that study (19). Rowley et al. (5) reported very high hsCRP concentrations in 171 participants in
a chronic disease study in an Aboriginal community in Western Australia, in association with high levels of vascular cell adhesion molecules (a measure of vascular inflammation) and an inverse correlation with plasma carotenoids. McDonald et al. (20) reported high hsCRP concentrations in association with high infectious load in another Australian Aboriginal community. Additionally, results from the Strong Heart Study of CVD in 3277 participants from American Indian communities (13) showed a median (interquartile range) hsCRP of 3.2 (1.8–5.1) mg/L with a relatively high proportion of people with hsCRP >10.0 mg/L (16% vs 24% at baseline in this cohort), consistent with abnormally high hsCRP concentrations in economically disadvantaged populations.

The stability of hsCRP after multiple freeze-thaw cycles and a lengthy period in storage has been reported (21, 22) and was also reproduced in our laboratory (data not shown). Technically, a number of assays can be used to determine hsCRP concentrations in blood. High levels of agreement among commercially available hsCRP measurement assays in categorizing patients into CVD-risk categories has been demonstrated (19, 23), indicating that such assays are comparable for clinical purposes. Physiologically, hsCRP is unaffected by diurnal variation and has a relatively long half-life (23). An additional advantage is that, like total and HDL cholesterol, hsCRP can be measured in nonfasting blood samples.

In conclusion, hsCRP appears to be useful for monitoring CVD risk, and possibly evaluating clinical and public health interventions in indigenous communities with high levels of cardiovascular disease risk.

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**Honoraria:** None declared.

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**References**


