Cystatin C as a Determinant of Fasting Plasma Total Homocysteine Levels in Coronary Artery Disease Patients With Normal Serum Creatinine

Andrew G. Bostom, Linda Bausserman, Paul F. Jacques, Gintaras Liaugaudas, Jacob Selhub, Irwin H. Rosenberg

Abstract—Serum creatinine, a surrogate for both renal function and homocysteine generation, is a determinant of fasting plasma total homocysteine levels in coronary artery disease (CAD) patients. We hypothesized that among stable-CAD patients with normal creatinine levels (ie, \( \leq 1.4 \text{ mg/dL} \)), serum cystatin C, a more sensitive indicator of glomerular filtration rate, would better predict fasting total homocysteine levels in comparison with serum creatinine. Fasting plasma total homocysteine, folate, vitamin \( B_12 \), and pyridoxal \( 5' \)-phosphate levels, along with serum cystatin C, creatinine, and albumin levels, were determined in 164 consecutive stable-CAD patients (mean \( \pm \text{SD age, } 61 \pm 9 \text{ years; } 78.7\% \text{ men} \) whose serum creatinine level was \( \leq 1.4 \text{ mg/dL} \). All subjects were examined at least 3 to 4 months after the widespread availability of cereal grain flour products fortified with folic acid. General linear modeling with ANCOVA revealed that serum cystatin C \( (P<0.001) \), \( B_12 \) \( (P<0.001) \), age \( (P=0.002) \), albumin \( (P=0.008) \), and sex \( (P=0.024) \) were independent determinants of fasting total homocysteine levels. Cystatin C alone determined over half of the variability (ie, \( R^2 \)) in total homocysteine levels accounted for by these 5 independent regressors. In contrast, creatinine, folate, and pyridoxal \( 5' \)-phosphate were not independently predictive of fasting total homocysteine levels \( (P>0.2) \). Consistent with the impact of folic acid fortification of cereal grain flour in the general population, only 1 of the CAD subjects (0.6%) had a plasma folate level <3 ng/mL. We conclude that serum cystatin C levels may reflect subtle decreases in renal function that independently predict fasting total homocysteine levels among stable-CAD patients with normal serum creatinine. (Arterioscler Thromb Vasc Biol. 1999;19:2241-2244.)

Key Words: coronary arteriosclerosis ■ renal function ■ homocysteine ■ determinants

Pooled observational data strongly suggest that mild hyperhomocysteinemia is an independent risk factor for coronary artery disease (CAD).\(^{1,2} \) Creatinine, a surrogate for both glomerular filtration rate\(^{3} \) and homocysteine (Hcy) generation,\(^{4} \) is a significant determinant of total homocysteine (tHcy) levels in CAD\(^{5,6} \) and general populations.\(^{7} \) Systemic arteriosclerosis\(^{8} \) and clinical\(^{9} \) as well as subclinical CAD\(^{10} \) have been associated with nephrosclerosis, specifically, renal arteriolar hyalinization.\(^{8-10} \) In cross-sectional analyses, creatinine may be a relatively insensitive marker of the mildly to moderately reduced glomerular filtration rates\(^{11} \) likely to be encountered among CAD patients with subclinical nephrosclerosis.

Cystatin C is a nonglycosylated 13-kDa basic protein produced at a stable rate by all investigated nucleated cells and whose serum concentration is primarily determined by the glomerular filtration rate.\(^{12} \) Consistent investigations now clearly indicate that serum cystatin C is superior to serum creatinine for the detection of early decreases in glomerular filtration rate.\(^{13-15} \) There is a strong, independent (inverse) association between glomerular filtration rate, directly determined by either iohexol clearance\(^{16} \) or \( {^{51} \text{Cr-EDTA clearance}} \), and fasting tHcy levels, which encompasses glomerular filtration rates throughout the normative range. These data further revealed that serum creatinine was not an independent predictor of fasting tHcy levels in models that included directly measured glomerular filtration rate.\(^{16,17} \) In accord with these findings, we have recently demonstrated that among renal transplant recipients with normal renal function (ie, serum creatinine \( \leq 1.5 \text{ mg/dL} \)), serum cystatin C was a much better predictor of fasting tHcy levels relative to serum creatinine.\(^{18} \) To examine the generalizability of our results in renal transplant recipients, we assessed fasting plasma tHcy, serum creatinine, and serum cystatin C, in

Received November 16, 1998; revision accepted January 26, 1999.

From the Divisions of General Internal Medicine and Cardiology, Memorial Hospital of Rhode Island, Providence, RI (A.G.B., G.L.); the Tufts–Jean Mayer USDA Human Nutrition Research Center on Aging, Boston, Mass (A.G.B., P.F.J., J.S., I.H.R.); and the Lipid Research Laboratory, The Miriam Hospital, Providence, RI (L.B.).

The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Reprint requests to Andrew G. Bostom, MD, MS, Division of General Internal Medicine, Memorial Hospital of Rhode Island, 111 Brewster St, Pawtucket, RI 02860. E-mail abostom@loa.com

© 1999 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

2241
conjunction with the other established determinants of tHcy levels (age, sex, B-vitamin status, and albumin), among 164 consecutive patients with clinically stable CAD whose serum creatinine level was \( \leq 1.4 \) mg/dL.

### Methods

The institutional review board at the Memorial Hospital of Rhode Island (Pawtucket) approved the study protocol, and all participants provided written, informed consent. Study participants were 164 stable-CAD patients (ie, they were at least 3 months after myocardial infarction or coronary angioplasty and/or at least 6 months after coronary artery bypass graft surgery) whose serum creatinine level was \( \leq 1.4 \) mg/dL. CAD status was confirmed by established 12-lead ECG and cardiac isoenzyme (ie, creatine phosphokinase MB) criteria for definite myocardial infarction and/or unstable angina with angiographically proven \( \geq 50\% \) stenosis of at least 1 major epicardial coronary artery. Participants lived in the Pawtucket and Providence, RI, metropolitan areas and were examined between October 1997 and October 1998. Information regarding prior vitamin supplement use was obtained by standardized interview, and subjects either were nonusers of any supplements containing folic acid or had abstained from using such supplements for at least 6 weeks by the time of their examination. However, all participants were examined at least 3 to 4 months after the widespread availability in New England (John Watson, President, Watson Foods, New Haven, Conn, personal communication) of cereal grain flour products fortified with folic acid at 140 \( \mu \)g per 100 g of flour.  

Overnight (10 to 14 hours) fasting blood samples were collected from each participant. Plasma tHcy levels were determined by high-performance liquid chromatography with fluorescence detection, and plasma pyridoxal 5'-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay, as reported earlier. Plasma folate and vitamin B\(_12\) levels were measured by radioassay (Bio-Rad Quantaphase II). Serum creatinine (Jaffe method) and albumin (bromcresol method) levels were determined using standard techniques adapted for automated clinical chemistry analyzers. Serum cystatin C levels were determined by particle-enhanced immunoturbidimetry.

All skewed continuous variables were (natural log) transformed to better approximate a normal distribution, and unadjusted correlations between continuous variables were assessed in a Pearson correlation matrix. General linear modeling with ANCOVA was then performed to determine the independent association between potential predictor covariates (ie, age, sex, folate, B\(_12\), albumin, PLP, creatinine, and cystatin C) and fasting tHcy levels. Reported probability value were based on 2-tailed calculations, and all statistical analyses were performed using SYSTAT (version 7.0.1) software.

### Results

Key subject characteristics expressed as means, geometric means, and complete ranges are depicted in Table 1. Only 1 subject (0.6%) had a plasma folate level <3 ng/mL. ANCOVA-adjusted (ie, for age, cystatin C, B\(_{12}\), and albumin) geometric mean fasting tHcy levels were greater in men \((n = 129)\) than in women \((n = 35)\); 8.6 versus 7.8 \( \mu \)mol/L, \( P = 0.024 \). Unadjusted Pearson correlations between the continuous variables examined are illustrated in Table 2. Creatinine was correlated with tHcy \((r = 0.162, P = 0.038)\), age \((r = 0.170, P = 0.029)\), cystatin C \((r = 0.157, P = 0.038)\), and folate \((r = 0.159, P = 0.041)\). Relative to creatinine, cystatin C was correlated more strongly with tHcy \((r = 0.400, P < 0.001)\) and age \((r = 0.236, P = 0.002)\) in these unadjusted analyses. General linear modeling with ANCOVA was performed with natural log fasting tHcy as the dependent variable and age, sex, natural log cystatin C, natural log creatinine, natural log folate, natural log B\(_{12}\), natural log PLP, and natural log albumin as the potential explanatory variables. Stepwise forward selection and backward elimination yielded the same set of 5 significant \((P < 0.10)\) regressors: cystatin C \((P < 0.001)\), B\(_{12}\) \((P < 0.001)\), age \((P = 0.002)\), albumin \((P = 0.008)\), and female sex \((P = 0.024)\). In contrast, creatinine, folate, and PLP levels were not independently predictive of fasting tHcy levels \((P > 0.2)\). Table 3 provides the standardized regression coefficients and partial \( R \) values for the 5 significant, independent regressors. As also noted in Table 3, cystatin C alone determined more than half of the variability in tHcy \((ie, R^2)\) accounted for by the “full” model containing, in addition, the 4 other independent regressors (ie, B\(_{12}\), age, sex, and albumin).

### Discussion

Prior studies\(^{13–15}\) have revealed that cystatin C is a more sensitive marker of mildly impaired glomerular filtration rate compared with creatinine and that glomerular filtration rate itself, well into the normative range, is a significant, independent predictor of fasting tHcy levels.\(^{16–17}\) An earlier analysis\(^{22}\) with cystatin C as a surrogate for glomerular filtration rate in healthy subjects had suggested that the well-characterized increase in plasma tHcy levels with advancing age might be due, in part, to an age-related decline in renal function. Moreover, we recently showed that cystatin C, but not serum creatinine, was an independent predictor of fasting tHcy levels in renal transplant recipients with normal renal function \(ie, serum creatinine levels \leq 1.5 \) mg/dL.\(^{18}\) Enlarging on these combined observations, we report the initial analysis, indicating that serum cystatin C levels may strongly and independently predict fasting tHcy levels among stable-CAD patients with normal serum creatinine levels.

The only independent determinants of fasting tHcy levels among the CAD patients, in addition to cystatin C, were plasma B\(_{12}\), age, albumin, and sex. All patients were examined at least several months after the widespread availability in southeastern New England of cereal grain flour products \(ie, all enriched wheat, corn, and rice flour products\) fortified with folic acid at 140 \( \mu \)g per 100 g of flour.\(^{21}\) Within a population-based sample of New England residents \(ie, the Framingham Offspring cohort\) who were nonusers of vitamin supplements, this fortification policy has doubled plasma folate levels while reducing the prevalence of low folate levels \(ie, <3 \) ng/mL \(by >90\%\) and the prevalence of fasting tHcy levels \(>13 \) \( \mu \)mol/L by nearly 50%.\(^{23}\) The very low prevalence of plasma folate <3 ng/mL \(0.6\%)\) in the CAD patients examined in the present study is completely consis-

### Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>N</th>
<th>164</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No./ % of women</td>
<td>35 (21.3%)</td>
</tr>
<tr>
<td>Age, y (mean±SD)</td>
<td>61±9 [30–76]</td>
</tr>
<tr>
<td>tHcy, ( \mu )mol/L</td>
<td>8.4* [3.8–18.2]</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>0.93* [0.43–1.87]</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.1* [0.6–1.4]</td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td>8.9* [2.9–41.1]</td>
</tr>
<tr>
<td>B(_{12}), pg/mL</td>
<td>391* [165–788]</td>
</tr>
<tr>
<td>PLP, nmol/mL</td>
<td>53.6* [9.2–285.3]</td>
</tr>
<tr>
<td>Albumin, mg/dL</td>
<td>4.4* [2.9–5.6]</td>
</tr>
</tbody>
</table>

*Values are geometric means.

The full range of values appears in brackets.

---

1. Framingham Offspring cohort.
2. Jaffe method.
3. Natural log transformation.
4. Pearson correlation coefficient.
5. Mean.
7. Median.
8. Interquartile range.
11. General linear modeling with ANCOVA.
12. Partial correlation coefficient.
13. Regression coefficient.
15. Full model R-squared.
17. Partial R-squared.
18. Partial R-squared.
20. Partial R-squared.
22. Partial R-squared.
23. Partial R-squared.
tent with the prevalence of folate <3 ng/mL (1.7%; 95% CI, 0.0% to 5.4%) among 248 nonusers of supplements in the Framingham Offspring Study, who were similarly examined after the advent of folic acid fortification. Accordingly, improved relatively “homogeneous” folate status secondary to cereal grain fortification may have contributed to the lack of association between plasma folate and fasting tHcy levels observed in the present study. In contrast, the median age of the CAD patient population (62 years) could have accentuated the impact of the age-related decline in vitamin B12 status on fasting plasma tHcy levels.24 Persistent mild hyperhomocysteinemia is characteristic of patients with chronic renal insufficiency and end-stage renal disease.25 The etiology of this hyperhomocysteinemia remains unknown, although it has been hypothesized to result from either the loss of intrarenal Hcy metabolism26 or uremia-induced extrarenal defects in Hcy metabolism.27 The current findings from clearly nonuremic subjects with, at most, only subclinical renal impairment might suggest that intrarenal Hcy metabolism is a major determinant of Hcy levels. These data, however, cannot rule out the possibility that subtle extrarenal defects in Hcy metabolism that may accompany even such mild reductions in renal function could account for the resulting increases in tHcy levels.

Autopsy data have revealed a significant association between both clinical9 and subclinical10 CAD and nephrosclerosis. We suggest that the strong, independent association between cystatin C, but not creatinine, and tHcy levels reported here among CAD patients reflects the ability of cystatin C to detect subtle decrements in glomerular filtration rate.13–15 Related most likely to subclinical nephrosclerosis. Additional studies in other clinical as well as general populations will be required to confirm the external validity of the present findings.

In summary, serum cystatin C levels may reflect mild, subclinical losses of renal function that strongly and independently predict fasting tHcy levels among stable-CAD patients with normal serum creatinine levels.

### Acknowledgments
Support for this work was provided in part by National Heart, Lung, and Blood Institute (Bethesda, Md) grant No. RO1-HL-56908-01A1 (to J.S.); the US Department of Agriculture, Agricultural Research Service contract 53-3KO6-01 (to J.S.); and a grant from Roche Vitamin Division (to A.G.B.). We thank Susan Ritter, Evelyn Tolbert, Marie Nadeau, Bonnie Souppa, and Roberta Dickenson, for their excellent technical assistance.

### References

---

**TABLE 2. Pearson Correlation Matrix (P Values) of Natural Log–Transformed Data**

<table>
<thead>
<tr>
<th></th>
<th>tHcy</th>
<th>Cystatin C</th>
<th>B12</th>
<th>Folate</th>
<th>PLP</th>
<th>Creatinine</th>
<th>Age*</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy</td>
<td>...</td>
<td>0.400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystatin C</td>
<td>&lt;0.001</td>
<td>...</td>
<td>-0.254</td>
<td>-0.290</td>
<td>-0.255</td>
<td>0.186</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td>B12</td>
<td>(&gt;0.2)</td>
<td>(0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLP</td>
<td>(&gt;0.2)</td>
<td>(0.002)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>(0.001)</td>
<td>(0.045)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td>(&gt;0.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Untransformed.

---

**TABLE 3. Multiple Regression of Fasting Plasma tHcy on Potential Independent Predictor Variables**

<table>
<thead>
<tr>
<th></th>
<th>Standardized Regression Coefficient</th>
<th>Adjusted Partial R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>0.350</td>
<td>0.379</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B12</td>
<td>-0.253</td>
<td>-0.290</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.255</td>
<td>-0.247</td>
<td>0.002</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.186</td>
<td>0.209</td>
<td>0.008</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.152</td>
<td>-0.178</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Model $R^2$ = 0.308

Full model: $R^2$ = 0.220

Full model minus Cystatin C: $R^2$ = 0.165

Cystatin C-only “model”: $R^2$ = 0.160

Age, sex, and albumin-adjusted model: $R^2$ = 0.103

B12-only “model”: $R^2$ = 0.053

This table shows significant ($P<0.100$) independent regressors of fasting plasma tHcy determined from stepwise (ie, forward and backward) multivari-

able linear regression modeling as described in the text.

*The (final) full model includes cystatin C, B12, age, albumin, and sex but not (ie, $P>0.150$) folate, PLP, or creatinine as the independent regressors.


Cystatin C as a Determinant of Fasting Plasma Total Homocysteine Levels in Coronary Artery Disease Patients With Normal Serum Creatinine
Andrew G. Bostom, Linda Baussnerman, Paul F. Jacques, Gintaras Liaugaudas, Jacob Selhub and Irwin H. Rosenberg

doi: 10.1161/01.ATV.19.9.2241
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/9/2241

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/