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References

Day-to-Day, Postprandial, and Orthostatic Variation of Total Plasma Homocysteine, Poul Thrupp and Suzanne Ekelnud (1 Department of Clinical Biochemistry, Hvidovre Hospital, DK-2650 Hvidovre, Denmark, and 2 Department of Clinical Biochemistry, Aalborg Hospital, DK-9100 Aalborg, Denmark; * author for correspondence: fax 45 3675 0977, e-mail hcy@forum.dk)

Increased concentrations of the amino acid homocysteine—hyperhomocysteinemia—are correlated with atherosclerotic and thrombotic diseases (1, 2). High concentrations can be lowered by diet and dietary supplements with vitamins B12, folate, and pyridoxine. Homocysteine is produced in the metabolism of the essential amino acid methionine and is converted by cystathionine β-synthase to cysteine and by methionine synthase back to methionine. These enzymatic reactions are dependent on sufficient concentrations of the vitamins B12, folate, and pyridoxine.

Knowledge of biological, postprandial, and orthostatic variations are important in judging significant changes in results and error sources in blood sampling conditions (3), and several studies on the biological variation of plasma homocysteine have been published (4–6).

Fasting blood samples traditionally have been recommended for plasma homocysteine measurement because postprandial changes produce a modest decrease in the first hours and an increase after 8 h (7, 8).

Orthostatic changes can also be important in the monitoring of homocysteine in patients with atherosclerotic and thrombotic diseases. Because most homocysteine is bound to albumin, the decrease with supine posture is expected to be 5–10%.

In this study we examined the day-to-day, postprandial, and orthostatic variations of plasma total homocysteine.

Blood samples were obtained from 19 healthy hospital employees (11 women and 8 men) ages 19–60 years (median age, 44 years). None of these individuals was or became pregnant or had medical diseases. The intake of oral contraceptives, intermittent asthma and allergy medicines, nonsteroidal antiinflammatory drugs, acetaminophen, and multivitamins was allowed during the study. Multivitamins were taken on a regular basis by four persons. Blood hemoglobin, erythrocyte folate, and serum cobalamin concentrations in all subjects were within the reference intervals.

Participants provided fasting blood samples after arrival for work (0800 to 1000) and nonfasting samples after lunch (1215 to 1500). Samples were collected daily for 5 days from subjects in an upright position. In addition, samples for plasma homocysteine, serum albumin, and
serum sodium were obtained from nine participants before and after 30 min of supine rest.

Samples were collected in K$_3$EDTA, and all plasmas were separated within 30 min (except four samples used for biological variation that were separated at 35, 50, 60, and 60 min). After separation, the samples were frozen at $-20$ °C and kept frozen until analysis. The samples were not kept on ice before separation to reflect routine sampling conditions. Samples were assayed in singlicate, and all samples from the same person were assayed in one batch.

The study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the Regional Scientific Ethics Committee. All participants gave informed consent.

The method used to determine total plasma homocysteine was HPLC and has been described previously (9). The within-run analytical CV was 4.9%, and the between-run CV was 4.5%. The material used for internal quality control was plasma from a healthy donor. An external quality-assurance program with participants from all of Scandinavia (10) has shown results with maximum differences of 6% from the mean value.

Sodium and albumin in serum were determined by the dry-chemistry methods on a Vitros system (Johnson & Johnson). The analytical CVs were 1% for sodium and 1.5% for albumin (total). The samples were analyzed using the same batch of slides.

The formulas used in the calculation of biological, postprandial, and orthostatic CVs are given below:

$$CV_B^2 = CV_i^2 + CV_G^2$$

$$CV_T^2 = CV_B^2 + CV_A^2$$

where $CV_B$ is the coefficient of biological variation, $CV_i$ is the coefficient of within-person biological variation, $CV_G$ is the coefficient of between-person (group) variation, $CV_A$ is the coefficient of analytical variation, and $CV_T$ is the coefficient of total variation. The $CV_I$s were calculated according to the mean-square successive difference method, which is less sensitive to trends than the more usual variance method (11).

Thus, the variance of fasting to postprandial values was calculated as:

$$SD^2 = \sum \left( (fasting\ day\ 1 - postprandial\ day\ 1)^2 + \ldots + (fasting\ day\ 5 - postprandial\ day\ 5)^2 \right) / 10$$

This can also be regarded as the within-day or $\sim 6$ h variation.

The variance of the orthostatic values was calculated as:

$$SD^2 = \sum (before - after\ supine\ rest)^2 / 2$$

The variance of within-person day-to-day values was calculated as:

$$SD^2 = \sum \left( (day\ 1 - day\ 2)^2 + (day:\ 2 - day\ 3)^2 + (day\ 3 - day\ 4)^2 + (day\ 4 - day\ 5)^2 \right) / 8$$

Mean SD = $\left[ (SD_i^2 + \ldots + SD_n^2) / n \right]^{1/2}$

$$CV = 100\% \times (SD / mean\ value)$$

Mean $CV_i = [(CV_i^2 + \ldots + CV_{in}^2) / n]^{1/2}$

The median $CV_i$ and the $CV_i$ at the 25th and 75th percentiles were calculated because of the heterogeneity of variance.

The index of individuality = $CV_i / CV_G$ (3).

The reference change value = $1.96 \times 2^{1/2} \times (CV_i^2 + CV_A^2)^{1/2}$, showing the change that can be explained by analytical and biological variation covering 95% of the changes (probability level, 95%; probability of false alarm, 0.05) (12).

The heterogeneity of variance was tested by calculating whether the $CV_i$ values were all estimates of the same true variance ($E_{CV}$), using the formula in Ref. (12).

Two-way ANOVA with replication was used to determine whether there was a systematic difference between fasting and postprandial values.

The mean fasting and postprandial plasma homocysteine values did not differ through the five weekdays (no trend over the week). The mean plasma homocysteine values for women were significantly lower than for men (8.7 vs 10.0 μmol/L; $P = 0.004$, two-tailed unpaired t-test).

The observed changes in homocysteine concentrations and calculated values are listed in Table 1. As can be seen in Table 1 and Fig. 1, the plasma homocysteine decreases after 30 min of supine rest were larger than can be explained by protein binding.

Our values for within-person biological variation were comparable to the $CV_i$ values obtained by others: 7.03% (weekly sampling) (4), 9.4% (sampling at 2-week intervals) (5), and 9% (sampling at 2-month interval) (6). There was no or very little seasonal variation in plasma homocysteine (6). Cobbaert et al. (5) observed $CV_i$ values between 0.0% and 26.1%, which are values very close to our $CV_i$ range. Guttormsen et al. (7) observed a day-to-day maximum change in plasma homocysteine, including postprandial values, between 15% and 39% (analytical imprecision was 2–4%). Santhosh-Kumar et al. (13) measured serum homocysteine 3 weeks apart and found that the second values were between 46% and 165% of the first values (analytical imprecision was 8%).

All blood samples for biological variation in our study were drawn with the subjects in an upright position so that orthostatic variation would not affect the result. Physical exertion before blood sampling was not standardized, but in general sampling was performed in a relaxed condition. In a recent publication, there was a slight (1.2 μmol/L, 11.5%) increase immediately after acute exercise on plasma homocysteine (14).

Postprandial values taken from 1215 to 1500 did not
differ significantly from fasting values taken in the morning from 0800 to 1000. The meals eaten were traditional, i.e., cereals in the morning and more protein-rich food at noon. It does not seem to be necessary to collect a homocysteine sample in a fasting condition, although the first hours after breakfast were not investigated in this study. The postprandial blood sample was collected 30 min to 3 h after lunch and 4–7 h after breakfast. Ubbink et al. (8) observed a mean decrease 2 h after breakfast of 0.57 μmol/L and 4 h after breakfast of 0.72 μmol/L, with a maximum decrease of ~1.8 μmol/L. These values are also small compared with the random unavoidable day-to-day variation (see Table 1).

The surprisingly large orthostatic variation might suggest a function of homocysteine in the vasomotor response, which is triggered by the centralization of the blood flow at supine rest. A connection between homocysteine and nitric oxide, a potent vasodilator, that might explain this variation has been found (15). Whatever the cause, the large orthostatic variation has practical implications in monitoring of patients, who may go from bedridden to non-bedridden and later, to outpatient status. The mean value of plasma homocysteine is ~30% lower at day 1 compared with day 3 in acute myocardial infarction (16). This might be explained by orthostatic changes instead of acute-phase reaction.

Because the index of individuality is 0.4 (0.6 for women), the population-based reference intervals are not very useful in monitoring individual patients and as screening procedure to pick up illness in a particular individual (3, 12). Persons with homocysteine values in the upper part of the reference interval have a higher risk of atherosclerotic events than persons with homocysteine values in the lower part (1), making the reference interval even less

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Table 1. Changes in and values for day-to-day, postprandial, and orthostatic variation of plasma homocysteine.

<table>
<thead>
<tr>
<th></th>
<th>Day-to-day fasting values</th>
<th>Day-to-day postprandial values</th>
<th>Day-to-day fasting and postprandial values combined</th>
<th>Fasting to postprandial</th>
<th>Orthostatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean plasma homocysteine, μmol/L</td>
<td>9.1</td>
<td>9.2</td>
<td>9.1</td>
<td></td>
<td></td>
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<tr>
<td>Plasma homocysteine range, μmol/L</td>
<td>4.5–16.4</td>
<td>5.1–17.4</td>
<td>4.5–17.4</td>
<td>6.8–17.4</td>
<td></td>
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<tr>
<td>Maximum change, μmol/L</td>
<td>4.4</td>
<td>4.8</td>
<td>4.8</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Maximum change, %</td>
<td>62.2</td>
<td>53.8</td>
<td>62.2</td>
<td>58</td>
<td>29.9</td>
</tr>
<tr>
<td>Mean change, μmol/L</td>
<td>(2.8 μmol/L)</td>
<td>(3.5 μmol/L)</td>
<td>(2.8 μmol/L)</td>
<td>(3.6 μmol/L)</td>
<td>(3.5 μmol/L)</td>
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<tr>
<td>Mean change, %</td>
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<td>1.3</td>
<td>1.1</td>
<td>2.1</td>
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<tr>
<td>Mean change, %</td>
<td>15.2</td>
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<td>14.7</td>
<td>13.7</td>
<td>19.0</td>
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<td>Mean SD, μmol/L</td>
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<td>1.1</td>
<td>1.1</td>
<td>0.7</td>
<td>1.0</td>
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<tr>
<td>SD range, μmol/L</td>
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<td>0.2–2.0</td>
<td>0.2–2.2</td>
<td>0.5–1.1</td>
<td>0.7–1.3</td>
</tr>
<tr>
<td>Mean CV%, %</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>7.5</td>
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<tr>
<td>Median CV%, %</td>
<td>9.0</td>
<td>9.6</td>
<td>9.8</td>
<td>7.7</td>
<td>8.9</td>
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<tr>
<td>CV range, %</td>
<td>0.0–25%</td>
<td>0.0–18%</td>
<td>0.0–25%</td>
<td>0.0–11%</td>
<td>3.0–13</td>
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<td>CV₀%, %</td>
<td>21</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
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<tr>
<td>Men and women separately</td>
<td>30 and 22</td>
<td>29 and 19</td>
<td>29 and 20</td>
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<tr>
<td>Index of individuality, CV₀/CV₀ ratio</td>
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<td>0.6</td>
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<tr>
<td>Men and women separately</td>
<td>0.4 and 0.6</td>
<td>0.4 and 0.6</td>
<td>0.4 and 0.6</td>
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<td></td>
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<tr>
<td>Reference change value, %</td>
<td>37.4</td>
<td>35.6</td>
<td>35.4</td>
<td>24.8</td>
<td>29.1</td>
</tr>
</tbody>
</table>

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* Changes are calculated on the numerical values.
* Morning to afternoon, ~6h. There was no systematic difference between fasting and postprandial values (ANOVA).
* Thirty minutes prior to and after supine rest.
* Minus the analytical imprecision (CV₀).
* CV₀ values at the 25th and 75th percentiles were 5.8% and 15%, respectively. ECV₀ = 0.25, indicating heterogeneity of variance.
useful. Risk calculation at a certain concentration of plasma homocysteine and assessment of significant response to treatment are the most important features.

In conclusion, the mean day-to-day change in fasting plasma homocysteine was 15.2% (1.3 μmol/L), and the maximum change was 62.2% (4.4 μmol/L). The mean CV was 13%, but there was heterogeneity of variance with CV values from 0% to 25%. The postprandial values did not differ systematically from the fasting values; therefore, it does not appear critical that the patient be in a fasting state when plasma homocysteine is measured. The orthostatic changes after 30 min were up to 29.9% (3.5 μmol/L), with a mean of 19% (2.1 μmol/L), and correlated only weakly to the albumin change. This might indicate a function of plasma homocysteine in the vaso-motor response, which needs further investigation.

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References

8. Mikkelsen and Susanne Øberg for excellent technical assistance.

Mannose-binding Lectin Gene Variation and Cardiovascular Disease in Canadian Inuit, Robert A. Hegele,1,7 Christopher P. Busch,1 T. Kay Young,2 Philip W. Connelly,3 and Henian Cao1 (1 Robarts Research Institute and Department of Medicine, University of Western Ontario, London, Ontario, Canada N6A 5K8; 2 Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW2; 3 Departments of Medicine and Biochemistry, St. Michael’s Hospital and University of Toronto, Toronto, Ontario, Canada M5B 1A6; * address correspondence to this author at: Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, 406-100 Perth Dr., London, Ontario, Canada N6A 5K8; fax 519-663-3789, e-mail robert.hegele@ri.rri.on.ca)

Canadian Inuit have an age-adjusted mortality from cardiovascular disease that is ~40% lower than the rest of Canada (1). This might result from the protective influence of certain environmental factors, such as the consumption of Arctic fish (1) or of certain genetic factors. For example, the thermolabile variant of the MTHFR gene, which encodes methylenetetrahydrofolate reductase, is one-sixth as prevalent in Inuit than in subjects of European origin (2). However, there are some inconsistent genetic findings in these people. For example, genetic variants that are associated with an increased risk of cardiovascular disease, such as the E4 allele of the APOE gene and the T235 allele of the AGT gene, are significantly more prevalent in Inuit than in whites (3). The resolution of such inconsistencies may come from the fact that several genes likely determine susceptibility to cardiovascular disease (4). It will thus be necessary to evaluate newer genetic determinants of cardiovascular disease risk in the Inuit.

One possible new genetic determinant for cardiovascular risk is the common coding sequence variation in the MBL gene, which encodes mannose-binding lectin (MBL) (5). MBL is an innate immune defense protein that binds mannose and other sugars on the surface of a variety of infectious agents, thereby facilitating phagocytosis and activation of the complement cascade (6, 7). MBL likely modulates the severity of infection with Chlamydia pneumoniae (6, 7), a pathogen linked by several lines of experimentation to the initiation and propagation of atherosclerosis (8). This might explain the association of genetic variation in MBL with severe atherosclerosis (5).

There are three common polymorphic sites in MBL. The Gly→Asp variant at MBL codon 54 (G54D) in exon 1, also called the “B allele”, has been associated with recurrent infections (6, 7). In addition, MBL G54D destabilizes the sixth collagen repeat of MBL, and the B-type chains of MBL expressed in vitro fail to activate complement (6, 7). Furthermore, subjects who are heterozygotes for the wild-type MBL A allele (G54) and the poorly functioning MBL B allele (D54) have a 20-fold reduction in MBL concentrations compared with G54 homozygotes because the MBL B allele product apparently acts as a dominant negative (6, 7). The two other polymorphisms, namely G57E (also called the “C allele”) and R52C (also called the “D allele”)