Validation of Abbreviated Oral Methionine-Loading Test

To the Editor:

Hyperhomocysteinemia 4 to 6 h after an oral methionine load may identify individuals at increased risk for atherothrombotic sequelae despite normal fasting total plasma homocysteine concentrations (1, 2). An excess prevalence of postmethionine-load hyperhomocysteinemia, in the absence of fasting hyperhomocysteinemia, has also been described in women with a history of giving birth to children with neural tube defects (3). Administration of the methionine-loading test in both epidemiologic and clinical settings is limited by the prolonged duration of existing protocols. In an effort to facilitate expanded application of the methionine-loading test, we attempted to validate a shortened (i.e., 2-h) protocol.

Three groups of adult volunteers were studied at The Framingham Study, or at the General Clinical Research Center at Tufts New England Medical Center (NEMC). The participants included 40 Framingham Study or NEMC employees (26 women, 14 men; mean age 43 years, age range 22–75); 6 obligate heterozygotes (3 women, 3 men; mean age 44 years, age range 29–53) for deficiency of cystathionine synthase (CS; EC 4.2.1.22) who were free of clinical or laboratory evidence of cardiovascular, renal, and hepatic disease; and 21 rheumatoid arthritis patients (14 women, 7 men; mean age 53 years, age range 35–76). Informed consent was obtained from all study participants.

Phlebotomy was performed after an overnight fast (10 to 14 h), and at 2 and 4 h after receiving an oral load (0.1 g per kg of body weight) of L-methionine (Ajinomoto, Teaneck, NJ) mixed in 200 mL of fruit juice. After the fasting phlebotomy and oral methionine load, those evaluated at the Framingham Study (i.e., 23 of 40 of the employee volunteers, and all 6 of the CS deficiency heterozygotes) received a standardized snack low in methionine (<15 mg); the remaining 17 employee volunteers and the 21 rheumatoid arthritis patients evaluated at NEMC were given only water. All whole-blood specimens for homocysteine analyses were drawn into evacuated blood-collection tubes containing EDTA, and without delay cooled to 4°C. Within 2 h of collection, the whole blood was centrifuged in a refrigerated centrifuge, and the separated EDTA plasma was aliquoted and cryopreserved at −70°C for <2 months until analysis.

Total plasma homocysteine was determined by HPLC with fluorescence detection in a modification of the method originally described by Araki and Sako (4). Inter- and intraassay CVs for this assay are routinely ≤7% and ≤5%, respectively.

Linear (Pearson r) and rank order (Spearman ρ) correlations between 2-h and 4-h plasma total homocysteine concentrations were respectively 0.97 and 0.92 for the rheumatoid arthritis patients (n = 21), 0.94 and 0.95 for the Framingham and USDA-HNRC employee volunteers (n = 40), each 0.99 for the CS deficiency heterozygotes (n = 6), and 0.96 and 0.94 for the pooled groups (n = 67). As shown in Fig. 1, the 2-h plasma homocysteine concentration accounted for >92% of the variability in 4-h plasma homocysteine concentration. In addition, the correlation between the 2-h value minus the fasting value and the 4-h value minus the fasting "delta" total plasma homocysteine concentrations for the pooled groups was r = 0.95. Four individuals with a normal fasting total plasma homocysteine (<16 μmol/L; see ref. 5) had a 4-h postmethionine-load total plasma homocysteine exceeding the 90th percentile distribution for the pooled groups, i.e., >50.6 μmol/L, a value consistent with increased risk for atherothrombotic sequelae (1). These same four individuals also had 2-h total plasma homocysteine concentrations exceeding the 90th percentile (i.e., >37.7 μmol/L), indicating 100% concordance in these four subjects for hyperhomocysteinemia at 2 and 4 h postmethionine loading.

In view of our combined experience with ~300 4-h methionine-loading tests (A. Bostom, unpublished observations), the 2-h loading test offers...