

New Technologies, Diagnostic Tools and Drugs

No added value of the methionine loading test in assessment for venous thrombosis and cardiovascular disease risk

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Summary

Homocysteine is a risk factor for cardiovascular disease and venous thrombosis. Clinical guidelines differ in their recommendation whether or not to measure homocysteine after methionine loading. In this study, we investigated the added value of the methionine loading test next to fasting homocysteine levels for identifying subjects at risk for venous thrombosis or cardiovascular disease, using Receiver Operating Characteristic (ROC) curves. The analysis was performed in 185 patients with recurrent venous thrombosis, 130 patients with cardiovascular disease and 601 controls. The discriminatory power of the fasting homocysteine measurement alone for identifying subjects at risk of venous thrombosis expressed as the area under the ROC curve (AUC) was 0.61 (95%CI 0.56–0.66). Using both a fasting

homocysteine measurement and a methionine loading test together yielded a similar AUC of 0.65 (95%CI 0.60–0.69), indicating no added value of methionine loading next to fasting homocysteine measurement in identifying subjects at risk for thrombosis. Similar results were found for cardiovascular disease, with an AUC of 0.62 (95%CI 0.57–0.67) for the fasting homocysteine measurement alone and an AUC of 0.62 (95%CI 0.57–0.67) for the combination of both the fasting and the post-load homocysteine measurement. The methionine loading test has no added value next to measuring fasting homocysteine levels for identifying subjects at risk for venous thrombosis or cardiovascular disease and for that reason should not be used in clinical practice.

Keywords

Methionine loading test, hyperhomocysteinemia, homocysteine, venous thrombosis, ROC curve

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Introduction

Hyperhomocysteinemia is a risk factor for venous thrombosis and cardiovascular disease (1, 2). Clinical guidelines differ in their recommendation whether or not to measure homocysteine after methionine loading, next to measuring the fasting concentration. Most of these guidelines recommend not to use the methionine loading test in routine clinical settings, because the test is cumbersome and there is insufficient evidence on the association between postload hyperhomocysteinemia and risk of vascular disease (3–6). However, some people advocate the use of the methionine loading test, because without a methionine loading test 27% to 55% of subjects with hyperhomocysteinemia will be missed (7–10).

The methionine loading test was first used for the detection of subjects heterozygous for cystathionine β -synthase (CBS) deficiency (11), who often had hyperhomocysteinemia after methionine loading. Methionine loading was used in those days to increase homocysteine concentrations, since basal homocysteine levels could not be accurately measured with the laboratory techniques of that time.

The observation that subjects with homocystinuria often suffer from severe cardiovascular disease and venous thrombosis lead to the hypothesis that mild hyperhomocysteinemia could also be associated with vascular disease (12). In 1976, Wilcken et al. (13) provided the first evidence that hyperhomocysteinemia after methionine loading was associated with cardiovascular disease. The first studies on hyperhomocysteinemia and cardiovas-

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ascular disease suggested that study participants with postload hyperhomocysteinemia were heterozygous for cystathionine β -synthase deficiency. Later, it was established that heterozygosity for cystathionine β -synthase deficiency is not, or only a minor cause of postload hyperhomocysteinemia (14).

In the 80s, the development of more sensitive and precise laboratory techniques made it possible to accurately measure fasting homocysteine levels. From then on, both measurement methods, i.e. fasting homocysteine measurement and after methionine loading, were applied in epidemiological studies on hyperhomocysteinemia and vascular disease, yielding similar risk estimates (1, 9, 10, 15–18). This has initiated the discussion whether or not to perform a methionine loading test in vascular disease, which has been obscured by the argument that subjects with isolated postload hyperhomocysteinemia will remain undiagnosed when this test is omitted (7–10).

In our opinion, the real question is whether the methionine loading test increases the risk prediction for venous thrombosis or cardiovascular disease. For that reason the added value of the methionine loading test should be determined by its ability to identify subjects at risk for venous thrombosis or cardiovascular disease. Most studies that assess the added value of the methionine loading test, use risk estimates for homocysteine levels above a certain cut-off point (either fasting or after methionine loading). Results therefore largely depend on the cut-off point that is chosen. In this study we try to clarify the discussion using Receiver Operating Characteristic (ROC) curves, because ROC curves enable us to assess the added value of the methionine loading test over a whole range of homocysteine values. We performed the analyses in 185 patients with recurrent venous thrombosis, 130 patients with cardiovascular disease and 601 control subjects.

Materials and methods

Subjects

For assessing the value of methionine loading in identifying subjects at risk for venous thrombosis and cardiovascular disease, we used the following study populations, which are described in detail elsewhere (16, 18, 19). We recruited 185 patients who had had two or more episodes of venous thrombosis through an anticoagulant clinic in The Hague (this group is further referred to as ‘patients with venous thrombosis’) (16, 18). 130 patients with coronary artery disease were recruited through the Zuiderziekenhuis Hospital in Rotterdam, The Netherlands (19). They had had a coronary angiography and had $\geq 90\%$ occlusion in one and $\geq 40\%$ occlusion in another additional major coronary artery (this group is further referred to as ‘patients with cardiovascular disease’). As a control group, we used 500 control subjects recruited through a general practice in The Hague (16, 18) and 101 control subjects recruited from the general population in Rotterdam (19).

For both the study on venous thrombosis and cardiovascular disease, the medical ethics committee approved the study protocol and informed consent was obtained from all study participants.

Methods

From all study participants, venous blood samples for total homocysteine determination were collected after an overnight fast. Hereafter, participants received an oral methionine load consisting out of a single dose of 0.1g L-methionine per kilogram of bodyweight. Six hours after the oral methionine load a second blood sample was obtained. In between, participants received only a low-protein meal (20). Total homocysteine concentrations (fasting and postload) were determined in EDTA plasma through the use of high-performance liquid chromatography and fluorescence detection (21). All determinations were performed at the Radboud University Nijmegen Medical Centre, The Netherlands.

Statistical analysis

Fasting and postload hyperhomocysteinemia was defined as a homocysteine concentration above the 90th percentile value of the distribution of the control group. ‘Delta’ homocysteine levels were defined as the postload minus the fasting homocysteine concentration.

ROC curves were established for the fasting homocysteine measurement alone, the postload homocysteine measurement alone, and for the two measurements combined. We used logistic regression analysis to find the optimal combination of both measurements. To compare the overall prognostic value of the fasting and the postload homocysteine levels, we used the area under the curve (AUC).

Although ROC-curves are not widely used in the analysis of case-control data, they provide a good insight in the additive value of a certain measurement (22). It should be clear that they should not be interpreted as diagnostic ROC-curves (i.e. we do not mean that homocysteine is a diagnostic tool to diagnose thrombosis or myocardial infarction (like D-dimers or troponine measurements). They should be interpreted as prognostic ROC-curves. As in case-control studies, we assume that the exposure measurement (homocysteine) is not affected by the disease (23).

Results

The group characteristics are given in Table 1. For the venous thrombosis group the age was higher compared to the control group, while the cardiovascular patients showed a higher percentage of males.

Number of hyperhomocysteinemic subjects identified by fasting and/or postload homocysteine measurement

The 90th percentile value in control subjects was 18.3 $\mu\text{mol/L}$ for fasting homocysteine levels and 59.5 $\mu\text{mol/L}$ for postload homocysteine concentrations.

We identified 173 (59 venous thrombosis patients, 21 cardiovascular patients and 93 controls) subjects with hyperhomocysteinemia (fasting, postload or both) of which 114 (66%) could be diagnosed by measuring only fasting homocysteine levels (Fig. 1, Table 2). The additional 59 (20 venous thrombosis patients, 6 cardiovascular patients and 33 controls) hyperhomocysteinemics had normal fasting homocysteine levels and elevated postload homocysteine concentrations, i.e. they had isolated postload hyperhomocysteinemia. This implies that without a

Table 1: General characteristics of the study populations.

	Patients with Venous Thrombosis N=185	Patients with Cardiovascular Disease N=130	Controls N=601
Age, y	61.1±14.6**	52.5±7.5	50.6±12.6
Sex, % male	50.8	84.7**	47.1
Body weight, kg	83.5±16.8**	82.2±12.3**	75.8±14.1
BMI, kg/m ²	-	26.8±3.0*	26.0±4.2
Systolic BP, mmHG	-	135±13	132±20
Diastolic BP, mmHG	-	81±8	83±11.3
Currently smoking, %	33%	25%**	34%
Total cholesterol	-	6.8±1.4**	6.1±1.2
Creatinine, μmol/L	85.8±29.2**	76.9±12.2	75.5±16.6

* p<0.05, **p<0.01

Table 2: Number of patients with venous thrombosis and cardiovascular disease and control subjects identified by fasting and/or postload homocysteine measurement. Hyperhomocysteinemia is defined as a homocysteine concentration above the 90th percentile.

	Patients with Venous Thrombosis N=185	Patients with Cardiovascular Disease N=130	Controls N=601
Fasting hyperhomocysteinemia	39 (21%)	15 (12%)	60 (10%)
Postload hyperhomocysteinemia	39 (10%)	13 (10%)	60 (10%)
Isolated postload [†] hyperhomocysteinemia	20 (11%)	6 (5%)	33 (5%)
Fasting and postload hyperhomocysteinemia	19 (10%)	7 (5%)	27 (4%)
Fasting or postload hyperhomocysteinemia	59 (32%)	21 (16%)	93 (15%)

[†] Subjects with hyperhomocysteinemia only detected by a methionine loading test

methionine loading test 33% (59 out of 173) of hyperhomocysteinemic subjects in this study would not be detected.

Receiver Operating Characteristic (ROC) curves

Because the purpose of the methionine loading test is not to diagnose hyperhomocysteinemia, but to identify subjects at risk for thrombosis, we used ROC-curve analysis to compare the prognostic value of fasting and post-load homocysteine concentrations. Figure 2 shows the ROC-curves for fasting and post-load homocysteine concentrations and for the combination in the venous thrombosis group. We found that the area under the curves were quite similar for the fasting homocysteine (AUC=0.61 [95%CI 0.56–0.66]) and the postload homocysteine measurement (AUC=0.64 [95%CI 0.59–0.69]), indicating that both the fasting test alone and the postload test alone are individually equally capable of identifying subjects at risk for venous thrombosis. Performing both the fasting homocysteine measurement and the methionine loading test together results in a similar area under the curve (AUC=0.65 [95%CI 0.60–0.69]), which

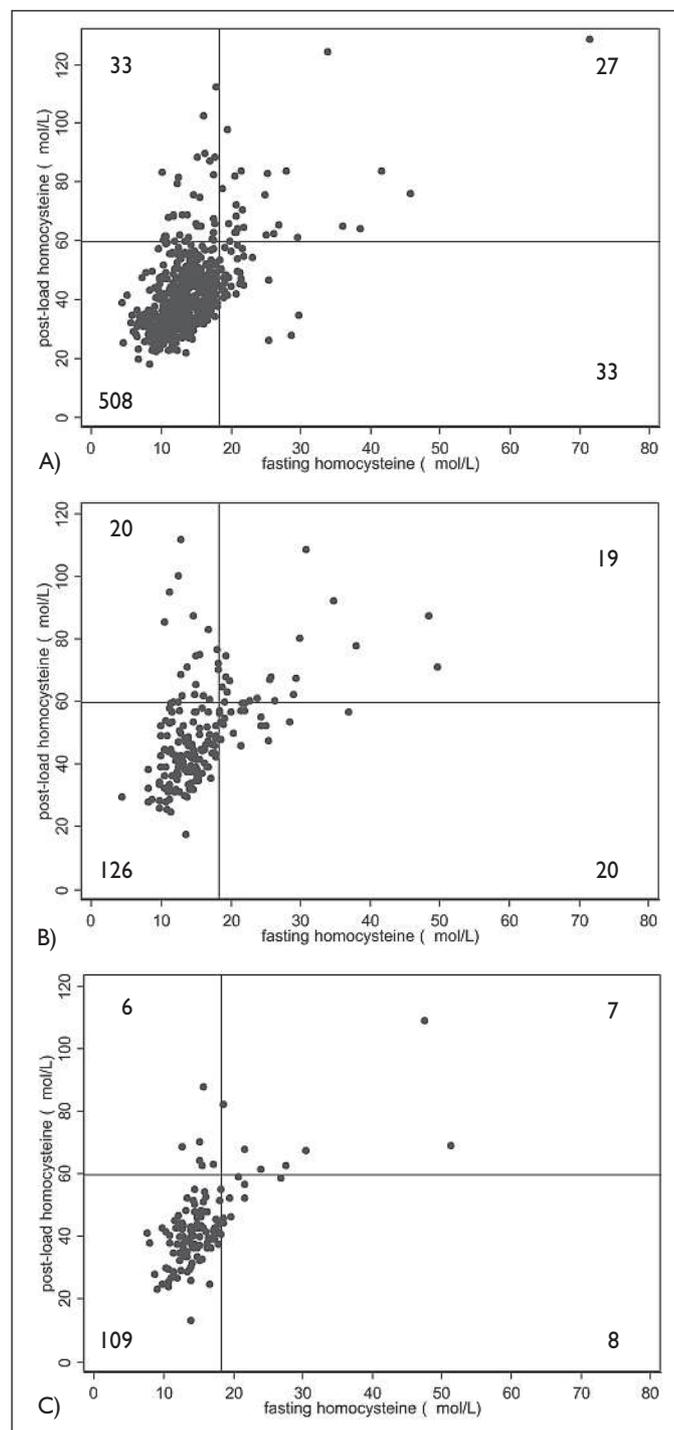


Figure 1: Scatter plot of individual homocysteine levels for control subjects and patients with venous thrombosis (B), indicating if they were identified as hyperhomocysteinemic by means of fasting or postload homocysteine measurement or both. The straight lines represent the 90th percentiles.

means that adding a methionine loading test next to a fasting homocysteine measurement does not decrease the amount of false-positives or false-negatives and therefore this test has no added value in identifying subjects at risk for venous thrombosis next to fasting homocysteine measurement.

Figure 3, shows ROC curves for the fasting homocysteine measurement alone (AUC=0.62 [95%CI 0.57–0.67]), the post-load homocysteine measurement alone (AUC=0.54 [95%CI 0.49–0.59]) and for both tests together in the cardiovascular patient group (AUC=0.62 [95%CI 0.57–0.67]). Also in this patient

group we did not find any added value of the methionine loading test.

As is shown in table 1, the venous thrombosis group was older than the control group while the cardiovascular patient group show a higher percentage of males. Therefore we did a

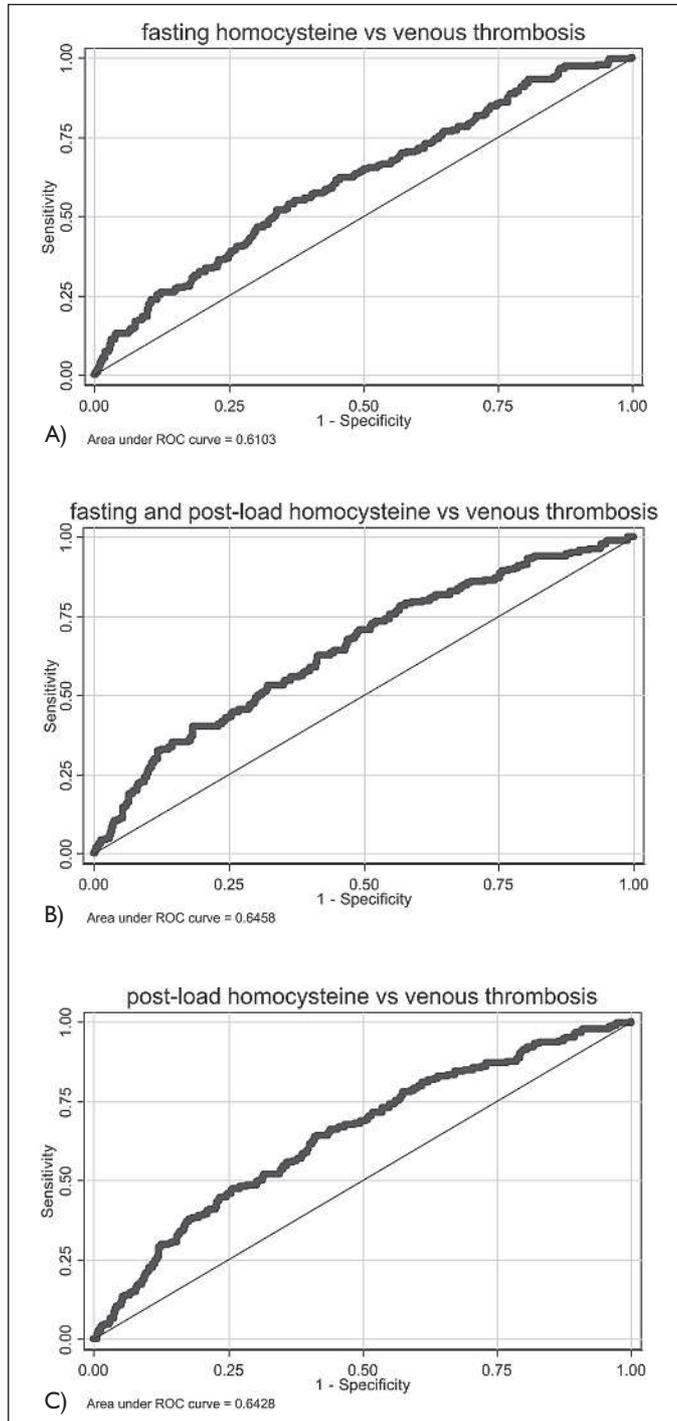


Figure 2: ROC curves indicating the capacity of the fasting homocysteine measurement alone (A), the postload homocysteine measurement alone (B) and for both measurements together (C), to identify subjects with venous thrombosis. The overall accuracy is expressed in the Area Under the Curve (AUC).

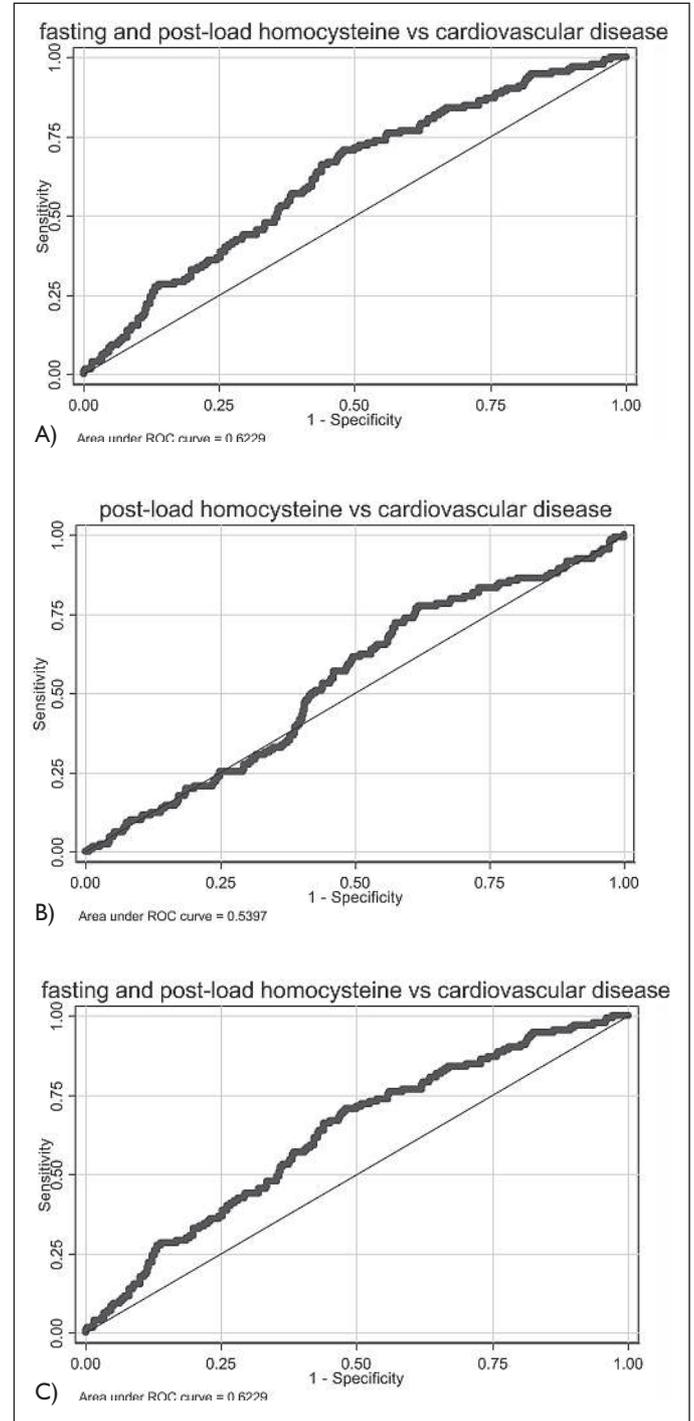


Figure 3: ROC curves indicating the capacity of the fasting homocysteine measurement alone (A), the postload homocysteine measurement alone (B) and for both measurements together (C), to identify subjects with cardiovascular disease. The overall accuracy is expressed in the Area Under the Curve (AUC).

subgroup analysis in males only and in a subgroup with a similar age distribution. Also in these subgroup analyses, there was no indication for an added value of the methionine loading test (data not shown).

“Delta hyperhomocysteinemia”

Instead of absolute postload homocysteine levels for detecting hyperhomocysteinemia one could also use “delta” homocysteine levels, i.e. the difference between fasting and postload homocysteine levels. For that reason, we also generated ROC curves to investigate the added value of determining delta homocysteine levels next to measuring only fasting homocysteine levels (Figures not shown). The AUC for delta homocysteine levels alone was 0.62 [95%CI 0.57–0.66]), which is the same as the AUC for absolute postload homocysteine levels alone (AUC=0.64 [95%CI 0.59–0.69]), indicating that both methods are equally capable of identifying subjects at risk of venous thrombosis. The combination of fasting homocysteine measurement and delta homocysteine levels together, yielded an AUC of 0.66 (95%CI 0.60–0.69), which means that calculating “delta” homocysteine levels after methionine loading has no added value in risk assessment for venous thrombosis. Also in the cardiovascular patient group there was no added value of calculating “delta” homocysteine levels (data not shown).

Discussion

We assessed the added value of the methionine loading test next to fasting homocysteine levels in risk assessment of venous thrombosis and cardiovascular disease by using ROC curves. We showed that the methionine loading test has no additional value as a prognostic tool for risk assessment of venous thrombosis or cardiovascular disease.

Most guidelines do not recommend the use of the methionine loading test in a routine clinical setting because there is not enough evidence on the association between postload hyperhomocysteinemia and vascular disease (3–6). Other drawbacks are that this test is more expensive, is inconvenient for the patient and for the laboratory and that the dose of methionine greatly exceeds the daily intake of methionine (24).

However, an argument that is often used in favour of the methionine loading is that 27% to 55% of subjects with hyperhomocysteinemia will not be detected if a methionine loading test is omitted (7–10). Based on this result, people advocate the use of the methionine loading test in all patients that are screened for hyperhomocysteinemia or in a subset of these patients based on the fasting homocysteine concentrations and thus lowering the total amount of methionine loading tests needed in clinical settings (25, 26).

Although it is true that a normal fasting homocysteine level does not exclude a abnormal post-load homocysteine level, the purpose of homocysteine measurements is not to identify subjects with hyperhomocysteinemia, but to identify subjects at risk for venous thrombosis or cardiovascular disease that may possibly benefit from vitamin supplementation. Therefore, we evalu-

ated the prognostic value of fasting and post-load homocysteine levels with ROC-curve analysis. Performing a methionine loading test in addition to a fasting homocysteine measurement did not increase the AUC for either venous thrombosis or cardiovascular disease, indicating no added value of the methionine loading test. We have found that the fasting homocysteine measurement alone and the methionine loading test alone are more or less equally capable of identifying subjects at risk for venous thrombosis and cardiovascular disease. This means that clinicians can choose to perform either a fasting homocysteine measurement alone or a methionine loading test alone in risk assessment for venous thrombosis and cardiovascular disease. However, because fasting homocysteine measurements are cheaper, more feasible, and less invasive for the patient, fasting homocysteine measurement will be preferred.

We also investigated the added value of “delta” homocysteine determination next to fasting homocysteine levels, which was nil for both risk assessment of venous thrombosis and cardiovascular disease. We choose to focus on absolute postload homocysteine levels in this paper on account of the paper by Ubbink et al. (27), stating that postload homocysteine levels should not be adjusted for fasting homocysteine levels because it results in an increased regression dilution due to higher analytical and biological variability.

Our evaluation of the methionine loading test was performed on homocysteine samples that were drawn 6 hours after the methionine load. Whether our results are also applicable to homocysteine samples drawn at another time-interval is uncertain. However, although time intervals for postload homocysteine measurement differ, most studies measure postload homocysteine levels either after 4 or 6 hours, and there is a high correlation between homocysteine samples drawn after a short time interval (2 or 3 hours) and those drawn after 4 to 6 hours (28, 29). So, it is very unlikely that other versions of the loading test will give more information next to fasting homocysteine levels. We also would like to point out that our study was restricted to venous thrombosis and cardiovascular disease. It does not apply to the value of methionine loading in assessment of other medical conditions, for instance cobalamin or folate deficiency, Alzheimer disease, vascular dementia, complications of pregnancy or neural tube defects (3).

Finally, it can be questioned whether homocysteine levels should be measured at all in clinical settings. Even if clinical trials show a beneficial effect of vitamin B supplementation, it is not likely that this effect is restricted to subjects with elevated homocysteine levels. Because there is a quite linear concentration-risk relationship between homocysteine levels and vascular disease (30), it is to be expected that, if there is any effect, every subject may benefit from vitamin supplementation independent from the initial pre-treatment homocysteine value.

In conclusion, the methionine loading test has no added value next to fasting homocysteine measurement in identifying subjects at risk for venous thrombosis or cardiovascular disease and for that reason should not be used in clinical practice.

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