High-normal serum homocysteine concentrations are associated with an increased risk of early atherosclerotic carotid artery wall lesions

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We read with great interest the paper by Winfried Willinek and colleagues in a previous issue of the journal [1]. They reported that high-normal serum homocysteine concentrations were associated with intima–media thickness (IMT) in the common carotid artery in 75 healthy subjects. This association was independent of age, body mass index, low density lipoprotein cholesterol and systolic blood pressure, and serum homocysteine explained 18% of the variability in intima–media thickness.

We have performed a similar study and obtained different results. In our study, 58-year-old men were randomly selected from all men in this age group in the County Council register. More than 800 men were screened for a study aimed at examining the relationship between insulin metabolism and intima–media thickness in the carotid artery [2]. Exclusion criteria were cardiovascular or other clinically overt disease, treatment with cardiovascular drugs that might disturb the measurements performed in the study and unwillingness to participate. The subjects had provided their informed consent and the study was approved by the local ethic’s committee. One hundred and three of the screened men with different degrees of insulin sensitivity were selected for more detailed substudies. This selection was based on a body mass index : blood glucose score that gave an estimate of insulin sensitivity [2].

Common carotid artery intima–media thickness was defined as the distance from the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall (10 mm segment in the carotid artery, 15 mm in the femoral artery). The images were measured using an automated analysing system, based on automatic detection of the echo structures in the ultrasound image, demonstrating a very high reproducibility [3]. The mean of four images from the left and right sides were used in the analyses [4].

Blood was drawn after an overnight fast and plasma was separated and frozen within 4 h at −70 °C. Plasma homocysteine concentration was measured by high-performance liquid chromatography (n = 103). The plasma concentrations of folates and vitamin B12 were assessed by using enzyme-ligand technique (CEDIA, Boehringer Mannheim, Germany).

The characteristics of the subjects are shown in Table 1. The results showed that plasma homocysteine was not associated with IMT (r = 0.032, P = 0.75) (Fig. 1).

Table 1 Characteristics of the study subjects (mean ± SD in 58-year-old men, n = 103)

| Characteristics                   | Mean ± SD |
|-----------------------------------|-----------|
| Body mass index (kg/m²)           | 26 ± 4.4  |
| Systolic blood pressure (mmHg)    | 137 ± 20  |
| Diastolic blood pressure (mmHg)   | 83 ± 11   |
| Serum LDL cholesterol (mmol/l)    | 4.1 ± 1.0 |
| Smoking status (yes/no)           | 24/80     |
| Serum folate (nmol/l)             | 15.3 ± 8.0|
| Serum vitamin B12 (pmol/l)        | 359 ± 117 |
| Plasma homocysteine (µmol/l)      | 13.0 ± 2.9 |
| Carotid intim-media thickness (mm)| 0.80 ± 0.12|

LDL, low density lipoprotein.

Fig. 1.

Relationship between intima–media thickness (IMT) of the common carotid artery and plasma homocysteine concentrations (n = 103, r = 0.032, P = 0.75).
However, the latter was associated with systolic blood pressure ($r = 0.20$, $P < 0.05$), apolipoprotein B ($r = 0.21$, $P < 0.05$) and smoking, described as cigarette years ($r = 0.22$, $P < 0.05$). Dividing plasma homocysteine into quartiles and comparing the proportion of patients with an IMT $> 0.80$ mm did not reveal any statistically significant relation between homocysteine and vessel wall characteristics (data not shown).

In summary, we performed a study that was based on a population-based sample of men and used a validated ultrasound method to examine the common carotid artery IMT [4]. We could verify that IMT was associated with established cardiovascular risk factors such as systolic blood pressure, apolipoprotein B, and smoking. However, there was no indication of an association between IMT and plasma homocysteine concentrations. The fact that we observed significant correlations between plasma homocysteine and serum levels of $B_{12}$ and folate indicate that the methods used for these measurements were valid [5].

There are a number of differences between the study by Willinek et al. and ours in addition to the fact that our study was larger. Willinek et al. recruited subjects by advertisements, whereas we included subjects from the general population. They recruited men and women with an age ranging from 20 to 80 years. We kept the age and sex factor constant by only examining 58-year-old men and selected a subgroup with different degrees of insulin sensitivity. Willinek et al. examined both the right and left artery, but selected the side with the most advanced IMT for further analyses, whereas we used the mean of measurements from both sides.

However, if we used the maximum IMT instead of mean common carotid artery IMT, the results did not change. Furthermore, we also measured IMT in the carotid bulb and in the common femoral artery and could not find any indications of an association between these measures of wall thickness in other vascular territories and plasma homocysteine concentrations (data not shown).

Our conclusion is that plasma homocysteine may not be associated with the atherosclerotic process in all populations. Meta-analyses have indicated a clear relationship between homocysteine and cardiovascular disease in cross-sectional and case–control studies [6,7]. However, the observation that results from prospective studies indicate less or no predicative ability for plasma homocysteine in cardiovascular disease has lead to a suggestion that elevated homocysteine levels may be an acute phase reactant that is more related to pre-existing disease [7]. Finally, it is important that negative or inconclusive studies are also published in order to avoid publication bias. In this respect, our negative study was larger and population-based compared to the positive study by Willinek et al. and we also examined several vascular beds.

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Reply
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We read with interest Björn Fagerberg’s comments on our recent paper [1] entitled ‘High-normal serum homocysteine concentrations are associated with an increased risk of early atherosclerotic carotid artery wall lesions in healthy subjects’ noting that, in contrast to our findings, his group [2] did not find any association between homocysteine concentration and carotid artery wall thickness. At present, the reason for this discrepancy remains unclear. The methods for ultrasound and homocysteine determination used by those authors were similar to ours. However, there are at least two essential differences between the studies.

The study by Hulthe et al. [2] aimed to examine the relationship between insulin metabolism and carotid intima media thickness, whereas our study was performed to investigate the influence of homocysteine on intima media thickness. Our randomly recruited, more general, study population was healthy, without pre-existing disease and with a broader age range (22–75 years); furthermore, 47 of the total number of 75 (63%) were aged younger than 58 years [1]. In the study by Hulthe et al. [2], only 58-year-old men were included. As a result of these differences, the study cohorts are poorly comparable. The study population of Fagerberg’s group was selected on the basis of a body mass...
index/blood glucose score, and systolic blood pressure was strongly associated with carotid intima–media thickness. The association between cardiovascular risk factors such as hypertension or diabetes and carotid intima–media thickening has been described [3,4].

In fact, in our healthy study population we found that serum homocysteine concentration was significantly associated with intima–media thickness after adjustment for other known cardiovascular risk factors, including systolic blood pressure. Furthermore, systolic blood pressure did not remain significantly correlated to carotid intima–media thickness after adjustment for homocysteine concentration, whereas serum homocysteine concentration accounted for a statistically significant proportion (18%) of the variation in intima–media thickness after adjustment for the other cardiovascular risk factors in our study population [1].

Our results are consistent with the findings of other cross-sectional, prospective and retrospective studies [5–8]. Recent meta-analyses of epidemiological data clearly support a dose-dependent, positive association between homocysteine concentration and risk for cardiovascular disease [9]. Nygard et al. [10] reviewed the biochemical, clinical, epidemiological and experimental data in order to question whether an increased homocysteine concentration is a causal factor for cardiovascular disease, and found homocysteine concentration to be a strong risk factor for such disease. However, they concluded that the underlying mechanisms remain uncertain.

We are well aware that the positive correlation between serum homocysteine concentration and carotid artery intima–media thickness observed in our study does not establish causality, but the significance of the contribution of homocysteine to carotid intima–media thickness in our healthy study participants may indicate the importance of homocysteine as a risk factor for early atherosclerotic wall lesions. Only results from large, prospective and randomized trials with homocysteine-decreasing treatments will demonstrate whether reduction of an increased homocysteine concentration may result in a reduction in the risk for cardiovascular disease.

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