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Serum homocysteine is weakly associated with von Willebrand factor and soluble vascular cell adhesion molecule 1, but not with C-reactive protein in type 2 diabetic and non-diabetic subjects – The Hoorn Study

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Abstract

Background Hyperhomocysteinaemia may constitute an independent risk factor for cardiovascular disease, but it is still unclear by which pathophysiological mechanisms homocysteine (tHcy) may promote atherothrombosis. The aim of this study was firstly to examine whether tHcy is associated with endothelial dysfunction, increased adherence of leukocytes, and/or chronic low-grade inflammation, as estimated from plasma levels of von Willebrand factor (vWf), soluble vascular cell adhesion molecule 1 (sVCAM-1) and C-reactive protein (CRP), respectively. Secondly we investigated whether the presence of type 2 diabetes modifies these associations.

Materials and Methods Six hundred and ten subjects of a general population of middle-aged and elderly subjects, 170 of whom had type 2 diabetes, participated in this cross-sectional study. Linear regression analyses were used to study whether tHcy was associated with vWf, sVCAM-1 and CRP, and whether the presence of diabetes modified these associations.

Results After adjustment for confounders, tHcy was significantly but weakly associated with vWf ($\beta = 0{\cdot}15$, $P = 0{\cdot}05$) and sVCAM-1 ($\beta = 0{\cdot}082$, $P = 0{\cdot}04$). tHcy was not significantly associated with CRP ($\beta = 0{\cdot}02$, $P = 0{\cdot}91$). The presence of diabetes did not significantly modify these associations.

Conclusions This study provides evidence that tHcy is, at most, weakly associated with endothelial dysfunction as estimated from plasma vWf, and with leukocyte adhesion as estimated from plasma sVCAM-1. tHcy was not significantly associated with chronic low-grade inflammation as estimated from plasma CRP. Our data thus suggest that the link between tHcy and atherothrombosis cannot be explained by associations of tHcy with vWf, sVCAM-1 or CRP.

Keywords Homocysteine, C-reactive protein, soluble vascular cell adhesion molecule 1, type 2 diabetes.
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Introduction

Many studies provide evidence for an independent association between hyperhomocysteinaemia and atherothrombotic disease, as recently reviewed [1]. However, the strength of the association between an elevated total homocysteine (tHcy) concentration and cardiovascular disease seems to vary among populations [2]. Four recent studies in populations with diabetic subjects demonstrated that hyperhomocysteinaemia is a strong risk factor for cardiovascular disease in subjects with type 2 diabetes [3–6]. For diabetic subjects, tHcy appeared to be a 1·6-fold stronger risk factor for cardiovascular disease than for non-diabetics [3].

tHcy is derived metabolically from the essential amino acid methionine. Homeostasis of methionine-homocysteine metabolism is complex and is influenced by demographic, genetic, and acquired factors. It is not fully understood by which pathophysiological mechanisms tHcy promotes atherothrombosis in humans. Recent studies suggest that tHcy is a vasculotoxic molecule that mediates its adverse vascular effects by causing endothelial damage [7–13]. Impairment of regulatory functions of the endothelium is a key event in current models of atherothrombosis [13]. In addition, tHcy may cause an increased adherence of leukocytes to the endothelium, mediated by an increased expression of adhesion molecules, as observed in early atherosclerosis [13–15]. An alternative hypothesis is that tHcy induces a chronic low-grade inflammation, thus promoting the atherogenetic process [13].

Previous studies concerning the pathophysiological mechanisms by which tHcy may promote atherothrombosis have either examined a study group that was not population-based or did not assess the strength of the association separately for type 2 diabetes and non-diabetics [7–12]. The latter is important because diabetes and tHcy seem to interact [3]. Therefore, we assessed, in a population-based study that was stratified for glucose tolerance [16], the association between tHcy level and endothelial dysfunction by measuring plasma levels of the endothelium-derived regulatory protein, von Willebrand factor (vWf). We also assessed the association between tHcy and the soluble form of vascular cell adhesion molecule 1 (sVCAM-1) as an estimate of the adherence and transendothelial migration of leukocytes; and finally, between tHcy and C-reactive protein, a marker of chronic low-grade inflammation. The use of these protein markers is supported by studies showing an association between high plasma or serum levels of these proteins and an increased risk of cardiovascular disease [17–26].

Methods

Subjects

The study population consisted of an age-, sex-, and glucose-tolerance-stratified sample of the Hoorn Study, a population-based study of glucose tolerance and other cardiovascular risk factors in a 50- to 75-year-old general Caucasian population conducted from 1989–1992, as described previously [16].

Briefly, 2484 subjects (71% of those invited) participated. All subjects, except previously diagnosed diabetic subjects treated with oral glucose-lowering agents or insulin, underwent an oral glucose tolerance test (OGTT) according to the WHO guidelines [27]. For reasons of efficiency, subjects with a 2-h postload glucose $\geq 7{\cdot}5\,\mathrm{mmol\,L^{-1}}$, all subjects with type 2 diabetes and a random sample of subjects with a 2-h postload glucose $< 7{\cdot}5\,\mathrm{mmol\,L^{-1}}$ stratified by age and sex were invited within 4 weeks for a second visit to investigate glucose-intolerance-related complications (709 invited, of whom 631 − 89% − participated). These subjects underwent a second OGTT (except those who already used blood glucose lowering agents; $n = 67$). On the basis of the mean of the two OGTTs, glucose tolerance was divided into three categories [16]: normal glucose tolerance ($n = 288$), impaired glucose tolerance ($n = 170$) and type 2 diabetes ($n = 173$). Subjects with normal or impaired glucose tolerance or type 2 diabetes in the present study population thus represented a stratified random sample of all subjects with normal or impaired glucose tolerance or type 2 diabetes in the initial cohort.

The Hoorn Study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit. Informed consent was obtained from all participants.

Laboratory and clinical assessments

After an overnight fast, blood was drawn from an antecubital vein. We measured tHcy, vWf, VCAM-1, CRP, glucose, creatinine, albumin, urea nitrogen and lipids. No plasma was available for 21 subjects for the measurement of vWf, sVCAM-1 and CRP. Concentrations of serum total (free plus protein bound) tHcy were assessed in frozen (− 20 °C) EDTA serum by using high-performance liquid chromatography with fluorescence detection [28]. The intra-and interassay coefficients were 2·1% and 5·1%, respectively. Plasma concentrations of von Willebrand factor (vWf) and C-reactive protein (CRP) were assessed as previously described [26]. VWf is expressed in IU $\mathrm{mL^{-1}}$ of vWf antigen by comparison to the 4th International Standard for vWf in plasma [26]. The intra-and interassay coefficients of variation of CRP were 3·8% and 4·7%, respectively. Concentrations of sVCAM-1 were assessed in deep frozen (− 70 °C) heparin plasma samples. Concentrations of sVCAM-1 were measured, in duplicate, by ELISA kits (Bender MedSystems, Wenen, Austria; reference plasma values in 111 healthy subjects, as provided by the manufacturer, $1090 \pm 237\,\mathrm{ng\,mL^{-1}}$; range 675–1693). We measured sVCAM-1 levels in 27 healthy volunteers and found a mean level of $966\,\mathrm{ng\,mL^{-1}}$ with an intra-and interassay coefficient of variation of 3·4% and 14%, respectively [23]. Serum total cholesterol, HDL cholesterol, and triglycerides levels were all measured as described elsewhere [3]. We estimated the glomerular filtration rate by

the method of Levey *et al.* which is thought to be more accurate than the Cockcroft–Gault formula [29]. Blood pressure was measured as the mean of four measurements performed on two different occasions, using a random-zero sphygmomanometer under standardized conditions. Hypertension was defined as a blood pressure ≥ 160 mmHg systolic and/or ≥ 95 mmHg diastolic and/or the current use of antihypertensive medication. Subjects were classified as current cigarette smokers or nonsmokers. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated as described elsewhere [16]. ABO blood groups were determined by standard agglutination techniques using commercial test erythrocytes.

Statistical analysis

Variables are presented as mean ± standard deviation (SD), number (percentage of the total), or, in case of a skewed distribution, the median and the interquartile range (IQR). To study whether tHcy is independently related to vWf, VCAM-1 and CRP, and whether this differs between subjects with type 2 diabetes and those with impaired or normal glucose tolerance, we constructed crude and adjusted regression models. Analyses adjusted only for the stratification variables age, sex and glucose tolerance (*i.e.* normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes) were used to study the relation between the outcome variables (vWf, VCAM-1, CRP) and possible determinants thereof: tHcy, glomerular filtration rate (GFR), smoking, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, systolic and diastolic blood pressure, hypertension, BMI and the WHR. For variables that did not have a normal distribution of the residuals, the logarithmically transformed data were used for a better fit of the data. Possible determinants measured on a continuous scale were used as such in the regression models. For each dependent variable three regression models were made. The regression analyses were primarily adjusted for the stratification variables, secondarily for all determinants with a P-value $< 0{\cdot}15$ in the crude analysis and current smoking, and thirdly for other variables of pathophysiological interest [30]. The residuals were checked for a normal distribution and a constant variance. In addition, to check whether there was a threshold in the relation between tHcy and the dependent variables, we performed analyses in which we divided tHcy into four categories ($\leq 10\,\mu\text{mol L}^{-1}$, $10{\cdot}1$–$15{\cdot}0\,\mu\text{mol L}^{-1}$, $15{\cdot}1$–$20{\cdot}0\,\mu\text{mol L}^{-1}$ and $\geq 20{\cdot}1\,\mu\text{mol L}^{-1}$, respectively). To ascertain whether the presence of cardiovascular disease at enrolment disturbed the results, we performed the same analysis as described above for the subjects with and without a history of cardiovascular disease. Cardiovascular disease was defined as coronary artery disease, cerebrovascular disease and or peripheral arterial disease as described before [3]. vWf depends on blood group, and therefore we

Table 1 General characteristics of the subjects

	All Subjects	Non-diabetic subjects	Diabetic subjects
Number	610	440	170
Men (%)	48·7	50·2	44·7
Age (yr)	64·3(7·2)	63·7(7·3)	65·9(6·7)
BMI (kg m^{2-1})	27·3(4·0)	26·7(3·5)	28·9(4·6)
WHR (men)	0·97(0·07)	0·96(0·07)	1·0(0·07)
WHR (women)	0·87(0·07)	0·86(0·07)	0·92(0·06)
Current smoker (%)	26·3	28·2	21·4
Systolic blood pressure (mmHg)	139·4(19·5)	137·6(19·5)	144·2(18·7)
Diastolic blood pressure (mmHg)	82·5(10·0)	82·3(9·9)	83·1(10·3)
Hypertension (%)	39·5	33·2	55·9
Total cholesterol (mmol L^{-1})	6·6(1·2)	6·7(1·2)	6·5(1·3)
HDL cholesterol (mmol L^{-1})	1·3(0·4)	1·3(0·4)	1·2(0·3)
LDL cholesterol (mmol L^{-1})	4·5(1·1)	4·6(1·0)	4·3(1·1)
Triglycerides (mmol L^{-1})	1·9(1·3)	1·7(0·9)	2·4(1·8)
Glomerular filtration rate ($\text{mL min}^{-1)}$*)	69·8(20·0)	69·3(18·8)	70·9(22·8)
Total homocysteine (μmol L†)	11·4 (9·3–14·1)	1·6 (9·4–14·4)	11·3 (9·3–13·6)
Von Willebrand factor (IU mL†)	1·22 (0·86–1·73)	1·12 (0·82–1·62)	1·56 (0·99–2·10)
Soluble vascular cell adhesion molecule (μglL†)	1304·8 (1090·8–1594·6)	1273·3 (1051·0–1563·7)	1402·3 (1184·3–1753·7)
C-reactive protein (mg L^{-1})	1·8 (0·83–3·8)	1·5 (0·7–3·2)	2·5 (1·3–4·7)

Data are presented as mean (SD), percentage of the total or median (interquartile range).
*Estimated by the method of Levey *et al.* [29], †median (interquartile range).
BMI, body mass index; WHR, waist-to-hip ratio; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol.

performed stratified analyses for the different blood groups to investigate if blood group modified the association between tHcy and vWf. Finally we investigated whether glucose tolerance was an effect modifier of the tHcy-outcome relationships by adding product terms to the analysis (*i.e.* product terms of tHcy with impaired glucose tolerance and tHcy with type 2 diabetes). With an *F*-test we checked whether these product terms significantly improved the model. All analyses were performed with the SPSS. Two-sided *P*-values less than 0·05 were considered to be statistically significant.

Results

Table 1 shows the characteristics of the participants. Twenty-one subjects had missing blood samples, so we obtained complete data in 610 participants, 170 (28%) of whom had type 2 diabetes. The median tHcy serum level was 11·4 (IQR: 9·3–14·1) μmol L^{-1}. One hundred 25 (21%) subjects had a tHcy level $\geq 15\,\mu$mol L^{-1}, the cut-off value frequently used to define a high tHcy level [1–31].

tHcy was significantly but weakly associated with vWf ($\beta = 0{\cdot}198$; $P = 0{\cdot}004$; Fig. 1a; Table 2a) after adjustment for the stratification variables. Adjustment for other possible confounders, especially GFR, weakened this association ($\beta = 0{\cdot}150$; $P = 0{\cdot}046$), but it remained significant. A 50% increase in the concentration of tHcy was associated with an increase in the concentration of vWf with 0·08 IU mL^{-1}. Thus, for example, when the concentration of tHcy increased from 10 to 15 μmol L^{-1}, the level of vWf increased with 0·08 IU mL^{-1} (95% confidence interval (C.I.), 0·01–0·22 IU mL^{-1}). In comparison, type 2 diabetes, as compared to normal glucose tolerance, was associated with a 0·39 IU mL^{-1} (95% C.I., 0·23–0·56 IU mL^{-1}) higher vWf level, after adjustment for age, sex, current smoking, LDL cholesterol and GFR, but the presence of diabetes did not modify the association between tHcy and vWf (*P*-interaction = 0·34 after exclusion of one diabetic subject with an extremely high tHcy level).

After adjustment for the stratification variables there was a significant but weak association between tHcy and sVCAM-1 ($\beta = 0{\cdot}130$; $P = 0{\cdot}001$). Adjustment for other possible confounders, especially GFR, weakened this association ($\beta = 0{\cdot}082$; $P = 0{\cdot}041$; Fig. 1b; Table 2b), but it remained significant. For example, when the concentration of tHcy increased from 10 to 15 μmol L^{-1}, the concentration of sVCAM-1 increased with 60·9 μg L^{-1} (95% C.I., 19·6–144·6 μg L^{-1}). In comparison, type 2 diabetes, as compared to normal glucose tolerance, was associated with a 146·2-μg L^{-1} (95% C.I. 30·9–270·8 μg L^{-2}) higher sVCAM-1 level, after adjustment for age, sex, current smoking, LDL cholesterol, HDL cholesterol, GFR, BMI and hypertension, but the presence of diabetes did not modify the association between tHcy and sVCAM-1 (*P*-interaction = 0·79).

There was no significant association of tHcy with CRP in any model (Fig. 1c; Table 2c). When the concentration of tHcy increased from 10 to 15 μmol L^{-1}, the concentration of CRP increased with 0·01 mg L^{-1} (95% C.I., −0·01–0·01 mg L^{-1}). In addition, the presence of diabetes did not modify the association between tHcy and CRP (P-interaction = 0·17).

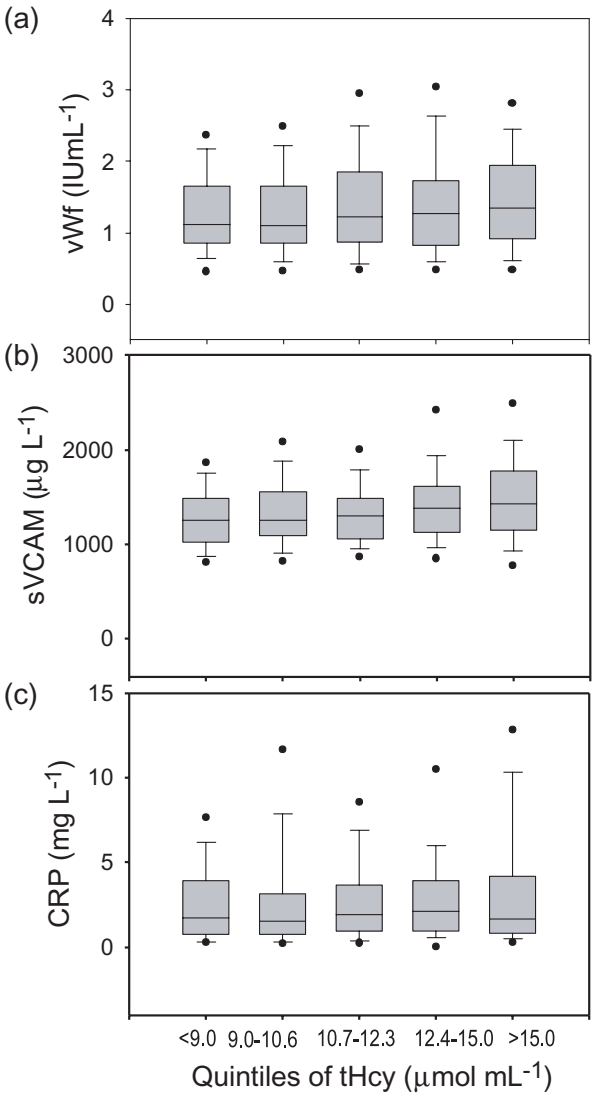

Figure 1 Plots with the 5th (dot), 10th, 25th, 50th, 75th, 90th and 95th (dot) percentiles as vertical boxes of the univariate relation between fasting concentrations of serum total homocysteine (in quintiles) and plasma von Willebrand factor, soluble vascular cell adhesion molecule 1 and C-reactive protein.

The results did not differ significantly between the subjects with and without cardiovascular disease at enrolment (data not shown). In addition, the relation between vWf and tHcy was not modified by blood group (data not shown).

Our analysis did not suggest any threshold in the relation between tHcy and the dependent variables (data not shown).

Table 2a Multiple linear regression analysis with ln von Willebrand factor (vWf in IU mL^{-1}) as the outcome variable and homocysteine (tHcy) as the independent variable of interest

Model	β ln tHcy (95% C.I.)	*P*-value	Percentage change (95% C.I.) in vWf (IU mL^{-1}) associated with a 50% increase in tHcy
1	0·198 (0·062–0·334)	0·004	8·36 (1·73–14·5)
2	0·150 (0·002–0·297)	0·046	6·27 (0·08–12·8)
3	0·149 (0·001–0·296)	0·049	6·23 (0·04–12·75)

Model 1: the independent variables were ln tHcy and the stratification variables glucose tolerance, age and sex. Model 2: as model 1 plus the variables with a *P*-value < 0·15 in the crude analysis (LDL cholesterol, glomerular filtration rate) and current smoking. Model 3: as model 2 plus other variables of interest (body mass index, waist-to-hip ratio, hypertension, HDL cholesterol).

Table 2b Multiple linear regression analysis with ln soluble vascular cell adhesion molecule 1 (sVCAM-1 in μg/L) as the outcome variable and tHcy as the independent variable of interest

Model	β ln tHcy (95% C.I.)	*P*-value	Percentage change (95% C.I.) in sVCAM-1 ($\mu g\,L^{-1}$) associated with a 50% increase in tHcy
1	0·130 (0·054–0·206)	0·001	5·41 (2·21–8·71)
2	0·082 (0·002–0·162)	0·041	3·38 (0·08–6·79)
3	0·080 (0·001–0·160)	0·045	3·3 (0·04–6·7)

Model 1: the independent variables were ln tHcy and the stratification variables glucose tolerance, age and sex. Model 2: as model 1 plus the variables with a *P*-value < 0·15 in the crude analysis (current smoking, LDL cholesterol, HDL cholesterol, glomerular filtration rate, body mass index, hypertension). Model 3: as model 2 plus other variables of interest (waist-to-hip ratio).

Table 2c Multiple linear regression analysis with ln C-reactive protein (CRP in mg L^{-1}) as the outcome variable and tHcy as the independent variable of interest

Model	β ln tHcy (95% C.I.)	*P*-value	Percentage change (95% C.I.) in CRP (mg L^{-1}) associated with a 50% increase in tHcy
1	0·082 (− 0·240–0·404)	0·611	3·39 (0–17·8)
2	0·017 (− 0·239–0·381)	0·912	0·69 (0–16·71)
3	0·017 (− 0·229–0·439)	0·530	0·69 (0–19·48)

Model 1: the independent variables were ln tHcy and the stratification variables glucose tolerance, age and sex. Model 2: as model 1 plus the variables with a *P*-value < 0·15 in the crude analysis (current smoking, body mass index, waist-to-hip ratio, HDL cholesterol, hypertension). Model 3: as model 2 plus other variables of interest (glomerular filtration rate, LDL cholesterol).

Discussion

This is the first study that evaluates the association between tHcy and markers of endothelial function, leukocyte adhesion and low-grade inflammation in a population-based study with a focus on diabetes.

Hyperhomocysteinaemia may predispose to atherothrombosis by injuring the vascular endothelium. We assessed endothelial function by measuring vWf, an endothelial protein, increased levels of which are associated with an adverse cardiovascular outcome [17–19,26,32–35]. We found that tHcy was associated with a significant, but small change in the vWf concentration. If we assume that this association is causal, a relatively large increase in tHcy (which is clearly associated with increased mortality in this population [36]) induces a relatively minor increase in vWf. Such minor increases in vWf were not strongly associated with increased mortality in the present population, because the association between vWf and mortality was non-linear and restricted to subjects with vWf > 1·56 IU/mL [26]. Accordingly, our results suggest that if tHcy does in fact induce endothelial dysfunction, this is not reflected by an increased level of vWf. Three previous studies concerning vWf and tHcy showed a positive correlation in subjects with peripheral arterial occlusive disease [7–9]. In contrast, a study in hyperlipidaemic male smokers showed a significant negative correlation between tHcy and vWf [37]. A recent study performed by de Roo *et al.* demonstrated a weak positive relation between tHcy and the endothelial proteins vWf and endothelin in healthy

postmenopausal women [38]. Taken together, these studies and the current data suggest that there is a weak association between tHcy and vWf in the general (mostly healthy) population, and that this association may be magnified in the presence of other factors, such as peripheral arterial occlusive disease. This would be consistent with studies suggesting that mild hyperhomocysteinaemia, at least in some populations, appears more strongly associated with the progression of atherothrombotic disease than with its initiation [39–41]. As the prevalence of cardiovascular disease was 10·6% [3], our study lacked power to investigate this issue, but this hypothesis clearly requires further study.

We examined whether hyperhomocysteinaemia was associated with an increased recruitment of leukocytes as reflected by plasma levels of sVCAM-1, a member of the immunoglobin superfamily that plays an important role in the recruitment of mononuclear leukocytes to the endothelium [14,15]. A 50% increase in tHcy was associated with a $60{\cdot}9\ \mu g\,L^{-1}$ increase in sVCAM-1 levels, which corresponds with a 6% increase in 7-year mortality risk [23]. This weak association between tHcy and sVCAM-1 does not provide strong evidence in favour of the hypothesis that tHcy is associated with an increased adherence of leukocytes to the endothelium, a hypothesis recently proposed by Dudman [42]. Our finding is in line with a recent study that demonstrated a significant association between sVCAM-1 and carotid intimal medial thickness, a surrogate measurement of early atherosclerosis, but not between sVCAM-1 and tHcy [21].

We further investigated whether tHcy was related to CRP. CRP is an acute phase reactant and increased levels may reflect a chronic low-grade inflammatory state. Increased CRP levels have been shown to be an independent predictor of atherothrombotic events [19,24–26,43–46]. In the population investigated, tHcy was not associated with CRP. This may imply that hyperhomocysteinaemia does not promote atherothrombosis by contributing to low-grade inflammation.

Kuller *et al.* have argued that the relation between tHcy and atherogenesis may not be causal, but, that conversely, vascular disease may induce an increase in the concentration of tHcy [47]. They suggest that the inflammation in atherosclerosis is associated with an increased mitotic activity and results in a shortage of folic acid, which is used in DNA synthesis. Folic acid is also essential in the metabolism of tHcy and the increased demand on folic acid would lead to a secondary elevation of tHcy levels. To test this theory we performed a multiple regression analysis with CRP as the independent and tHcy as the dependent variable. However, this analysis also did not suggest a relation between tHcy and CRP (data not shown).

A shortcoming of this study might be that folate, vitamin B6 and B12 status were not measured. Although there is no evidence that inadequate B-vitamin status can directly cause a raise in plasma vWf, sVCAM-1 or CRP, we cannot fully exclude this.

In conclusion, this study provides evidence that, in a general population of middle-aged and elderly subjects with and without type 2 diabetes, tHcy is, at most, weakly associated with endothelial dysfunction as estimated from plasma vWf, and with leukocyte adhesion as estimated from plasma sVCAM-1. tHcy was not associated with chronic low-grade inflammation as estimated from plasma CRP. Our data thus suggest that the link between tHcy and atherothrombosis cannot be explained by associations of tHcy with vWf, sVCAM-1 or CRP. We cannot exclude that these associations may be stronger in specific patient groups. The present data do provide evidence against the hypothesis that the chronic low-grade inflammation that is part of the atherosclerotic process is itself a determinant of tHcy levels.

References

1 Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999;**354**:407–13.

2 Stehouwer CDA, Weijenberg MP, van den Berg M, Jakobs C, Feskens EJM, Kromhout D. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly man. *Arterioscler Thromb Vasc Biol* 1998;**18**:1895–901.

3 Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs J, Bouter LM *et al.* Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1998;**18**:133–8.

4 Stehouwer CDA, Gall MA, Hougaard P, Jakobs C, Parving HH. Plasma homocysteine concentration predicts mortality in non-insulin dependent diabetic patients with and without albuminuria. *Kidney Int* 1999;**55**:308–14.

5 Smulders YM, Rakic MR, Slaats EH, Treskes M, Sijbrands EJG, Odekerken DAM *et al.* Fasting and post-methionine homocysteine levels in NIDDM. *Diab Care* 1999;**22**:125–32.

6 Kark JD, Selhub J, Adler B, Rosenberg IH. Plasma homocysteine and all-cause mortality in diabetes. *Lancet* 1999;**353**:1936–7.

7 Van den Berg M, Boers GHJ, Franken DG, Blom HJ, Van Kamp GJ, Jakobs C *et al.* Hyperhomocysteinemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 1995;**25**:176–81.

8 De Jong SC, Stehouwer CDA, van den Berg M, Vischer UM, Rauwerda JA, Emeis JJ. Endothelial marker proteins in hyperhomocysteinemia. *Thromb Haemost* 1997;**78**:1332–7.

9 Mercié P, Seigneur M, Conri C, Boisseau MR. Hyperhomocysteinaemia and endothelial dysfunction in peripheral arterial disease. *Thromb Res* 1999;**93**:97–9.

10 Chambers JC, McGregor A, Jean-Marie J, Kooner JS. Acute hyperhomocysteinaemia and endothelial dysfunction. *Lancet* 1998;**351**:36–7.

11 Woo KS, Chook P, Lolin YI, Cheung BN, Chan LT, Sun YY *et al.* Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* 1997;**96**:2542–4.

12 Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilatation in humans. *Circulation* 1997;**95**:1119–21.

13 Ross R. Atherosclerosis – an inflammatory disease. *N Eng J Med* 1999;**340 (2)**:115–26.

14 Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature* 1995;**376**:517–9.
15 Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M *et al*. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1993;**92**:1866–74.
16 Beks PJ, Mackaay AJC, De Vries H, De Neeling JND, Bouter LM, Heine RJ. Carotid artery stenosis is related to blood glucose level in an elderly Caucasian population. *The Hoorn Study Diabetologia* 1997;**40**:290–8.
17 Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, den Ottolander GJH. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992;**340**:319–21.
18 Blann AD, Miller JP, McCollum CN. Von Willebrand factor and soluble E-selectin in the prediction of cardiovascular disease progression in hyperlipidaemia. *Atherosclerosis* 1997;**132**:151–6.
19 Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden death in patients wih angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *N Eng J Med* 1995;**332**:635–41.
20 Wallen NH, Held C, Rehnqvist N, Hjemdahl P. Elevated serum intercellular adhesion molecule-1 and vascular adhesion molecule-1 among patients with stable angina pectoris who suffer cardiovascular death or non-fatal myocardial infarction. *Eur Heart J* 1999;**20**:1039–43.
21 Rohde LE, Lee RT, Rivero J, Jamacochian M, Arroyo LH, Briggs W *et al*. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 1998;**18**:1765–70.
22 Tsakiris DA, Tschöpl M, Jäger K, Haefeli WE, Wolf F, Marbet GA. Circulating cell adhesion molecules and endothelial markers before and after transluminal angioplasty in peripheral arterial occlusive disease. *Atherosclerosis* 1999;**142**:193–200.
23 Jager A, van Hinsbergh VWM, Kostense PJ, Emeis JJ, Nijpels G, Dekker JM *et al*. Increased levels of soluble vascular cell adhesion molecule 1 are associated with risk of cardiovascular mortality in type 2 diabetes. *Diabetes* 2000;**49**:485–91.
24 Haverkate F, Thompson SG, Pyke SDM, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and instable angina. *Lancet* 1997;**349**:462–6.
25 Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT-nested case-control study. *Am J Epidemiol* 1996;**144**:537–47.
26 Jager A, van Hinsbergh VWM, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G *et al*. Von Willebrand factor, C-reactive protein and five year mortality in diabetic and non-diabetic subjects. *The Hoorn Study Arterioscler Thromb Vasc Biol* 1999;**19**:3071–8.
27 Study group on diabetes mellitus. Technical report series No 727. World Health Organisation, Geneva, Switzerland: (1985).
28 Ubbink JB, Vermaak WJH, Bissbort S. Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chrom* 1991;**565**:441–6.
29 Levey AS, Bosch JP, Breyer Lewis J, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999;**330 (130)**:877–84.
30 Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM *et al*. Plasma homocysteine as a risk factor for vascular disease. *JAMA* 1997;**277**:1775–81.
31 Welch GM, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;**338**:1042–50.
32 Jansson JH, Nilsson TK, Johnson O. Von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. *Br Heart J* 1991;**66**:351–5.
33 Jansson JH, Nilsson TK, Johnson O. Von Willebrand factor, tissue plasminogen activator, and dehydroepiandrosterone sulphate predict cardiovascular death in a 10 year follow up of survivors of acute myocardial infarction. *Heart* 1998;**80**:334–7.
34 Cortellaro M, Boschetti C, Cofrancesco E, Zanussi C, Catalano M, de Gaetano G *et al*. Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. *Arterioscler Thromb* 1992;**12**:1063–70.
35 Catto AJ, Carter AM, Barrett JH, Bamford J, Rice PJ, Grant PJ. Von Willebrand factor and factor VIII. C in acute cerebrovascular disease. Relationship to stroke, subtype and mortality. *Thromb Haemost* 1997;**77**:1104–8.
36 Hoogeveen EK, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ *et al*. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hoorn Study. *Circulation* 2000;**101**:1506–11.
37 Brude IR, Finstad HS, Seljeflot I, Drevon CA, Solvoll K, Sandstad B *et al*. Plasma homocysteine concentration related to diet, endothelial function and mononunclear cell gene expression among male hyperlipidaemic smokers. *Eur J Clin Invest* 1999;**29**:100–8.
38 Valk de-Roo de GW, Stehouwer CDA, Lambert J, Schalkwijk CG, van der Mooren MJ, Kluft C *et al*. Plasma homocysteine is weakly correlated with plasma endothelin and von Willebrand factor, but not with endothelium-dependent vasodilatation in healthy postmenopausal women. *Clin Chem* 1999;**45**:1200–5.
39 Van den Berg M, Stehouwer CD, Bierdrager E, Rauwerda JA. Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. *Arterioscler Thromb Vasc Biol* 1996;**16**:165–71.
40 De Jong SC, Stehouwer CDA, Mackaay AJC, van den Berg M, Bulterijs EJ, Visser FC *et al*. High prevalence of hyperhomocysteinemia and asymptomatic vascular disease in siblings of young patients with vascular disease and hyperhomocysteinemia. *Arterioscler Thromb Vasc Biol* 1997;**17**:2655–62.
41 De Jong SC, Stehouwer CDA, van den Berg M, Geurts TW, Bouter LM, Rauwerda JA. Normohomocysteinemia and vitamin-treated hyperhomocysteinemia are associated with similar risks of cardiovascular events in patients with premature peripheral arterial occlusive disease. A prospective cohort study. *J Intern Med* 1999;**246**:87–96.
42 Dudman NPL. An alternative view of homocysteine. *Lancet* 1999;**354**:2072–4.
43 Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB *et al*. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med* 1994;**331**:417–24.
44 Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M *et al*. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: Results from the

Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol* 1997;**17**:1121–7.
45 Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;**336**:973–9.
46 Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;**98**:731–3.
47 Kuller LH, Evans RW. Homocysteine, vitamins, and cardiovascular disease. *Circulation* 1998;**98**:196–948.