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published in
Journal of Internal Medicine
2005

DOI (link to publisher)
10.1111/j.1365-2796.2005.01578.x

document version
Publisher's PDF, also known as Version of record

Journal of Internal Medicine 2005; **258**: 536–543 doi:10.1111/j.1365-2796.2005.01578.x

Homocysteine levels are not associated with cardiovascular autonomic function in elderly Caucasian subjects without or with type 2 diabetes mellitus: the Hoorn Study

A. M. E. SPOELSTRA-DE MAN[1,2], Y. M. SMULDERS[1,2], J. M. DEKKER[3], R. J. HEINE[3], L. M. BOUTER[3], G. NIJPELS[3] & C. D. A. STEHOUWER[1,3,4]

From the [1]Institute for Cardiovascular Research, [2]Department of Internal Medicine and [3]Institute for Research in Extramural Medicine, VU University Medical Center, Amsterdam; and [4]Department of Internal Medicine, University Hospital Maastricht, Maastricht; the Netherlands

Abstract. Spoelstra-de Man AME, Smulders YM, Dekker JM, Heine RJ, Bouter LM, Nijpels G, Stehouwer CDA (VU University Medical Center, Amsterdam; and University Hospital Maastricht, Maastricht; the Netherlands). Homocysteine levels are not associated with cardiovascular autonomic function in elderly Caucasian subjects without or with type 2 diabetes mellitus: the Hoorn Study. *J Intern Med* 2005; **258**: 536–543

Objective. Homocysteine and cardiovascular autonomic function are both predictors of cardiovascular disease and death, particularly in patients with diabetes. The mechanism by which homocysteine causes disease is unknown. The objective of our study was to determine whether hyperhomocysteinaemia is associated with impaired cardiovascular autonomic function in an age-, sex-, and glucose tolerance-stratified sample of an elderly Caucasian population.

Methods. We studied 609 subjects, 252 with normal glucose metabolism, 173 with impaired glucose metabolism, and 184 with type 2 diabetes. Cardiac cycle duration (RR interval) and continuous finger arterial pressure were measured under three conditions: during (i) spontaneous breathing, (ii) six deep breaths over 1 min, and (iii) an active change in position from lying to standing. From these readings, 10 parameters of autonomic function were assessed (three Ewing tests, six heart rate variability tests and one test of baroreflex sensitivity). These 10 measurements were summarized in a single cardiovascular autonomic dysfunction score (CADS).

Results. Comparing values of autonomic function measures in the lowest versus the highest quartile of homocysteine revealed no significant association between homocysteine level and autonomic function in the whole study group, nor in the individual glucose tolerance groups. Multiple adjustment for age, sex, waist-to-hip ratio, serum creatinine, use of antihypertensives and fasting insulin, confirmed this result. We found no evidence of effect modification of glucose tolerance status on the association between homocysteine and autonomic dysfunction (P for interaction for CADS = 0.79).

Conclusions. There is no evidence for an association between homocysteine levels and cardiovascular autonomic function in either diabetic or nondiabetic subjects. Cardiovascular autonomic dysfunction does not help explain why hyperhomocysteinaemia is related to cardiovascular mortality.

Keywords: autonomic nervous system diseases, diabetes mellitus type 2, glucose metabolism disorders, homocysteine, hyperhomocysteinaemia.

Introduction

Hyperhomocysteinaemia is a risk factor for mortality in the general population [1]. Homocysteine is a stronger risk factor for mortality in type 2 diabetic patients than in nondiabetic subjects [2]. The mechanism underlying the greater susceptibility of diabetic individuals to the adverse effects of

hyperhomocysteinaemia has not been elucidated. Autonomic dysfunction might play a role, as cardiovascular autonomic dysfunction, even if subclinical, is associated with sharply increased cardiovascular mortality [3], particularly in diabetic patients, but also in the nondiabetic population [4, 5]. Hyperhomocysteinaemia might contribute to the pathogenesis of autonomic dysfunction. Neurotoxicity of increased homocysteine levels may be caused by direct toxic effects, or by dysfunction of the vasa nervorum [6–8].

Previously, only two studies, both in diabetic subjects, have specifically addressed the association between hyperhomocysteinaemia and autonomic dysfunction. However, one study was small, involving only 65 subjects [6], and in the other study the diagnosis of autonomic neuropathy was based on only a single test [9]. Moreover, as both studies only included diabetic patients, possible effect modification of glucose tolerance with regard to the association between homocysteine and autonomic dysfunction could not be addressed.

In view of the unclear pathogenesis of hyperhomocysteinaemia-related cardiovascular mortality, the possible association between homocysteine and cardiovascular autonomic function is important. In this population-based study, we investigated whether an association exists between hyperhomocysteinaemia and cardiovascular autonomic function in subjects without and with type 2 diabetes.

Materials and methods

All participating individuals were involved in the Hoorn Study, the baseline measurements of which were conducted from 1989 to 1992. For this study, a random sample of all Caucasian individuals aged 50–75 years was drawn from the municipal population registry office of Hoorn (the Netherlands); 2484 subjects participated (response rate 71%). An extensive cardiovascular investigation was performed in an age-, sex-, and glucose tolerance-stratified random subsample [$n = 708$, of whom 631 responded (89%)] as described in detail elsewhere [10]. The Hoorn Study was carried out in accordance with the Declaration of Helsinki (2000) and was approved by the Ethical Review Committee of the University Hospital 'Vrije Universiteit' Amsterdam. Informed consent was obtained from all participants.

Measurements

Height and weight were measured barefoot wearing light clothes only. Double readings of systolic and diastolic (Korotkoff V) blood pressure were obtained on two separate occasions on the right arm with the subject in a sitting position. 'Actual' hypertension was defined as a mean systolic blood pressure of ≥160 mmHg and/or a mean diastolic pressure of ≥95 mmHg, with or without anti-hypertensive medication. Impaired glucose metabolism and type 2 diabetes mellitus were diagnosed according to the 1999 World Health Organisation criteria applied to the mean of two standard oral glucose tolerance tests [11]. We determined levels of fasting serum total cholesterol, HDL cholesterol and triglycerides by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany). The Friedewald formula was used to calculate the LDL cholesterol concentration, except in subjects with serum triglyceride levels >8.0 mmol l^{-1} ($N = 3$). HbA_{1c} was determined by means of ion exchange high-performance liquid chromatography (HPLC). The waist-to-hip ratio was measured as previously described [10]. Body mass index was calculated as weight divided by height squared (kg m^{-2}). All laboratory measurements were carried out by technicians unaware of the subjects' history of cardiovascular disease and glucose tolerance status.

Measurement of serum total homocysteine

Fasting blood samples were centrifuged within 1 h after drawing blood. Serum was stored at −20 °C for 6 years. There is good evidence that, under these conditions, serum total homocysteine levels are stable for many years [12]. Serum total (free plus protein bound) homocysteine level was measured by using tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate as the thiol-specific fluorochromophore, followed by HPLC with fluorescence detection [13]. The intra- and interassay coefficients are 2.1% and 5.1% respectively.

Autonomic function tests

For the assessment of cardiovascular autonomic function, study participants were asked to refrain from smoking and drinking coffee for 2 h prior to

the examination. The tests took place between 8.30 a.m. and 4 p.m., at least 1 h after a light meal. A quiet ambience was ensured, with a room temperature between 19 and 22 °C. All tests were performed by a single investigator, who was unaware of the individual's glucose tolerance status.

The duration of one cardiac cycle (RR interval) and continuous finger arterial pressure were measured under three conditions: (i) during spontaneous breathing for 3 min in supine position, (ii) during 1 min with six deep breaths in supine position, and (iii) during an active position change from lying to standing. The correct breathing frequency at six breaths per minute was controlled by the investigator. The record was discarded if off-line spectral analysis showed that breathing was not performed at the appropriate frequency. After a resting period of at least 10 min, testing was started, whilst each separate test started with a resting period of at least 1 min. During the tests, RR intervals and blood pressure were continuously recorded on a PC-based data-acquisition system. We obtained RR intervals from an electrocardiogram by a hardware QRS detector with an accuracy of one millisecond (ms). Blood pressure was recorded using the Finapres™ method (Finger Arterial Blood Pressure, Ohmeda BP2000, Englewood, CO, USA) [14]. Systolic blood pressure values were obtained from the sampled continuous blood pressure signal by means of an automatic procedure, which was verified by visual inspection.

We computed 10 measures of cardiovascular autonomic function from the RR interval and the SBP recordings under the three respective conditions, with computerized data analysis using a system developed locally by the Department of Medical Physics, Vrije Universiteit, Amsterdam, the Netherlands, as previously described in detail [15]. Table 1 gives the definition and the computational information of the 10 measures [3, 16–21].

Individual data were missing for the following reasons: an incomplete test schedule, insufficient

Table 1 Overview of the 10 measures of cardiovascular autonomic function

Measure	Unit	Definition
During spontaneous breathing over 3 min in supine position		
Mean NN	ms	The mean of all normal to normal, i.e. sinus rhythm, RR intervals
SDNN	ms	The standard deviation of all normal to normal, i.e. sinus rhythm, RR intervals
LF power	ms^2	Low frequency power, in absolute units: energy in the power spectrum between 0.004 and 0.12 Hz
HF power	ms^2	High frequency power, in absolute units: energy in the power spectrum between 0.12 and 0.40 Hz
LF/(LF + HF)		The ratio of low frequency power to the sum of the low and high frequency power
During six deep breaths over 1 min in supine position		
EI difference	ms	The mean expiration–inspiration difference in RR intervals over the six consecutive breaths
BRS	ms $mmHg^{-1}$	A measure of baroreflex sensitivity, computed as gain, i.e. ratio of the energy in the cross-spectrum of systolic blood pressure and RR intervals, and the energy in the power spectrum of the RR interval; all between 0.05 and 0.15 Hz and with a squared coherence (γ^2) of 0.5 or higher
During an active change in position from lying to standing		
RR max	ms	The difference between the mean RR interval during 1 min of rest prior to standing up and the minimum RR interval within 15 s after standing up
RR max/min		Maximum RR interval between 15 and 30 s after standing up divided by minimum RR interval within 15 s after standing up
SBP difference	mmHg	Systolic blood pressure after standing up (mean of 1.5–2 min after standing) minus systolic blood pressure in supine position

Mean NN, mean of all sinus rhythm (normal-to-normal) RR intervals;
SDNN, standard deviation of all sinus rhythm (normal-to-normal) RR intervals;
LF power, low frequency power in the RR-interval spectrum between 0.04–0.12 Hz;
HF power, high frequency power in the RR-interval spectrum between 0.12–0.40 Hz;
LF/(LF + HF), ratio of low frequency power to the sum of low and high frequency power in the RR-interval spectrum;
EI difference, expiration-inspiration difference in RR intervals during breathing at 6/min;
BRS, baroreflex sensitivity;
RR max, maximal change in RR interval after standing up;
RR max/min, maximal RR interval between 15s and 30s after standing up divided by the minimal RR interval within 15s after stading up;
SBP difference, systolic blood pressure 1.5–2 min after standing up minus systolic blood pressure in supine position.

quality data for processing (a poor blood pressure signal or dysrhythmias), more than 10% non-sinus beats in the total number of recorded beats or if standing up took more than 10 s.

Statistical analysis

Routine parametric and nonparametric bivariate tests were used, as appropriate, to test for group differences. Based on the 10 measures of cardiovascular autonomic function, a summary 'cardiovascular autonomic dysfunction score' (CADS) was constructed as follows: the results of each measurement were divided into quartiles. A subject was assigned 0 point if the result was in the most normal quartile, 1 point if in the second quartile, 2 points if in the third quartile, and 3 points if the test outcome was in the most abnormal quartile. If all 10 measures were completed successfully, the scores of each were added together. If one or two results were missing (52 of 609 subjects), it was replaced by the median score for this measurement. When three or more results were missing the patient was excluded for calculation of CADS (79 subjects). The result is a CADS ranging from 0 (good) to 30 (poor). We used *t*-tests to compare values of the autonomic function measures in the lowest versus the highest quartile of homocysteine, both in the whole group as well as in the three separate glucose tolerance groups. Multiple linear regression analysis was used to identify independent determinants of CADS. Because the study population was stratified by age, gender and glucose tolerance, these three variables were entered into the regression model, even when they did not significantly contribute after other variables were entered. In addition, we adjusted for the determinants of autonomic function we have previously described [15]. A two-sided *P*-value of <0.05 was considered statistically significant. All analyses were performed with SPSS 11.0 for Windows 98 (SPSS Inc., Chicago, IL, USA).

Results

In 22 subjects, no autonomic function tests were available, so final analyses were based on 609 of the 631 subjects. The group of individuals excluded ($n = 22$) was significantly older (mean 69 years vs. 64 years; $P = 0.001$ by Student's *t*-test) than those included. In addition, they more often had diabetes (45% vs. 26%, $P < 0.001$) and less often normal glucose metabolism (32% vs. 46%, $P < 0.05$ both by chi-square analysis).

Table 2 shows demographic and clinical data of the study population in three categories of glucose tolerance. Only a small number (29) of patients had impaired fasting glycaemia. Therefore, we analysed patients with impaired fasting glycaemia together with the patients with impaired glucose tolerance, as the impaired glucose metabolism group. Amongst the noticeable findings in Table 2 is the absence of an association between glucose tolerance categories on the one hand, and renal function and homocysteine concentrations on the other. As described previously, autonomic function parameters in this population were most strongly associated with glucose tolerance status and moderately associated with age, sex, waist-to-hip ratio, use of antihypertensives and fasting insulin [15] (data not shown).

Comparing values of autonomic function measures in the lowest versus the highest quartile of homocysteine revealed no significant association between homocysteine level and autonomic function in the whole study group (Table 3). Likewise, analyses of homocysteine levels below and above commonly used cut-off levels of both 12 and 15 $\mu mol\ l^{-1}$ revealed no positive association between hyperhomocysteinaemia and autonomic function, nor was there any indication in the data of a threshold value of homocysteine above which a positive association with autonomic function became apparent. The three separate glucose tolerance groups did not differ with regard to the absence of any correlation between the autonomic function tests and homocysteine levels (data not shown). In 79 subjects, the combined autonomic function score CADS could not be calculated because of three or more missing measurements. Therefore, analyses on CADS were based on 530 subjects. As illustrated in Fig. 1, no crude correlation between homocysteine quartiles and CADS was apparent. Multiple linear regression analysis, both crude and with adjustment for glucose tolerance category, age, sex, waist-to-hip ratio, serum creatinine, use of antihypertensives and fasting insulin, confirmed that no significant relationship existed between homocysteine levels and both the individual autonomic function measures as well as the combined score (Table 4). We found no evidence of effect modification of glucose tolerance status on the

Table 2 Demographic and clinical data

	NGM ($n = 252$)		IGM ($n = 173$)		DM2 ($n = 184$)	
Variable	Men	Women	Men	Women	Men	Women
N	122	130	89	84	81	103
Age (years)	62 (8)	64 (7)	63 (7)	65 (7)	64 (7)	67 (6)
Anthropometric factors						
Body mass index (kg m^{-2})	25.4 (2.6)	26.3 (3.6)	27.1 (3.5)	27.8 (3.9)	27.7 (3.3)	29.6 (4.9)
Waist-to-hip ratio	0.94 (0.06)	0.84 (0.07)	0.98 (0.07)	0.88 (0.07)	0.99 (0.07)	0.91 (0.07)
Blood pressure						
Systolic (mmHg)	130 (17)	135 (19)	141 (19)	144 (21)	143 (17)	145 (19)
Diastolic (mmHg)	81 (10)	80 (10)	84 (9)	84 (10)	85 (10)	82 (10)
Hypertension (%)	28	21	44	39	59	46
Metabolic factors						
HbA_{1c} (%)	5.3 (0.5)	5.3 (0.5)	5.5 (0.5)	5.6 (0.5)	6.8 (1.6)	7.2 (1.8)
Fasting insulin	76 (30)	77 (37)	102 (50)	96 (52)	114 (52)	126 (64)
Total cholesterol (mmol l^{-1})	6.5 (1.0)	6.8 (1.2)	6.5 (1.2)	6.9 (1.0)	6.2 (1.2)	6.9 (1.3)
HDL cholesterol (mmol l^{-1})	1.2 (0.3)	1.5 (0.3)	1.2 (0.4)	1.4 (0.4)	1.1 (0.3)	1.2 (0.3)
Triglycerides (mmol l^{-1})	1.4 (0.5–5.1)	1.2 (0.5–4.2)	1.6 (0.6–10.5)	1.6 (0.8–4.9)	1.9 (0.4–6.7)	2.1 (0.6–14.0)
Homocysteine (μmol l^{-1})	13.5 (7.2)	11.6 (4.2)	14.0 (6.0)	12.0 (3.8)	12.0 (4.1)	11.9 (7.5)
Creatinine (μmol l^{-1})	99 (13)	84 (13)	98 (14)	84 (10)	102 (21)	84 (27)
Autonomic function measures						
BRS (ms $mmHg^{-1}$)	8.6 (0.8–35.8)	8.1 (1.7–27.8)	7.3 (0.6–25.6)	8.1 (1.8–40.1)	6.8 (0.8–18.8)	6.6 (1.1–23.0)
EI difference (ms)	196 (29–577)	156 (46–443)	176 (34–556)	145 (31–463)	130 (30–564)	124 (30–539)
HF power (ms^2)	217 (7–7393)	198 (7–4521)	150 (8–3103)	136 (7–1877)	144 (4–5657)	101 (10–7116)
LF power (ms^2)	344 (13–8093)	205 (7–3666)	247 (15–3441)	195 (27–1572)	265 (7–2471)	133 (3–2478)
Mean NN (ms)	979 (164)	973 (131)	950 (151)	920 (137)	945 (153)	870 (137)
LF/(LF + HF)	0.58 (0.17)	0.52 (0.19)	0.59 (0.19)	0.52 (0.20)	0.59 (0.19)	0.51 (0.19)
RR max (ms)	251 (87–944)	237 (60–487)	244 (60–514)	211 (75–493)	236 (60–547)	182 (63–525)
RR max/min	1.2 (1.0–2.1)	1.2 (1.0–2.1)	1.2 (1.0–1.9)	1.2 (1.0–1.6)	1.2 (1.0–1.8)	1.2 (1.0–1.8)
SBP difference (mmHg)	−4 (13)	−5 (15)	−4 (13)	−8 (15)	−6 (17)	−9 (16)
SDNN (ms)	37 (11–118)	35 (9–86)	31 (12–90)	31 (12–74)	31 (8–129)	24 (7–124)
CADS	12 (5)	13 (5)	13 (6)	15 (5)	14 (6)	17 (5)

Data are presented as mean (SD) or median (range). NGM, normal glucose metabolism, IGM, impaired glucose metabolism; DM2, diabetes mellitus type 2; CADS, combined score of the ten autonomic function scores. For explanation of autonomic function tests and their abbreviations, see Table 1.

Table 3 Median values of autonomic function tests in the whole group for each quartile of homocysteine and *t*-test comparing values of lowest and highest quartile of homocysteine

Homocysteine quartiles	*n*	0–25% (<9.3 μmol l^{-1})	25–50% (9.3–11.4 μmol l^{-1})	50–75% (11.4–14.1 μmol l^{-1})	75–100% (>14.1 μmol l^{-1})	*P*-value highest versus lowest quartile
BRS (ms $mmHg^{-1}$)	534	7.8	7.4	7.8	7.6	0.32
EI difference (ms)	568	161	150	146	144	0.24
HF power (ms^2)	564	165	183	156	150	0.17
LF power (ms^2)	564	240	192	184	230	0.43
Mean NN (ms)	564	950	923	920	936	0.76
LF/(LF + HF)	564	0.57	0.54	0.53	0.60	0.45
RR max (ms)	570	230	227	231	216	0.34
RR max/min	570	1.22	1.22	1.22	1.18	0.31
SBP difference (mmHg)	570	−6.3	−6.1	−6.3	−5.2	0.78
SDNN (ms)	564	35	31	34	35	0.10
CADS	530	13	15	14.5	13	0.81

CADS, combination score of the 10 autonomic function measures; *n*, number of subjects on which the analysis was performed. For explanation of autonomic function tests and their abbreviations, see Table 1.

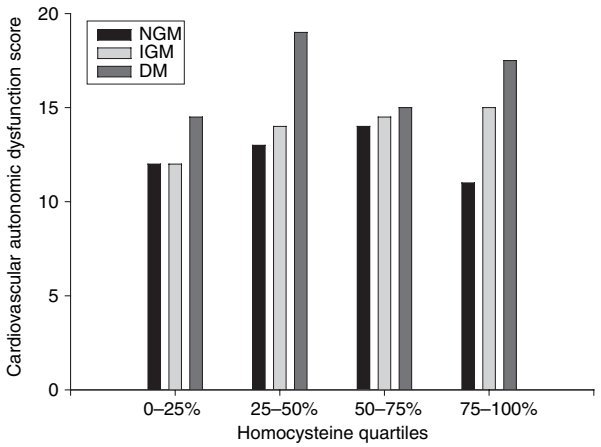

Fig. 1 Cardiovascular autonomic dysfunction scores in each homocysteine quartile for all three glucose tolerance groups. NGM, normal glucose metabolism; IGM, impaired glucose metabolism; DM, diabetes mellitus.

Table 4 Multiple linear regression analysis with autonomic function tests as dependent variable and homocysteine levels as independent variable after adjustment for age, gender, glucose tolerance status, waist-to-hip ratio, serum creatinine, use of antihypertensives and fasting insulin

Autonomic function test	*n*	*B*	95% CI for *B*
LnBRS	534	−0.004	−0.013 to 0.004
LnEI difference	568	−0.006	−0.014 to 0.003
LnHF power	564	−0.009	−0.026 to 0.01
LnLF power	564	−0.003	−0.019 to 0.013
Mean NN	564	−0.94	−3.10 to 1.21
LF/(LF + HF)	564	0.001	−0.001 to 0.004
LnRR max	570	−0.005	−0.01 to 0.001
LnRR max/min	570	0.0004	−0.001 to 0.002
SBP difference	570	−0.14	−0.37 to 0.08
LnSDNN	564	−0.002	−0.009 to 0.004
CADS	530	0.03	−0.05 to 0.11

CADS, combination score of the 10 autonomic function measures; *n*, number of subjects on which the analysis was performed. For explanation of autonomic function tests and their abbreviations, see Table 1.

association between homocysteine and autonomic dysfunction (P for interaction for CADS = 0.79).

Discussion

In this study, we observed no association between plasma homocysteine levels and cardiovascular autonomic function in either diabetic or non-diabetic subjects of an age-, sex- and glucose tolerance-stratified random sample of an elderly Caucasian population. These results do not support the concept of an intermediate role for cardiovascular autonomic dysfunction in a causal relationship between hyperhomocysteinaemia and cardiovascular mortality, either in non-diabetic or in diabetic individuals.

Most previous studies addressing this topic investigated peripheral sensorimotor neuropathy and showed no association with hyperhomocysteinaemia [22–25]. Only in two previous studies were data on autonomic neuropathy collected. Ambrosch *et al.* [6] investigated 65 patients with type 2 diabetes, and found a positive association between homocysteine levels and presence of neuropathy, but analysed patients with peripheral sensorimotor neuropathy, peripheral autonomic and/or cardiovascular autonomic neuropathy as a single group. Separate data on autonomic neuropathy were not presented, thus making comparison with our data difficult. In the Appropriate Blood pressure Control in Diabetes (ABCD) trial, Cohen *et al.* [26] did perform separate analyses of autonomic and sensorimotor neuropathy and found an independent association between hyperhomocysteinaemia and diabetic autonomic neuropathy, but not with diabetic sensorimotor peripheral neuropathy. Two differences between this study and ours are noteworthy. First, in the ABCD trial only a single cardiovascular autonomic test was performed (the heart rate response to deep breathing). In addition, differences in serum creatinine, although small, were not accounted for in the multivariate analyses, and may have confounded the outcome, as minor increases in serum creatinine may represent significant loss of glomerular filtration rate, which is associated with increases in homocysteine level [27] and with autonomic dysfunction [28–30]. In sum, most studies [22–25, and the present study] found no association between homocysteine levels and presence of neuropathy, but two [6, 9] did, and the reasons for this discrepancy remain to be clarified.

The strengths of our study are that it was large and population-based, included both diabetic and non-diabetic patients, and employed a number of validated cardiovascular autonomic function tests. The size of our study group allowed for the use of subgroup analyses and multivariable regression modelling. Inclusion of all three glucose tolerance groups made it possible to address possible effect

modification of glucose tolerance with regard to a possible association between hyperhomocysteinaemia and autonomic dysfunction. The 10 different measures of autonomic function reflect both parasympathetic and sympathetic function, and we were therefore able to analyse specifically cardiovascular autonomic function rather than of a mixture of sensorimotor neuropathy and peripheral and cardial autonomic neuropathy.

Limitations of our study were the moderate reproducibility of the test parameters (reliability coefficient around 50% [31]), reducing the power of the study and increasing the risk of a type II error. To enhance power, we constructed a total score combining the results of all 10 tests, as a single autonomic function measurement will by definition show a lower reproducibility than the combination of several tests. However, such a score has limitations in that there is a lack of knowledge of how autonomic function measures are interrelated and whether different weights should be given to separate tests. An important issue in this respect is distinction between sympatic and parasympatic function. Although several of the employed tests are thought to reflect predominantly either type of autonomic function [32], most authors agree that a clear distinction cannot be made [33]. The results of the combination score were in accordance with the individual tests. Furthermore, we did not analyse the results of the autonomic function tests as dichotomous variables for 'normal' and 'abnormal' results, as has been done in several previous studies. We chose this approach because abnormal values for autonomic function tests have been defined on the basis of statistical abnormality in a healthy control population, rather than on the basis of pathophysiological alterations. There is no evidence to indicate that these statistically abnormal results have pathophysiological significance in the context of a possible association with hyperhomocysteinaemia. Finally, B-vitamin levels were not analysed in this study. The absence of a correlation between homocysteine and autonomic function does not fully exclude negative confounding by B-vitamins (i.e. an association becoming apparent only after adjustment for B-vitamin levels), but we feel this would be unlikely, because this would imply that high B-vitamin levels are related to autonomic neuropathy. However, theoretically this is implausible and there are no studies showing such an association.

In conclusion, there is no evidence for an association between homocysteine levels and cardiovascular autonomic function in either diabetic or non-diabetic subjects. Cardiovascular autonomic dysfunction thus does not help explain why hyperhomocysteinaemia is related to cardiovascular mortality.

Conflict of interest statement

No conflict of interest was declared.

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Correspondence: Dr C.D.A. Stehouwer, Professor and Chair, Department of Medicine, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, the Netherlands.
(fax: +3143 3875006; e-mail: csteh@sint.azm.nl).