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## Correlations between plasma homocysteine and folate concentrations and carotid atherosclerosis in high-risk individuals: baseline data from the Homocysteine and Atherosclerosis Reduction Trial (HART)

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**Abstract:** Homocysteine has been proposed as a risk factor for atherosclerosis. The association between plasma total homocysteine (tHcy) concentration and carotid atherosclerosis has not been thoroughly studied in high-risk populations with vascular disease. For this study, carotid atherosclerosis was assessed by measurements of carotid intima-media thickness (IMT) and plaque calcification in 923 patients with vascular disease or diabetes. Associations with tHcy and plasma folate concentrations were examined. The mean and single maximum carotid IMT were  $1.27 \pm 0.34$  mm and  $2.41 \pm 0.83$  mm, respectively. The mean segment plaque calcification score was 27.8%. tHcy correlated with mean ( $r = 0.13$ ;  $p < 0.001$ ) and single maximum ( $r = 0.12$ ;  $p < 0.001$ ) carotid IMT. There was a progressive increase in mean and single maximum carotid IMT across quartiles of tHcy ( $p < 0.0001$  for trend). These associations were no longer significant after adjusting for other CV risk factors. A trend towards an inverse association between plasma folate and mean max carotid IMT was found in both univariate and multivariable analyses. However, the plaque calcification score increased across quartiles of tHcy ( $p < 0.01$ ) and decreased across quartiles of plasma folate concentrations ( $p < 0.05$ ) after multiple adjustments. In conclusion, in high-risk individuals, tHcy and low folate concentrations were only weakly associated with carotid IMT. In contrast, we found an independent association with the plaque calcification score, a measure of more advanced atherosclerosis. The effect of tHcy lowering on carotid atherosclerosis and stroke prevention warrants further investigation.

**Key words:** carotid intima-media thickness; folate; homocysteine; plaque

### Introduction

Homocysteine has been proposed as a risk factor for atherosclerosis.<sup>1</sup> Epidemiological studies suggest that mild to moderate elevations in plasma total homocysteine (tHcy) concentrations are associated with an increased risk for atherosclerotic vascular diseases.<sup>2–5</sup> Overall, these observational studies have concluded the relationship to be continuous,

graded, and independent of other cardiovascular (CV) risk factors.<sup>5</sup> While earlier retrospective epidemiological studies may have overestimated the strength of this association for myocardial infarction, a modest but significant association between tHcy and myocardial infarction is shown in prospective cohort studies and the relationship to stroke remains substantial in these more recent prospective observational evaluations,<sup>6</sup> as well as in mendelian randomization studies.<sup>7</sup> In experimental studies, hyperhomocysteinemia leads to increased oxidative stress, inhibition of nitric oxide synthase, proliferation of smooth muscle cells, endothelial dysfunction and promotes thrombosis.<sup>8–10</sup>

Therapy with folic acid or combined folic acid and vitamins B6 and B12 lowers tHcy levels and was suggested as a treatment to reduce the risk of CV events. To date, four completed trials in high-risk populations have generally failed to demonstrate the clinical benefits.<sup>11–14</sup> However, it was suggested that

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these trials do not necessarily disprove the 'homocysteine theory of atherosclerosis', and that their results may relate to the tHcy-lowering independent effects of the administration of high-dose B vitamins and ensuing complex metabolic consequences.<sup>15</sup>

The Heart Outcomes Prevention Evaluation (HOPE)-2 study, the largest B vitamin intervention trial completed to date, reported no significant reduction in overall major CV events in a high-risk population treated with folic acid and vitamins B<sub>6</sub> and B<sub>12</sub>.<sup>12</sup> However, the trial showed a reduction in the risk for stroke. The Homocysteine and Atherosclerosis Reduction Trial (HART) is a substudy of the HOPE-2 trial and aims to evaluate the effects of homocysteine-lowering vitamin therapy on the progression of carotid atherosclerosis, as evaluated by serial B-mode quantitative ultrasound (US) examinations.

Carotid intima-media thickness (IMT) is an accepted surrogate marker for atherosclerosis disease burden. Several case-control and cross-sectional studies have evaluated the association between tHcy and carotid IMT in healthy individuals, in individuals with homocysteinuria or specific CV risk factors, the majority being small or moderate in size and with inconsistent findings.<sup>16</sup> In high-risk populations with vascular disease, the associations have been examined only in a few and rather small studies, generally showing trends towards significant relationships.<sup>16</sup> Low plasma folate levels may also impact on atherosclerotic disease burden, as suggested by a recent study of healthy middle-age individuals, in which low folate concentrations were independently associated with increased carotid IMT.<sup>17</sup>

The effect of tHcy-lowering B vitamins on atherosclerosis progression is currently unclear. HART is the largest randomized trial evaluating the effects of B vitamins on carotid IMT. The current report outlines baseline characteristics and examines associations between baseline tHcy, folate and B vitamins and carotid IMT and to a plaque calcification score.

## Methods

The design and results of the HOPE-2 study have been described in detail previously.<sup>12,18</sup> A total of 5522 patients were randomized at 145 centers in 13 countries to daily therapy with combined folic acid 2.5 mg, vitamins B<sub>6</sub> 50 mg and B<sub>12</sub> 1 mg or to placebo. Median follow-up was 5 years. The primary study outcome was the composite of CV death, myocardial infarction and stroke. The HART substudy of the HOPE-2 trial is a prospective trial, conducted in 923 patients and aims to assess the effects of the study intervention on atherosclerosis progression.

Patients had yearly carotid ultrasound examinations and the effect of homocysteine-lowering B vitamins on carotid IMT will be assessed. The HART study will be published separately. Both the HOPE-2 and the HART trials were sponsored by the Canadian Institutes of Health Research and coordinated by the Population Health Research Institute, McMaster University. The current report provides the baseline characteristics of the HART study participants and evaluates associations between tHcy, folate and B vitamin concentrations and carotid IMT and plaque calcification at baseline.

## Patients

Between January 18 and December 31 2000, 923 patients were recruited from 10 Canadian HOPE-2 study centers. Clinical eligibility criteria for the HART study were identical to those for the main parent HOPE-2 trial.<sup>12,18</sup> Thus, men and women 55 years or older with stable coronary, cerebral or peripheral artery disease or diabetes and at least one additional CV risk factor (smoking, hypertension, dyslipidemia and/or microalbuminuria) were included, irrespective of their baseline tHcy levels. Exclusion criteria included current use of vitamin supplements containing folic acid > 200 µg/day, known adverse reactions to folic acid and/or vitamins B<sub>12</sub> or B<sub>6</sub>, planned revascularization or other clinically significant CV or non-CV disease, expected to limit any patient's adherence and ability to complete the trial. In addition, HART study patients had to have an adequate carotid IMT examination at baseline, defined as clear imaging of at least four predefined carotid artery segments. The study was conducted in accordance with the Helsinki declaration and was approved by the ethics committee of each participating center. All patients provided written informed consent.

## Laboratory measurements

Plasma tHcy, folate and B vitamin concentrations were measured at baseline in all study patients. For tHcy, the Abbott IMX immunofluorescence method (Abbott, Mississauga, Canada) was used, folate using the Roche chemiluminescence method (Roche Diagnostics, Mannheim, Germany), B<sub>6</sub> (pyridoxal) was measured by HPLC using the Chromsystems kit (Instruments and Chemicals, GmbH, Munchen, Germany) and B<sub>12</sub> by immunoassay using the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The coefficients of variation for these analyses were all within acceptable ranges.

## Carotid ultrasonography

All scans were performed by five sonographers (who underwent training and certification) at three

laboratories who had specific expertise and used high-resolution imaging systems. A standardized, validated imaging protocol was used,<sup>19</sup> aimed at recording the maximum carotid IMT in each of 12 predefined 1-cm long carotid artery segments, including the near and far walls of the internal, the bifurcation and the common left and right carotid arteries. All measurements were performed at the Core US Laboratory in Hamilton, Ontario by one of two specifically trained and experienced readers. A standardized and validated US reading protocol<sup>19</sup> and the Image-ProPlus software were used (Media Cybernetics, Silver Spring, MD, USA). A minimum of three readings/segment were performed and the highest reading was selected for each segment. Primary and secondary carotid US measurements were defined *a priori* as the sum of the maximum IMT for each segment, divided by the number of visualized segments (mean max carotid IMT) and the single segment maximum IMT (single max carotid IMT), respectively. For each carotid segment the presence or absence of plaque calcification, defined as bright echoes with acoustic shadowing, was evaluated and a plaque calcification score was computed for each patient, defined as the percentage of segments with calcification among the total number of segments visualized. Scanning and reading reproducibility were high; within and between the sonographer intraclass correlation coefficient (ICC) for repeat scanning they were > 0.90 and within and between the reader ICC they were 0.95 and 0.96, respectively. There were no temporal drifts in scanning or reading.

### Statistical analysis

Continuous variables are expressed as mean  $\pm$  SD or in quartiles unless otherwise stated. Logarithmic transformation was used for carotid IMT and other variables that were not normally distributed. The transformed IMT values were then back transformed for presentation on the original scale. Univariate associations between risk factors and carotid IMT were explored using Pearson's and Spearman rank correlations for continuous variables and analysis of variance (ANOVA) for categorical variables. Univariate comparisons between the plaque calcification score and categorical variables were conducted using logistic regression. Two-sided *p*-values  $\leq$  0.05 were considered to be statistically significant for all analyses. Age and sex-adjusted and multivariable models were used to evaluate the associations between tHcy and folate concentrations and carotid atherosclerosis, using stepwise linear regression for IMT measurements and stepwise logistic regression for the plaque calcification score. All variables with a *p*-value < 0.1 in univariate analyses were tested in the multivariable models. All statistical analyses were performed using the Statistical

Analysis Software, version 8.2 (SAS Institute, 1999; Cary, NC, USA).

## Results

### Patient characteristics

Patient characteristics are presented in Table 1. Study patients had a mean age of 68.6 years and were at high risk for vascular disease events. Indeed, over 85% had coronary, peripheral or cerebral artery disease. Over half of the patients had a history of hypertension and one third had diabetes. Most patients were treated for vascular disease and secondary prevention with aspirin, beta blockers, angiotensin-converting enzyme inhibitors and statins. Major CV risk factors, including serum lipid profile, and systolic and diastolic blood pressure were generally well controlled and within the targets recommended by clinical practice guidelines. High-sensitivity (hs)-CRP levels were elevated. Mean creatinine levels were within normal limits. tHcy levels were elevated:  $12.1 \pm 5.4$   $\mu\text{mol/l}$ ; and plasma folate levels were within the normal range:  $30.4 \pm 9.9$  nmol/l.

In multivariable analyses, age ( $p < 0.0001$ ), plasma folate levels ( $p < 0.01$ ), sex ( $p < 0.001$ ), vitamin B12 levels ( $p < 0.0001$ ), creatinine ( $p < 0.0001$ ), triglycerides ( $p < 0.05$ ) and total cholesterol/HDL-cholesterol ratio ( $p < 0.01$ ) were independent predictors of the tHcy concentration and accounted for approximately 27% of the variability of tHcy.

Most patients had evidence of carotid atherosclerosis, with both high mean and single max carotid IMT. The mean plaque calcification score was 27.8% (range 0–100%), indicative of advanced atherosclerosis (Table 1). The majority of the study patients, 81%, had a plaque calcification recorded in at least one carotid segment.

Compared with women, men had a higher mean max carotid IMT ( $p < 0.001$ ) and single max carotid IMT ( $p < 0.001$ ) (Table 2) and higher average plaque calcification scores (Table 3). Patients with a history of hypertension and current smoking had greater mean max carotid IMT than those without ( $p < 0.01$ ). There was no difference in mean or single max carotid IMT in patients with or without a history of diabetes. The mean plaque calcification score was higher among patients with a history of smoking ( $p < 0.01$ ) and hypertension ( $p < 0.01$ ) as compared to those without (Table 3).

### Univariate and multivariable correlations between risk factors and carotid IMT

In univariate analysis, age showed the strongest association with mean max carotid IMT ( $r = 0.28$ ;  $p < 0.0001$ ). Systolic blood pressure ( $r = 0.16$ ;  $p < 0.0001$ ) and serum creatinine ( $r = 0.10$ ;

**Table 1** Baseline characteristics of the HART study patients (*n* = 923)

Age (years)	68.6 ± 6.6
Female, number (%)	221 (23.9)
Vascular disease by history, number (%)	
Coronary artery disease	814 (88.2)
Myocardial infarction	554 (60.0)
Stable angina	568 (61.5)
Unstable angina	283 (30.7)
CABG	372 (40.3)
PCI	231 (25.0)
Stroke/TIA	98 (10.6)
Intermittent claudication	35 (3.8)
Peripheral artery surgery or PTA	46 (5.0)
Carotid endarterectomy	27 (2.9)
Risk factors by history, number (%)	
Hypertension	466 (50.5)
Diabetes mellitus	335 (36.3)
Low HDL cholesterol	122 (13.2)
Current smoking	88 (9.5)
Medication use, number (%)	
Aspirin	753 (81.7)
Antiplatelets	47 (5.1)
Beta-blockers	493 (53.4)
Lipid-lowering drugs	660 (71.5)
ACE inhibitors (history)	595 (64.5)
Angiotensin receptor blockers	54 (5.9)
Calcium channel blockers	171 (18.5)
Diuretics	185 (20.0)
Oral hypoglycemic agents	218 (23.6)
Insulin	121 (13.1)
Hormone replacement therapy	62 (28.1)
Multivitamins	153 (16.6)
Physical exam (mean ± SD)	
Heart rate (beats/min)	65.3 ± 10.8
Systolic blood pressure (mmHg)	131.5 ± 16.1
Diastolic blood pressure (mmHg)	76.0 ± 9.3
Body mass index (kg/m <sup>2</sup> )	28.9 ± 4.7
Waist-to-hip ratio	0.90 ± 0.07
Laboratory results (mean ± SD)	
Total cholesterol (mmol/l)	4.8 ± 0.9
LDL-cholesterol (mmol/l)	2.7 ± 0.8
HDL-cholesterol (mmol/l)	1.2 ± 0.3
Triglycerides (mmol/l)	2.0 ± 1.2
Plasma glucose (mmol/l)	6.6 ± 2.5
Creatinine (μmol/l)	92.7 ± 30.8
hs-CRP (mg/l)	4.1 ± 7.1
Ultrasonographic measurements	
IMT, mean max, mm (mean ± SD)	1.26 ± 0.34
IMT, single max, mm (mean ± SD)	2.42 ± 0.83
Plaque calcification, % (95% CI)	27.8 (27.0–28.7)

CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; PTA, percutaneous transluminal angioplasty; ACE, angiotensin converting enzyme; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness.

$p < 0.01$ ) were modestly but significantly correlated to carotid IMT. Established risk factors such as LDL-cholesterol, HDL-cholesterol and triglycerides were not significantly associated with carotid IMT in univariate analysis, but 72% of the patients were on lipid-lowering therapy. There was no significant association between hs-CRP and mean max carotid IMT.

In multivariable analysis, traditional risk factors such as age ( $p < 0.0001$ ), sex ( $p < 0.0001$ ), history of smoking ( $p < 0.0001$ ), systolic blood pressure ( $p < 0.0001$ ), body mass index ( $p < 0.01$ ) and triglycerides ( $p < 0.001$ ) were all independently associated with mean max carotid IMT and accounted collectively for approximately 20% of the variability in carotid IMT.

**Table 2** Mean and single maximum carotid IMT in males and females and in patients with selected cardiovascular risk factors

	Mean max carotid IMT (mm)	<i>p</i>	Single max carotid IMT (mm)	<i>p</i>
Males	1.25 (1.23–1.28)	<0.001	2.35 (2.29–2.41)	<0.0001
Females	1.13 (1.10–1.17)		2.08 (1.99–2.17)	
Diabetes	1.22 (1.19–1.26)	0.86	2.24 (2.16–2.33)	0.33
No diabetes	1.23 (1.20–1.25)		2.30 (2.23–2.36)	
Hypertension	1.25 (1.22–1.27)	0.005	2.33 (2.27–2.39)	0.01
No hypertension	1.19 (1.15–1.22)		2.20 (2.12–2.28)	
Current smoker	1.33 (1.26–1.40)	0.003	2.40 (2.22–2.57)	0.19
Not current smoker	1.21 (1.19–1.24)		2.27 (2.22–2.32)	
Ever smoked	1.25 (1.23–1.27)	0.0001	2.30 (2.24–2.36)	0.13
Never smoked	1.16 (1.12–1.20)		2.21 (2.12–2.31)	

All IMT values are mean (95% CI), mm.

The *p*-values denote differences between groups.

**Table 3** Percent plaque calcification in males and females and in patients with selected cardiovascular risk factors

	Plaque calcification (%)	<i>p</i>
Males	28.2 (27.3–29.2)	0.088
Females	26.5 (24.8–28.3)	
Diabetes	28.0 (26.6–29.5)	0.713
No diabetes	27.7 (26.6–28.8)	
Hypertension	28.9 (27.8–30.0)	0.002
No hypertension	26.0 (24.6–27.4)	
Current smoker	31.6 (28.7–34.7)	0.008
Not current smoker	27.4 (26.6–28.4)	
Ever smoked	28.6 (27.6–29.6)	0.002
Never smoked	25.5 (23.9–27.2)	

Values are mean (95% CI).

The *p*-values denote differences between groups.

### Association between homocysteine and carotid IMT

Mean tHcy levels were elevated,  $12.1 \pm 5.4 \mu\text{mol/l}$ . In univariate analyses, tHcy correlated significantly with both single max ( $r = 0.12$ ;  $p < 0.001$ ) and mean max ( $r = 0.13$ ;  $p < 0.001$ ) carotid IMT. There was a significant and gradual increase in both mean and single max carotid IMT ( $p < 0.0001$  for both variables) across quartiles of tHcy (Table 4 and Figure 1). However, after age and sex adjustment and in multivariable analysis, the association was no longer significant (Table 4). When evaluating men and women separately, there were significant univariate associations between tHcy and mean max ( $p < 0.0001$ ) and single max ( $p < 0.01$ ) carotid IMT in men, but after age adjustment the relationship was no longer significant. In women, no significant associations between tHcy and carotid IMT

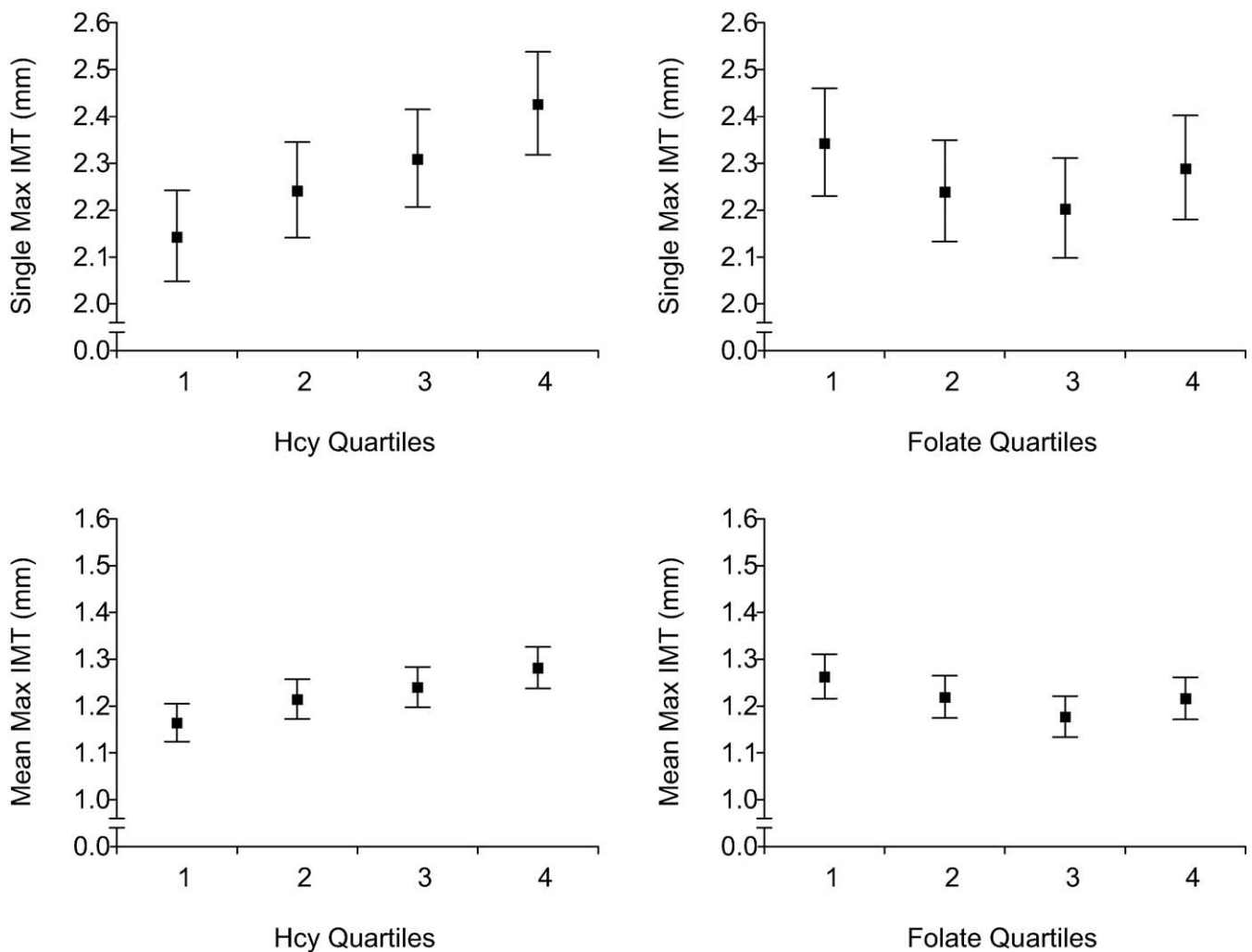
**Table 4** Carotid IMT and percent plaque calcification by quartile of plasma Hcy and folate concentration

Quartiles of Hcy ( $\mu\text{mol/l}$ )	Q1	Q2	Q3	Q4	<i>p</i> for trend	<i>p</i> age and sex adjusted	<i>p</i> multivariable
	< 9.4	9.4–11.2	11.2–13.4	> 13.4			
Mean max carotid IMT (mm)	1.16	1.21	1.24	1.28	<0.0001	0.17	0.22
Single max carotid IMT (mm)	2.14	2.24	2.31	2.43	<0.0001	0.10	0.11
Carotid plaque calcification (%)	24.0	27.0	28.8	30.8	<0.0001	<0.01	<0.01
Quartiles of folate (nmol/l)	Q1	Q2	Q3	Q4	<i>p</i> for trend	<i>p</i> age and sex adjusted	<i>p</i> multivariable
	< 23.0	23.0–28.5	28.5–39.4	> 39.4			
Mean max carotid IMT (mm)	1.26	1.22	1.18	1.22	0.08	0.12	0.05
Single max carotid IMT (mm)	2.34	2.24	2.20	2.29	0.43	0.64	0.33
Carotid plaque calcification (%)	30.2	26.5	26.4	26.9	<0.05	<0.05	<0.05

Values are means for each quartile.

The *p*-values denote trend across quartiles.

Variables with a *p*-value <0.1 in univariate analyses were tested in multivariable models.



**Figure 1** Mean maximum carotid IMT and single maximum carotid IMT across quartiles of plasma total homocysteine and plasma folate concentrations.

were found, although the statistical power of this analysis was lower.

#### Associations between homocysteine and plaque calcification

The plaque calcification score increased gradually across quartiles of tHcy ( $p < 0.0001$  for trend; Figure 2). This association remained significant after adjustment for age and sex ( $p < 0.01$ ) and in multivariable analysis ( $p < 0.01$ ).

#### Association between plasma folate levels and carotid IMT

The mean baseline folate concentration was  $30.4 \pm 9.9$  nmol/l. There was a trend towards a gradual decrease in mean max carotid IMT across quartiles of plasma folate in both univariate and multivariable analyses. The association was observed from the lowest to the third fourth of the folate distribution. There was no clear relationship to carotid mean max IMT in the highest fourth of the folate

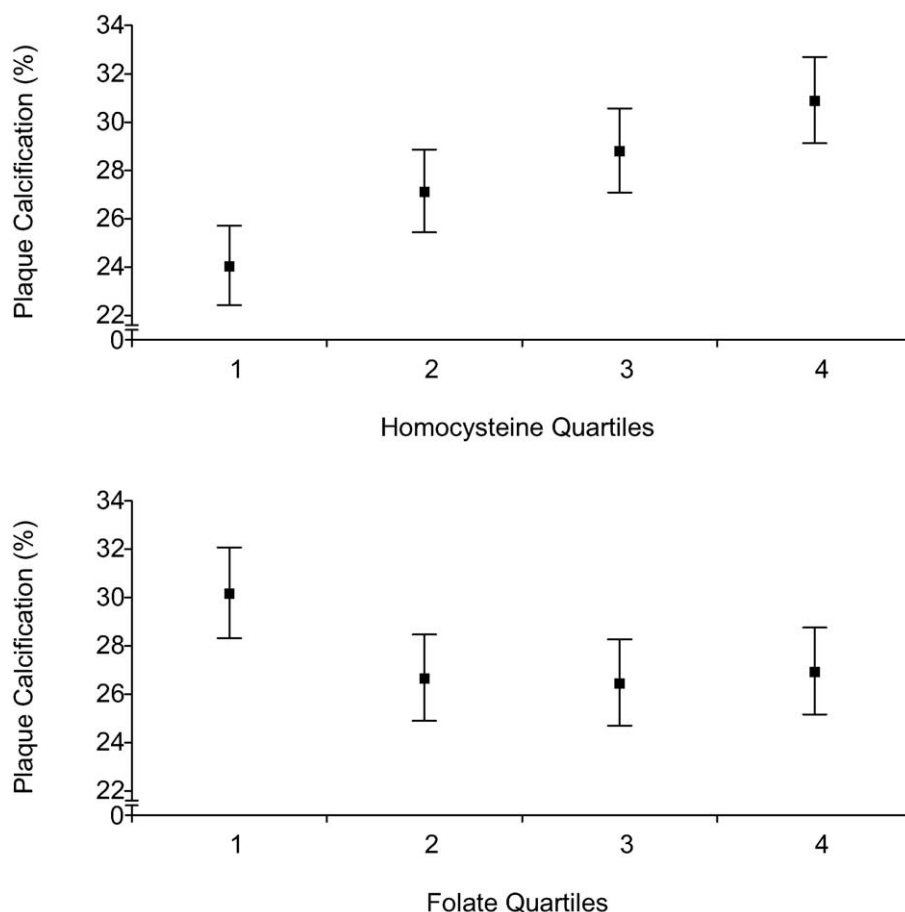
distribution (Table 4 and Figure 1). No significant association between folate concentration and single max IMT was noted.

#### Association between folate and plaque calcification

The plaque calcification score decreased gradually across quartiles of folate ( $p < 0.05$  for trend; Figure 2 and Table 4). This relationship remained statistically significant after age and sex adjustment ( $p < 0.05$  for trend) and in multivariable analysis ( $p < 0.05$  for trend; Table 4).

#### Associations between vitamin B6 and B12 levels and carotid atherosclerosis

Mean baseline vitamin B6 and B12 concentrations were  $65.8 \pm 81.2$  nmol/l and  $310 \pm 144$  pmol/l, respectively. There were no significant correlations between the concentrations of vitamins B6 and B12 and carotid IMT (mean max carotid IMT or single max carotid IMT) or plaque calcification score (data not shown).



**Figure 2** Carotid artery plaque calcification score (%) across quartiles of plasma total homocysteine ( $p < 0.01$  for trend in multivariable analysis) and plasma folate concentrations ( $p < 0.05$  for trend in multivariable analysis).

## Discussion

We report associations between tHcy, folate and vitamins B6 and B12 concentrations and carotid atherosclerosis in a large cohort of patients at high risk for CV events. We observed a gradual and consistent increase in mean and single max carotid IMT across quartiles of tHcy. However, this relationship was weak and not significant after adjusting for age, sex and other traditional risk factors. There was also a trend towards an inverse relationship between plasma folate concentration to mean max carotid IMT, although this association did not reach statistical significance. In contrast to the associations with IMT, both tHcy and low folate concentrations were independently related to carotid plaque calcification across quartiles, even after accounting for other CV risk factors in multivariable analyses.

Our findings are consistent with other studies in patients with established vascular disease, which in general showed weak associations between tHcy levels and carotid IMT, as recently reviewed,<sup>16</sup> although these studies were smaller. Observational studies on other high-risk individuals, such as those with uremia, hypercholesterolemia, type 2 diabetes,

insulin resistance, immune disorders or neurological abnormalities have generally failed to demonstrate consistent associations between tHcy levels and carotid IMT. In a previous large study on individuals at higher than average coronary heart disease (CHD) risk, which enrolled siblings of CHD patients and other high-risk individuals, a significant increase in carotid IMT across quintiles of tHcy was reported. This association was weak and significant only in those  $> 55$  years.<sup>20</sup> Similarly, in a large cross-sectional study in a general population with mildly elevated tHcy levels, a significant but weak correlation between tHcy and carotid IMT was seen.<sup>21</sup>

Whether raised homocysteine concentrations may cause ischemic heart disease and stroke is still under debate.<sup>22</sup> In our study, the association between tHcy and low folate and plaque calcification score was more robust than the association with carotid IMT. Calcification of plaques is a result of increased atherosclerotic disease burden and more advanced disease. However, studies evaluating the clinical importance of plaque calcification as a marker of risk for CV events have been inconsistent and controversial.<sup>23,24</sup>



We observed trends towards an inverse association to carotid IMT. A previous study<sup>17</sup> suggested that this association was only seen at very low serum folate levels. Our study was initiated after the legislated implementation of food supplementation with folic acid in Canada<sup>25,26</sup> and indeed most patients in our study had normal plasma folate levels. Thus, in folate-replete individuals with vascular disease, as represented by our population, serum folate levels may not be predictive of carotid IMT measurements. However, low folate concentration was more strongly related to plaque calcification, as was also found for tHcy.

There were no significant associations between plasma vitamin B6 and B12 levels and either carotid IMT or plaque calcification in our population. This observation is interesting in the light of recent data, suggesting a modest effect of vitamin B6 and B12 supplementation on carotid IMT levels in vascular disease<sup>27</sup> and renal transplant patients.<sup>28</sup> Our patients had normal baseline vitamin B6 and B12 concentrations. It is possible that vitamin B6 and B12 supplementation affects carotid IMT only in patients who have B vitamin deficiencies.

While recent randomized controlled clinical trials have failed to identify a reduction in major vascular events with tHcy-lowering B vitamins,<sup>11–14</sup> homocysteine lowering requires further study, particularly with regards to stroke. There still remains a debate on the interpretation of available epidemiological data and the trial results,<sup>22</sup> but in a late meta-analysis<sup>29</sup> there was no significant benefit of folic acid supplementation on the risk of CHD. The epidemiological association between tHcy and stroke is stronger than for coronary heart disease.<sup>6,7</sup> Also, a revised analysis of the VISP trial suggested a possible treatment benefit in subsets of people at high risk of stroke with B12 vitamin abnormalities<sup>30,31</sup> and in the HOPE-2 trial tHcy lowering reduced stroke rates. A recent meta-analysis on eight trials<sup>32</sup> concluded an 18% reduction in the risk of stroke. Therefore, the HART trial remains relevant and is expected to provide additional important information on the effects of tHcy lowering on carotid atherosclerosis.

In conclusion, in this population of patients at high CV risk, tHcy concentration was only weakly associated with carotid IMT. In contrast, tHcy was independently associated to plaque calcification, a measure of more advanced atherosclerosis. Although plasma folate and B vitamin levels were predictive of tHcy levels, there was no clear and consistent relationship demonstrated between plasma levels of folate, vitamins B6 and B12 and carotid IMT. However, low levels of folate were independent predictors of plaque calcification. Overall, associations between both Hcy and folate to plaque calcification were stronger than to carotid

IMT, which may have clinical importance since this parameter may be a better marker of advanced atherosclerosis in this population.

The HART study will evaluate the effects of tHcy-lowering therapy with combined folic acid 2.5 mg and vitamins B6 50 mg and B12 1 mg administered as a combined pill once daily for an average of 5 years versus matching placebo on the progression of carotid IMT and the plaque calcification score. The associations between tHcy and folate and carotid atherosclerosis and the awaited treatment results of the HART trial are highly relevant, considering the results on stroke in the HOPE-2 trial, which are in contradistinction with the neutral results of the trial on the main primary outcome, and the results of the VISP and NORVIT trials.

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