

# Effect of homocysteine-lowering treatment with folic acid plus vitamin B<sub>6</sub> on cerebrovascular atherosclerosis and white matter abnormalities as determined by MRA and MRI: a placebo-controlled, randomized trial

E. G. J. Vermeulen, C. D. A. Stehouwer, J. Valk, M. van der Knaap, M. van den Berg, J. W. R. Twisk\*, W. Prevoe and J. A. Rauwerda

‘Vrije Universiteit’ Medical Center (VUMC), Institute for Cardiovascular Research (IcaR-VU), \*Institute for Research in Extramural Medicine (EMGO), Amsterdam, the Netherlands

## Abstract

**Background** A high plasma homocysteine concentration is an independent risk factor for large and possibly small vessel disease. We investigated the effects of homocysteine-lowering treatment with folic acid plus vitamin B<sub>6</sub> on markers of cerebrovascular atherosclerosis and cerebral microangiopathy.

**Materials and Methods** Using 158 healthy siblings (mean age 46.0 ± 7.6 years) of patients with premature atherosclerotic disease, we performed a randomized, placebo-controlled trial using 5 mg of folic acid plus 250 mg of vitamin B<sub>6</sub> daily (*n* = 78) or placebo medication (*n* = 80). Participants were followed for 2 years with magnetic resonance angiography (MRA) (carotid stenosis; carotid and/or vertebral elongation) and magnetic resonance imaging (MRI) (white matter abnormalities; cerebral atrophy).

**Results** Seventeen (10.8%) subjects refused MRA/MRI owing to claustrophobia and were excluded. From the remaining 141 participants, 68 received vitamin and 73 received placebo medication [42 (61.8%) and 48 (65.8%) had postmethionine hyperhomocysteinaemia, respectively]. Twenty-four participants (15.2%; 10 in the treatment and 14 in the placebo group) did not complete both years of the trial. Vitamin treatment was associated with an increase in plasma folate (13-fold vs. placebo; *P* < 0.001) and vitamin B<sub>6</sub> (8.8-fold; *P* < 0.001). Fasting and postmethionine total homocysteine concentrations decreased 38.7% (95% CI, 27.4–50.0) and 29.1% (95% CI, 19.2–39.0) vs. placebo (all *P* < 0.001). During follow up six individuals in the vitamin-treated and 11 in the placebo-treated group deteriorated in their outcome measurements. Vitamin treatment, as compared with placebo, was associated with nonsignificantly improved outcomes on both MRA and MRI outcome measurements (odds ratio 0.48; 95% CI 0.17–1.41; *P* = 0.18 and 0.48; CI 0.14–1.60; *P* = 0.23, respectively).

**Conclusions** These results could indicate a possible favourable effect of homocysteine-lowering treatment on cerebrovascular atherosclerosis and cerebral microangiopathy among healthy siblings of patients with premature atherosclerotic disease, but larger trials are required to establish this with certainty.

**Keywords** Cerebral atherosclerosis, folic acid, homocysteine, MRI, Vitamin B<sub>6</sub>.  
*Eur J Clin Invest* 2004; 34 (4): 256–261

Departments of Vascular Surgery (E. G. J. Vermeulen, J. A. Rauwerda, M. van den Berg), Internal Medicine (C. D. A. Stehouwer), Radiology (J. Valk, W. Prevoe) and Neurology (M. van der Knaap), and the Institute for Cardiovascular Research (C. D. A. Stehouwer, J. A. Rauwerda), ‘Vrije Universiteit’ Medical Center; Institute for Research in Extramural Medicine (J. W. R. Twisk), Amsterdam, the Netherlands.

Correspondence to: Dr E. G. J. Vermeulen, Department of Vascular Surgery; Gelre Hospitals Apeldoorn, PO Box 9014, 7300 DS Apeldoorn, the Netherlands. Tel.: +31–55–5818181; fax: +31–55–5818131; e-mail: EGJ.Vermeulen@gelre.nl

Received 8 January 2004; accepted 15 February 2004

## Introduction

A high plasma total homocysteine concentration is an independent risk factor for stroke, cerebrovascular atherosclerosis and cerebral microangiopathy [1–5].

Total homocysteine concentrations can effectively be lowered by 30–50% using folic acid, vitamin B<sub>6</sub> and/or B<sub>12</sub> [6–9]. Prospective, nonrandomized cohort studies have suggested that such homocysteine-lowering treatment may have beneficial effects on the progression of atherosclerosis, including cerebrovascular atherosclerosis [7,10–13].

Recently we have reported a randomized trial on the effects of homocysteine-lowering treatment with folic acid plus vitamin B<sub>6</sub> on surrogate markers of subclinical atherosclerosis [6]. We here report on the effects on progression of cerebrovascular atherosclerosis and cerebral microangiopathy as estimated by magnetic resonance angiography (MRA) and magnetic resonance imaging (MRI).

## Subjects and methods

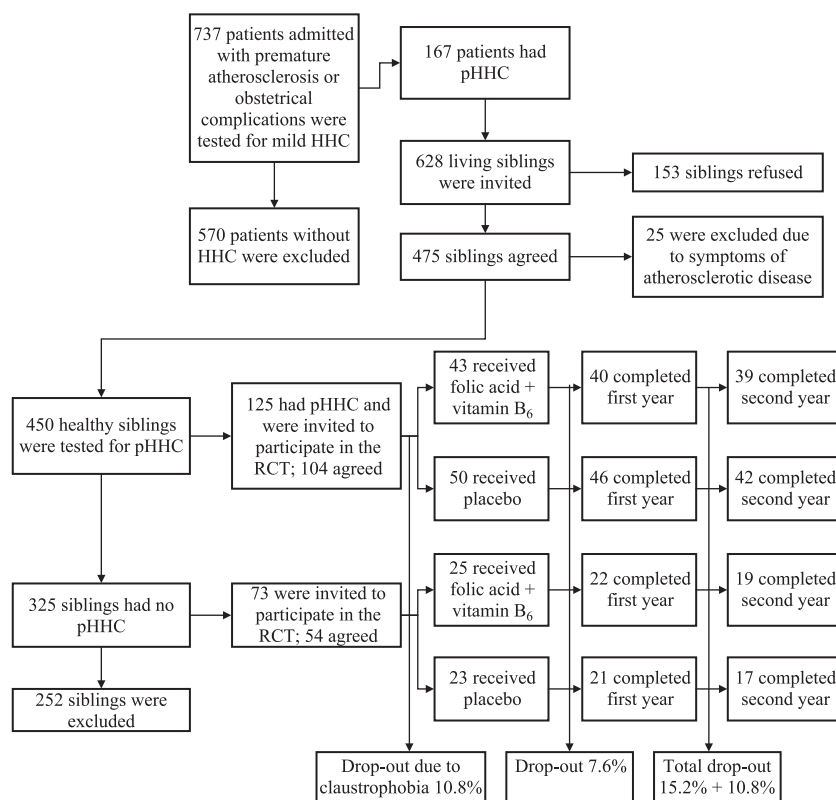
### Study design

After selection, as described in detail elsewhere [6], 158 healthy asymptomatic siblings [18 > age < 56 years; 104 with postmethionine hyper- and 54 with normohomocysteinaemia) of patients with clinical atherosclerotic disease

(i.e. index patient) met the inclusion criteria of the study. Participants were stratified according to family of origin ( $n = 66$ ) and the presence of postmethionine hyperhomocysteinaemia. Within each stratum, subjects were randomized by random digit sampling using a computer. Treatment assignment was generated by the study statistician. Reference values (i.e., values  $\leq 2SD$  greater than the mean of apparently healthy control individuals) for fasting postmethionine total homocysteine in our laboratory were less than 18  $\mu\text{mol L}^{-1}$  and less than 54  $\mu\text{mol L}^{-1}$  in men, less than 15  $\mu\text{mol L}^{-1}$  and less than 51  $\mu\text{mol L}^{-1}$  in premenopausal women, and less than 19  $\mu\text{mol L}^{-1}$  and less than 69  $\mu\text{mol L}^{-1}$  in postmenopausal women, respectively.

All participants were then treated orally with folic acid (5 mg daily) plus vitamin B<sub>6</sub> (250 mg daily) or placebo medication for 2 years in double-blind fashion (Figure 1). The hospital pharmacist prepared placebo and vitamin capsules to have the same appearance and taste, and executed the treatment assignment. Additional vitamin intake as food supplements was discouraged in all participants. At baseline and after 1 and 2 years, we collected demographic and clinical data and performed a MRA of the carotid and vertebral arteries to determine the extent of cerebrovascular atherosclerosis, and a MRI of the brain as a marker of the extent of cerebral microangiopathy (see below). Methionine-loading tests were performed at baseline and after 20 months (median).

The trial was approved by the Ethical Review Committee of the University Hospital 'Vrije Universiteit' and informed consent was obtained from all participants.



**Figure 1** Trial profile. HHC = hyperhomocysteinaemia; pHHC = postmethionine hyperhomocysteinaemia; RCT = randomized clinical trial.

### General demographic, clinical and laboratory data

These were recorded as described in details elsewhere [6].

### MRA and MRI outcome variables

Imaging was performed with a Siemens 1.5 Tesla Vision Scanner (Siemens AG, Erlangen, Germany), and included axial dual-echo spin-echo images (4000/19 & 93 or 16 & 98/1 [TR/TE/excitations]), with 5-mm slices and a 1-mm in-plane resolution. The MRA consisted of 3D time-of-flight (TOF) gradient echo sequences, with both a single thick slab and a so-called Multi Overlapping Thin Slab Acquisition (MOTSA) technique. The MOTSA sequence was performed with 29–35/6–7/1 (TR/TE/excitations; depending on the use of a magnetization transfer [MT] pulse, which was applied after the scanner upgrade), and a flip angle of 20–25 degrees, using 3–7 slabs, 1-mm effective slice thickness and a 1-mm in-plane resolution. The single-slab 3D TOF technique (33/7–8/1; flip-angle 20 degrees, no MT) used a slab thickness of 75 mm, an effective slice thickness of 1 mm, and a 0.5 × 1-mm in-plane resolution.

The consecutive MRA and MRI scans of each individual were independently assessed by two radiologists unaware of clinical data and treatment allocation, according to a prefixed protocol.

The MRA protocol classified the degree of stenosis of the carotid bifurcation into five grades (none, 0% to 30%, 30% to 70%, 70% to 99% or occlusion). Percent stenosis was computed by measuring the residual lumen diameter and the original diameter at the site of the maximal stenosis, and dividing the difference of the two by the latter (analogous to European Carotid Surgery Trial criteria) [14]. A change of stenosis was defined as an increase or decrease of at least one grade on either side. In addition, the degree of tortuosity (elongation) of the carotid and vertebral arteries was graded as straight, curve > 90°, curve > 180° or multiple curves. Progression or regression was defined as a change of tortuosity of at least one grade on either side. At 1 and 2 years the changes of grades of all arteries in each individual were summed and scored as unchanged, progression or regression and further evaluated.

For assessing microangiopathic changes a MRI protocol was used analogous to other studies [15,16]. Briefly, distinction was made between white matter hyperintensities directly adjacent to the ventricles (periventricular lesions) and punctate or confluent white matter hyperintensities at some distance from the ventricles (focal lesions). Small caps on the horns of the lateral ventricles and pencil-thin lining around the ventricles were considered normal. Lesions appearing as lacunar infarctions were not included in the study. On the basis of these criteria the total number of white matter lesions was determined. Compared with baseline, individual changes after 1 and 2 years were expressed as unchanged or increased (no decrease was noted). This dichotomized parameter of the MRI measurements was used for analysis.

Inter-observer agreement was 97% for the MRI and 98% for the MRA investigations. After further review,

discrepancies were easily resolved, and agreement was 100%.

### Statistics

Descriptive data are given as number (%), as mean ± SD or as median [interquartile range]. Skewed data were logarithmically transformed before analysis. Continuous variables were tested by a Student's *t*-test (for means) and percentages by Chi-square tests. Generalized estimating equations [17] were used to analyze the influence of therapy with folic acid plus vitamin B<sub>6</sub> on the longitudinal development of the outcome variables. In all analyses, a 5% significance level was used. All generalized estimating equations analyses were carried out with the Statistical Package for Interactive Data Analysis (version 6).

Analyses were performed on a per-protocol basis after exclusion of the claustrophobic participants. Initial analyses indicated that the baseline postmethionine total homocysteine concentration did not importantly affect the effect of placebo/active treatment on the main outcome variables, and therefore subjects receiving placebo and subjects receiving active treatment were pooled (Fig. 1). Subsequently, we compared the main outcome variables between the placebo- and the vitamin-treated groups, both without further adjustments (model 1) and with adjustments for age, sex and baseline postmethionine total homocysteine concentration (model 2); for the variables in model 2 plus presence of hypertension and diabetes mellitus, smoking habits and total cholesterol:HDL-cholesterol ratio (model 3); and for the variables in model 3 plus baseline concentrations of folic acid, vitamin B<sub>6</sub> and fasting homocysteine. We further assessed the influence of baseline postmethionine hyperhomocysteinaemia by adding an interaction term (with treatment) to the above-mentioned models.

This study had 80% power to detect a 5.5% point difference between the treatment and placebo groups with regard to dichotomous variables.

### Results

Table 1 shows that the vitamin-treated and placebo-treated groups were comparable with regard to homocysteine and vitamin status and other clinical variables. The median time from baseline to the first examination and from the first to the second examination was 13 months in both cases.

Seventeen (10.8%; 10 in the active treatment and seven in the placebo group) individuals continued other trial investigations [6], but refused magnetic resonance imaging owing to claustrophobia. Of the remaining 141 participants, a further 24 (15.2%) did not complete the trial (Fig. 1). These subjects declined further participation for the following reasons: development of colon malignancy (*n* = 3), declined further contact (*n* = 5), anxiety (*n* = 2), general malaise (*n* = 1), prolonged hospital admittance for hip infection (*n* = 1) and trial investigations too time-consuming (*n* = 12). None of

**Table 1** Baseline clinical and laboratory characteristics

	Vitamin-treated ( <i>n</i> = 68)	Placebo-treated ( <i>n</i> = 73)
Age (years)	45.8 ± 7.0	46.2 ± 8.1
Gender: male/female	36/32	32/41
Fasting HHC, <i>n</i> (%)	16 (23.5)	19 (26.0)
Post-methionine HHC, <i>n</i> (%)	42 (61.8)	48 (65.8)
Smoking, <i>n</i> (%)	34 (50.0)	30 (41.1)
Diabetes, <i>n</i> (%)	0 (0)	1 (1.4)
Body mass index (kg m <sup>-2</sup> )	25.6 ± 3.7	25.1 ± 4.6
Hypertension, <i>n</i> (%)	11 (16.2)	13 (17.8)
Systolic blood pressure (mmHg)	129.5 ± 17.6	134.1 ± 19.6
Diastolic blood pressure (mmHg)	82.6 ± 8.9	84.2 ± 9.1
Total cholesterol (mmol L <sup>-1</sup> )	5.9 ± 1.4	5.8 ± 1.2
Triglycerides (mmol L <sup>-1</sup> )	1.5 ± 0.7	1.5 ± 1.1
HDL-cholesterol (mmol L <sup>-1</sup> )	1.2 ± 0.3	1.3 ± 0.4
LDL-cholesterol (mmol L <sup>-1</sup> )*	4.2 ± 1.1	3.9 ± 1.1
Total cholesterol: HDL ratio	5.2 ± 1.9	4.9 ± 1.8
Lipoprotein (a) (mg L <sup>-1</sup> )	115 [35–312]	119 [29–324]

Data are given as number (percentage), as mean ± SD or as median [interquartile range].

HHC = hyperhomocysteinaemia as defined in Methods.

\*Calculated by Friedewald's formula.

**Table 2** Homocysteine and vitamin concentrations at baseline and after treatment

	Vitamin-treated ( <i>n</i> = 68)	Placebo-treated ( <i>n</i> = 73)	<i>P</i> -value
Fasting tHcy (μmol L <sup>-1</sup> )			
Baseline	14.1 ± 7.6	14.9 ± 9.1	
At 20 months	7.3 ± 1.9*	11.9 ± 5.3	< 0.001
Post-methionine load tHcy (μmol L <sup>-1</sup> )			
Baseline	63.5 ± 27.2	63.8 ± 25.4	
At 20 months	35.1 ± 11.2*	49.5 ± 17.2	< 0.001
Delta tHcy <sup>†</sup>			
Baseline	49.4 ± 25.0	48.9 ± 22.4	
At 20 months	27.8 ± 10.9*	37.6 ± 16.3	< 0.001
Plasma folate (nmol L <sup>-1</sup> )			
Baseline	12.7 ± 10.6	12.5 ± 4.4	
1 year	160.4 ± 207.8*	12.2 ± 5.9	< 0.001
2 years	218.5 ± 313.4*	30.0 ± 127.9	< 0.001
Plasma vitamin B <sub>6</sub> (nmol L <sup>-1</sup> )			
Baseline	44.7 ± 57.1	39.7 ± 51.9	
1 year	409.5 ± 252.5*	46.1 ± 42.8**	< 0.001
2 years	420.2 ± 216.8*	64.5 ± 96.0**	< 0.001

\**P* < 0.001 and \*\**P* < 0.01 vs. baseline value.

<sup>†</sup>Difference between postmethionine and fasting total homocysteine concentrations.

All other comparisons with baseline had *P* > 0.10.

tHcy = total homocysteine concentrations.

the participants developed clinical complaints of impaired cerebral circulation and treatment was well-tolerated.

Table 2 shows that at follow up vitamin treatment was associated with an increase in plasma folate (12.6-fold vs. baseline and 13-fold vs. placebo; *P* < 0.001) and in plasma vitamin B<sub>6</sub> (9.2-fold and 8.8-fold; *P* < 0.001). Fasting and postmethionine total homocysteine concentrations decreased

48.2% and 44.7% vs. baseline, and 38.7% (95% CI, 27.4–50.0) and 29.1% (95% CI, 19.2–39.0) vs. placebo (all *P* < 0.001). Compliance with vitamin treatment, as defined in Subjects and Methods, was 100%. Compliance with placebo medication was 100% at 1 year, but 95.0% at 2 years, probably as a result of the additional vitamin use in the placebo-treated group.

Table 3 shows the number of abnormal MRA and MRI outcome measurements. During follow up the MRA score deteriorated in six individuals in the vitamin-treated group and remained unchanged in 57, vs. 11 and 52 in the placebo-treated group. The MRI score deteriorated in six individuals in the vitamin-treated group and remained unchanged in 57, vs. 11 and 52 in the placebo-treated group.

Table 4 shows the analyses of the outcome variables. Vitamin treatment, as compared with placebo, was associated with a nonsignificantly improved outcome with regard to the MRA score (odds ratio 0.48; 95% CI 0.17–1.41; *P* = 0.18) and the MRI score (OR 0.48; CI 0.14–1.60; *P* = 0.23). Results were similar when adjusted for different covariates (models 2–4). Further adjustments for concentrations of lipoprotein (a) and the type of vascular disease of the index patient did not affect the outcomes (data not shown).

Analysis with an interaction term (treatment × presence of baseline postmethionine hyperhomocysteinaemia) was not different from those without an interaction term. The interaction term was not statistically significant in any of the analyses (data not shown).

## Discussion

In this 2-year trial, homocysteine-lowering treatment with folic acid plus vitamin B<sub>6</sub>, compared with placebo, was associated with a nonsignificantly improved outcome in MRA

**Table 3** Number of participants with abnormal test results by treatment group

	Sum of abnormal test results on MRA								Number of white matter abnormalities on MRI								
	0	1	2	3	4	5	6	11	0	1	2	3	4	5	7	10–20	> 20
<b>Therapy</b>																	
Baseline	51	7	3	2	3		1	1	54	7	3				1		3
1 year	46	4	5	2	3		1	1	51	5	3				1		3
2 years	44	2	3	4	3		1	1	45	6	4				1		3
<b>Placebo</b>																	
Baseline	51	6	8	3	3	2			61	7	2	2			1		
1 year	41	9	5	2	4	2			47	9	4	2					2
2 years	37	5	8	3	3	2			44	8	3		1	1			2

Each number represents the number of participants with that specific sum of points of abnormal test results (see Subjects and Methods). Numbers are not shown if not present for the participants.

**Table 4** Effect of 2-year treatment with folic acid and vitamin B<sub>6</sub> on the outcome variables of 141 healthy individuals

	OR	95% CI	P-value
Sum of MRA outcome measurements (MRA score)			
Model 1	0.48	0.17–1.41	P = 0.18
Model 2	0.57	0.19–1.73	P = 0.32
Model 3	0.60	0.22–1.65	P = 0.32
Model 4	0.58	0.21–1.62	P = 0.30
Sum of MRI outcome measurements (white matter lesions; MRI score)			
Model 1	0.48	0.14–1.60	P = 0.23
Model 2	0.53	0.16–1.81	P = 0.32
Model 3	0.56	0.16–2.00	P = 0.37
Model 4	0.52	0.15–1.77	P = 0.30

OR = odds ratio; 95% CI = 95% confidence interval.

Model 1 = without adjustments; model 2 = model 1 plus adjustments for age, sex and baseline postmethionine total homocysteine concentration; model 3 = model 2 plus adjustments for presence of hypertension and diabetes mellitus, smoking habits and total cholesterol. HDL-cholesterol ratio; model 4 = model 3 plus adjustments for baseline concentrations of folic acid, vitamin B<sub>6</sub> and fasting homocysteine.

and MRI parameters (odds ratio 0.48; 95% CI 0.17–1.41; P = 0.18 and OR 0.48; CI 0.14–1.60; P = 0.23, respectively).

Elevated concentrations of homocysteine are known to be an independent risk factor for stroke and cerebral atherosclerosis [1–3,7]. There is also some evidence that elevated concentrations of homocysteine are associated with white matter abnormalities and/or cerebral microangiopathy [3,5,18,19], which may be analogous to the associations of high homocysteine levels with diabetic retinopathy [20,21] or nephropathy (microalbuminuria) [22,23], i.e. retinal and renal microangiopathy.

A MRA of the carotid artery and circle of Willis was performed with a 3D TOF technique. Compared with angiography, the sensitivity in the detection of carotid stenosis ranges from 92 to 100% with a specificity from 64 to 100% [24,25]. Absence of need of contrast is an obvious advantage, but overestimation of a stenosis because of signal loss, owing to slow flow and turbulence, remains a major pitfall.

This overestimation has to be taken into account in the clinical decision-making for individual patients, but is not important in a randomized trial. In the same trial, we have reported on the absence of effect of homocysteine-lowering treatment on the carotid arteries as determined with duplex scanning [6]. In the present study, most of the carotid stenoses observed with MRA were less than 50%, and in this range duplex scanning cannot be used reliably. This explains the different findings of the two research modalities used in both studies, although it must of course be noted that the present study did not show statistically significant differences either.

High-intensity signal abnormalities on magnetic imaging of the cerebrum, caused by sclerosis of small cerebral arteries and arterioles [26], are thought to be the neuroradiological substrate for microangiopathic changes [3,27–29], and their relationship with high homocysteine levels may be caused by mechanisms similar to those responsible for the development of diabetic retinopathy [20,21] or nephropathy (microalbuminuria) [22,23]. Our results provide the first (albeit inconclusive) evidence that homocysteine-lowering treatment may affect cerebral microangiopathy.

This trial was relatively small and of short duration. The study investigated surrogate markers of atherosclerotic and microvascular cerebral disease by magnetic resonance imaging. The drop-out, especially due to claustrophobia, was considerable, which further decreased the already limited power of the study to find significant differences between the groups. Although the demographic data show a successful randomization, there was a considerable difference in the number of carotid bifurcation stenosis present between the groups. However, as we measured progression or development of stenosis within each participant, this should not have affected the results. In favour of the trial is the high doses of vitamin B<sub>6</sub> plus folic acid given to maximally induce biologically meaningful effects. Furthermore, this vitamin treatment was well tolerated and compliance with treatment allocation was very high.

In this study, homocysteine-lowering treatment of healthy siblings of patients with premature atherosclerotic disease was associated with improved outcomes with regard to MRA and MRI measurements, which were not statistically

significant, but the point estimates suggest that such treatment effects may be substantial (OR 0.48; 95% CI 0.17–1.41;  $P = 0.18$  and OR 0.48; CI 0.14–1.60;  $P = 0.23$ , respectively). Our data thus provide a basis for larger trials of the effect of homocysteine-lowering treatment on both large and small vessel cerebral disease.

## Acknowledgements

This study was supported by the Dutch 'Praeventiefonds' (Prevention Fund), grant number 282272.

## References

- Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG *et al.* Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 1995;346:1395–8.
- Yoo JH, Chung CS, Kang SS. Relation of plasma homocyst(e)ine to cerebral infarction and cerebral atherosclerosis. *Stroke* 1998;29:2478–83.
- Evers S, Koch HG, Grottemeyer KH, Lange B, Deufel T, Ringelstein EB *et al.* Features, symptoms, and neurophysiological findings in stroke associated with hyperhomocysteinemia. *Arch Neurol* 1997;54:1276–82.
- Ducloux D, Chalopin JM. Homocysteine in cerebral macroangiopathy and microangiopathy. *Lancet* 1999;354:1029–30.
- Fassbender K, Mielke O, Bertsch T, Nafe B, Froschen S, Hennerici M *et al.* Homocysteine in cerebral macroangiography and microangiopathy. *Lancet* 1999;353:1586–7.
- Vermeulen EGJ, Stehouwer CDA, Twisk JWR, van den Berg M, de Jong SC, Mackaay AJC *et al.* Effect of homocysteine-lowering treatment with folic acid plus vitamin B<sub>6</sub> on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. *Lancet* 2000;355:517–22.
- Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM *et al.* Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. *JAMA* 1997;277:1775–81.
- Olszewski AJ, Szostak WB, Bialkowska M, Rudnicki S, McCully KS. Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin in atherosclerosis. *Atherosclerosis* 1989;75:1–6.
- van den Berg M, Franken DG, Boers GH, Blom HJ, Jakobs C, Stehouwer CDA *et al.* Combined vitamin B<sub>6</sub> plus folic acid therapy in young patients with arteriosclerosis and hyperhomocysteinemia. *J Vasc Surg* 1994;20:933–40.
- Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049–57.
- Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D *et al.* A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA* 1992;268:877–81.
- Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M *et al.* Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;337:230–6.
- Peterson JC, Spence JD. Vitamins and progression of atherosclerosis in hyper-homocyst(e)inaemia. *Lancet* 1998;351:263.
- European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. *Lancet* 1991;337:1235–43.
- Sierra C, La SA, Mercader J, Gomez-Angelats E, Urbano-Marquez A, Coca A *et al.* Silent cerebral white matter lesions in middle-aged essential hypertensive patients. *J Hypertens* 2002;20:519–24.
- Breteler MM, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout HJ *et al.* Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology* 1994;44:1246–52.
- Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42:121–30.
- Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R *et al.* Homocysteine, silent brain infarcts, and white matter lesions. The Rotterdam Scan Study. *Ann Neurol* 2002;51:285–9.
- Hogervorst E, Ribeiro HM, Molyneux A, Budge M, Smith AD. Plasma homocysteine levels, cerebrovascular risk factors, and cerebral white matter changes (leukoaraiosis) in patients with Alzheimer disease. *Arch Neurol* 2002;59:787–93.
- Hoogeveen EK, Kostense PJ, Eysink PE, Polak BC, Beks PJ, Jakobs C *et al.* Hyperhomocysteinemia is associated with the presence of retinopathy in type 2 diabetes mellitus: the Hoorn study. *Arch Intern Med* 2000;160:2984–90.
- Vaccaro O, Ingrosso D, Rivellesse A, Greco G, Riccardi G. Moderate hyperhomocysteinemia and retinopathy in insulin-dependent diabetes. *Lancet* 1997;349:1102–3.
- Hoogeveen EK, Kostense PJ, Jager A, Heine RJ, Jakobs C, Bouter LM *et al.* Serum homocysteine level and protein intake are related to risk of microalbuminuria: the Hoorn Study. *Kidney Int* 1998;54:203–9.
- Jager A, Kostense PJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM *et al.* Serum homocysteine levels are associated with the development of (micro) albuminuria: the Hoorn study. *Arterioscler Thromb Vasc Biol* 2001;21:74–81.
- Anderson CM, Saloner D, Lee RE, Griswold VJ, Shapeero LG, Rapp JH *et al.* Assessment of carotid artery stenosis by MR angiography: comparison with X-ray angiography and color-coded Doppler ultrasound. *Am J Neuroradiol* 1992;13:989–1003.
- Litt AW, Eidelman EM, Pinto RS, Riles TS, McLachlan SJ, Schwartzberg S *et al.* Diagnosis of carotid artery stenosis: comparison of 2DFT time-of-flight MR angiography with contrast angiography in 50 patients. *AJNR Am J Neuroradiol* 1991;12:149–54.
- Ghika J, Bogousslavsky J. Pathophysiology, diagnosis and management. In: Ginsberg M, Bogousslavsky J, editors. *Subcortical Arteriosclerotic Encephalopathy (Binswanger's Disease)*. MA: Blackwell Malden;1998.pp.1755–71.
- Ludolph AC, Ullrich K, Bick U, Fahrenndorf G, Przyrembel H. Functional and morphological deficits in late-treated patients with homocystinuria: a clinical, electrophysiologic and MRI study. *Acta Neurol Scand* 1991;83:161–5.
- Mirowitz SA, Sartor K, Prenskey AJ, Gado M, Hodges FJ III. Neurodegenerative diseases of childhood: MR and CT evaluation. *J Comput Assist Tomogr* 1991;15:210–22.
- van den Berg M, van der Knaap MS, Boers GH, Stehouwer CDA, Rauwerda JA, Valk J *et al.* Hyperhomocysteinemia; with reference to its neuroradiological aspects. *Neuroradiology* 1995;37:403–11.