

Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia¹⁻³

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ABSTRACT

Background: Evidence supports an independent association between plasma total homocysteine concentrations and the risk of vascular disease. Recent epidemiologic studies reappraised the possibility that vascular risk factors might play a role in the pathogenesis not only of vascular dementia (VaD) but also of Alzheimer disease (AD).

Objective: The objective was to investigate the relations of mild cognitive impairment, AD, and VaD with blood homocysteine, folate, and vitamin B-12.

Design: The study population consisted of 314 consecutive subjects, 228 of whom were eligible for analyses. Plasma total homocysteine, serum folate, and serum vitamin B-12 concentrations were measured in 55 nondemented elderly control subjects, 81 mildly cognitively impaired subjects (Clinical Dementia Rating: 0.5), and 92 demented patients prevalently in a mild disease stage and with a clinical diagnosis of AD ($n = 74$) or VaD ($n = 18$).

Results: Subjects in the lowest folate tertile had significantly higher adjusted odds ratios (ORs) for mild cognitive impairment (OR: 3.1; 95% CI: 1.2, 8.1) and dementia (3.8; 1.3, 11.2). Hyperhomocysteinemia was significantly associated with dementia (adjusted OR: 4.3; 1.3, 14.7) and AD (adjusted OR: 3.7; 1.1, 13.1). In subjects with a Clinical Dementia Rating of 0.5, the mean (\pm SE) Mini-Mental State Examination score was significantly lower ($P < 0.05$) in the highest homocysteine tertile (24.5 ± 0.5) than in the lowest tertile (26.6 ± 0.5). No significant associations were found between minimum medial temporal lobe thickness or leukoaraiosis and any biochemical measure in the dementia and AD groups.

Conclusions: These findings suggest that relative folate deficiency may precede AD and VaD onset. Hyperhomocysteinemia might also be an early risk factor for cognitive decline in the elderly, but its role in dementia development must be addressed in future longitudinal studies. *Am J Clin Nutr* 2004;80:114–22.

KEY WORDS Folate, vitamin B-12, homocysteine, mild cognitive impairment, Alzheimer disease, vascular dementia

INTRODUCTION

In recent years, epidemiologic and neuropathologic evidence of an association between Alzheimer disease (AD) and indicators of atherosclerosis (1) or presence of brain infarcts (2) have again brought into question the direct or additional contribution of cerebrovascular abnormalities and lesions to cognitive impairment and to the pathogenesis and progression of AD. Major

difficulties in accurately diagnosing mixed forms of dementia in life (3–5) well illustrate how unclear the relation between AD and vascular pathologies still is. Putative vascular risk factors not only for vascular dementia (VaD) but also for AD have been investigated in several epidemiologic studies (6).

Elevated total homocysteine concentrations were first implicated in the pathogenesis of arteriosclerosis in 1969 by McCully (7), who described in a clinicopathologic study 2 children with homocystinuria, hyperhomocysteinemia, and extensive arterial damage. Over the next 2 decades, a large number of epidemiologic studies, mainly retrospective case-control and cross-sectional studies, have supported this hypothesis by showing a positive relation between homocysteine concentrations and the risk of cardiovascular, cerebrovascular, and peripheral vascular diseases (8). Recently, 2 meta-analyses of prospective observational studies reported significant, independent associations between elevated total homocysteine concentrations and ischemic heart disease or stroke, but reached different conclusions about the strength of the evidence: “strong” for Wald et al (9) while “at most modest” for the Homocysteine Studies Collaboration (10). Although basic research has proposed multiple mechanisms by which raised homocysteine concentrations could promote vascular damage, whether hyperhomocysteinemia is a risk factor for vascular disease or a consequence of tissue damage or repair is still a matter of discussion (9, 11–15).

The possible adverse effect of hyperhomocysteinemia has also been investigated in AD and VaD. Heterogeneity of design and difference in methodologic soundness aside, most cross-sectional studies have reported significantly higher plasma homocysteine concentrations in demented patients than in control

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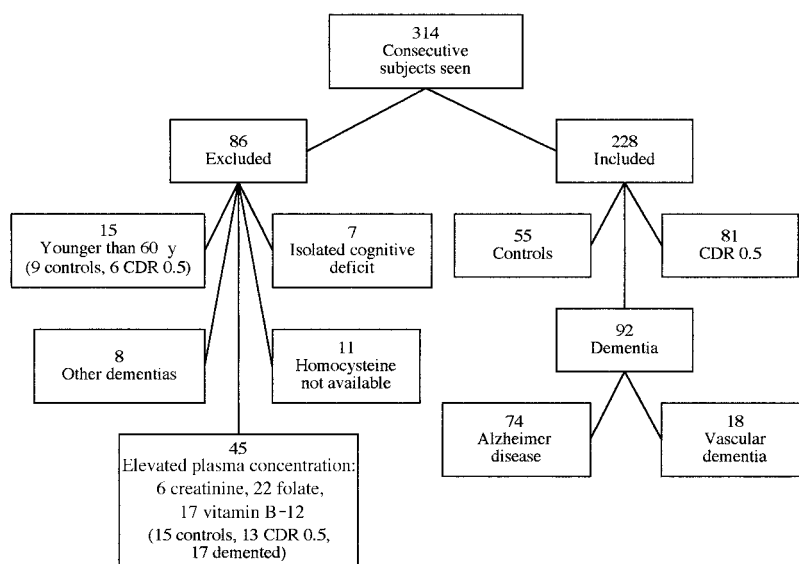


FIGURE 1. Study population: control subjects, subjects with Alzheimer disease, subjects with vascular dementia, and subjects with a Clinical Dementia Rating of 0.5 (CDR 0.5), which indicates mild cognitive impairment.

subjects (16–21), but a few found no significant association (22–25). Conflicting results were also reported in 2 longitudinal, observational studies with different outcomes: cognitive decline over time (26) and incident dementia or AD (27). B vitamins were also reported to be associated with cognitive variation in old age (28). Here too, whether high homocysteine and low B-vitamin concentrations have a causal role in the disease pathogenesis or are the consequences of an inadequate dietary intake secondary to the illness remains an open issue.

Absent from any alimentary source, homocysteine is a sulfur-containing amino acid produced by the demethylation of dietary methionine. Homocysteine is back-recycled into methionine through a remethylation pathway involving vitamin B-12 and folate as cofactors and cosubstrates. When methionine is in excess or cysteine is required, homocysteine is converted to cysteine via a pathway entailing vitamin B-6 as coenzyme, although a reduced activity of cystathionine β -synthase and the doubtful presence of γ -cystathionase in the brain (29) suggest “that, at best, this pathway is an inefficient means of disposing of homocysteine in the human brain” (30). Several determinants increase plasma total homocysteine concentrations: age, male sex, menopause, lifestyle, renal impairment, genetic defects, and many diseases and drugs (31). However, an inadequate dietary intake of nutritional factors such as B-vitamins has a major effect on homocysteinemia: some 65% of the hyperhomocysteinemia cases among the Framingham elderly population-based cohort could be accounted for by inadequate folate, or, to a lesser extent, inadequate vitamin B-12 or vitamin B-6 status and intake (32).

The current study aimed to examine the associations of plasma total homocysteine, serum folate, and vitamin B-12 concentrations with mild cognitive impairment, AD, and VaD, independently of other vascular and nonvascular risk factors. Aware of cross-sectional data limits, by investigating the preclinical and the early phases of dementia in a well-defined population of subjects, we tested the hypothesis that the rise in homocysteine or the reduction in folate or vitamin B-12 might already appear in the predementia phase. The relations between biochemical variables and neuroradiologic evidence of cortical atrophy and white

matter changes were used to inquire into the nature of the pathologic mechanisms underlying the aforementioned potential associations.

SUBJECTS AND METHODS

Subjects

The study population consisted of 314 consecutive subjects seen at the Memory Clinic of the Ospedale della Beata Vergine, Mendrisio, Switzerland. Subjects younger than 60 y ($n = 15$), with an isolated cognitive deficit ($n = 7$), affected by dementias other than AD or VaD ($n = 8$), or whose plasma total homocysteine concentrations were not available ($n = 11$) were not included. Because vitamin B-12 or folate supplementation reduces homocysteine concentrations and impaired renal metabolism increases them, subjects with serum concentrations of vitamin B-12 >600 pmol/L ($n = 17$), folate >30 nmol/L ($n = 22$), or creatinine >180 μ mol/L ($n = 6$) were also excluded (Figure 1).

The remaining 228 subjects were included in the analysis and, according to clinical diagnosis, were divided into 3 groups: 55 elderly control subjects free of cognitive impairment, 81 mildly cognitively impaired subjects, and 92 demented patients (Figure 1). This last group was made up of 74 patients who met the criteria of the National Institute of Neurological and Communicative Disorders–Alzheimer’s Disease and Related Disorders Association and of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) for probable or possible AD (33, 34) and 18 patients with a diagnosis of VaD based on CERAD criteria (1994) and a Hachinski Ischemic Score modified according to Loeb and Gandolfo (35) >4 . Mild cognitive impairment was defined clinically as a level of 0.5 on the Clinical Dementia Rating (CDR) scale (36). CDR 0.5 is a stage derived by published rules (37) indicating a condition of questionable dementia in which subjects exhibit a cognitive impairment insufficient to fulfill clinical criteria for dementia. Diagnoses were based on CERAD protocol, which includes a clinical history from both the subjects and a collateral source, physical and neurologic exam-

inations, neuropsychological assessment, laboratory tests, and computed tomography, magnetic resonance imaging scans of the brain, or both. Following the method described by Jobst et al (38), medial temporal lobe measurements by computed tomography were also made in 156 subjects (32 control subjects, 60 CDR 0.5 patients, 53 AD patients, and 11 VaD patients). Neuroradiologists were blind to the diagnoses and the clinical severity of the subjects.

Disease stage was rated by means of the CDR scale (36, 37), whereas the Mini-Mental State Examination (MMSE) (39)—a brief cognitive test widely used in clinical practice and epidemiologic studies—was administered to grade the subjects' global cognitive impairment. The study protocol was submitted to and approved by the local research ethics committee (Canton Ticino, Switzerland).

Laboratory measures

Nonfasting blood samples were collected from consenting patients into evacuated tubes containing EDTA, centrifuged within 10 min, and stored at or below -20°C until analyzed. Plasma total homocysteine concentrations were determined by using fluorescence polarization immunoassay technology (IMX System; Abbott Laboratories, Abbott Park, IL). The CV for this assay was 4.4% (normal values) and 2.2% (high values). Serum concentrations of folate and cyanocobalamin (vitamin B-12) were measured with a radioimmunoassay kit with the use of ^{57}Co and ^{125}I , respectively (SimulTRAC-SNB; ICN Pharmaceutical, Orangeburg, NY). Within-run CVs for these assays were 7.1% for serum folate and 12.3% for cyanocobalamin.

Statistical analyses

Baseline values were compared between control subjects and CDR 0.5 and dementia groups with analysis of variance followed by Dunnett's test or with a chi-square test followed by a Bonferroni-corrected pairwise comparison test. For these post hoc comparisons, the level of significance was set at $P < 0.05$. Because of the slightly skewed distribution of homocysteine, folate, vitamin B-12, and creatinine, these variables were log-transformed before analyses. The cumulative frequency distributions of total homocysteine and folate concentrations in CDR 0.5 and demented subjects were compared with those in control subjects by using the Kolmogorov-Smirnov test.

Univariate logistic regression analysis was used to estimate the crude odds ratios (ORs) of being in the questionable dementia, dementia, or AD group in the 3 tertile concentrations of vitamin B-12, folate, and total homocysteine. The tertiles were subdivided according to the distribution of concentrations of these biochemical variables in the control group. Reference tertiles were the top ones for vitamin B-12 and folate, and the bottom one was for total homocysteine. CDR 0.5, dementia, and AD groups were each compared against the control group in all analyses involving ORs.

Total cholesterol, systolic blood pressure, body mass index, creatinine, presence of hypertension (ie, a systolic blood pressure ≥ 160 mm Hg or a diastolic blood pressure ≥ 95 mm Hg, or the use of antihypertensive drugs) or diabetes, high alcohol intake, and smoking status were tried in multivariate logistic analyses, to

look for a possible effect on the relation between dementia, AD, and questionable dementia and total homocysteine, serum folate, and serum vitamin B-12. Of these potential confounding factors, only creatinine had an effect.

Multivariate logistic regressions—in which creatinine was entered together with age, sex, and education as covariates—were used to calculate the adjusted ORs of dementia, AD, and questionable dementia of the 3 putative risk factors. In a subsequent analysis, homocysteine, folate, and vitamin B-12 were put together in a single model. For all subjects whose apolipoprotein E genotyping was available ($n = 187$), this last logistic model was separately redone including this variable.

Linear correlations between the 3 log-transformed biochemical variables were assessed by using the Pearson r correlation coefficient. To study the associations between minimum medial temporal lobe thickness and vitamin B-12, folate, and total homocysteine concentrations, analysis of covariance was applied to test for differences among tertiles of each biochemical variable. Logistic regression was used to detect the presence of associations between leukoaraiosis and biochemical factors.

Because the few VaD patients showed characteristics either similar to or more pronounced than those shown by AD patients, comparisons between VaD and other groups were not subjected to formal statistical testing but were only descriptively inspected. All analyses were done by using SAS software (SAS Institute, Cary, NC).

RESULTS

Demographic, clinical, and biochemical characteristics of the 3 groups (control, mild cognitive impairment, and dementia) and 2 dementia subgroups (AD and VaD) into which the study population was divided are shown in **Table 1**. Groups were well balanced for demographic and clinical variables, although the demented patients were slightly older and less educated than were the control subjects. The vast majority of demented patients had mild disease severity: 78% had a CDR of 1, 20% had a CDR of 2, and only 2% had a CDR of 3.

The mean folate concentration was significantly lower in the CDR 0.5 group than in the control group ($P < 0.05$). The dementia group had significantly ($P < 0.05$) lower mean folate and higher mean homocysteine concentrations than did elderly control subjects, whereas no significant difference was found for vitamin B-12 concentrations. Also the proportion of subjects with a higher than normal homocysteine concentration (>14.6 $\mu\text{mol/L}$) was significantly ($P < 0.05$) greater in the dementia group than in the control subjects, with an increasing trend from elderly control (31%) to mildly cognitively impaired (42%) and to demented subjects (56%; 55% in the AD and 61% in the VaD subgroups).

The cumulative frequency distributions of total homocysteine and folate concentrations in the 3 groups are shown in **Figures 2** and **3**, respectively. Although the difference was greater for the distribution of folate concentrations, the cumulative frequency of both homocysteine and folate appeared distinctly disjointed in the control and dementia groups. In the CDR 0.5 group, the frequency distribution of folate grew together with that in the dementia group, whereas the frequency distribution of homocysteine—although tending to lie in between those in control and

TABLE 1
Demographic, clinical, and biochemical characteristics of the study groups¹

| | Control subjects (n = 55) | CDR 0.5 subjects (n = 81) | Demented subjects | | |
|--|------------------------------|------------------------------|----------------------------|----------------|-----------------|
| | | | All (AD + VaD) (n = 92) | AD (n = 74) | VaD (n = 18) |
| Demographic characteristics | | | | | |
| Age (y) ² | 75.6 ± 8.5 ³ | 76.1 ± 7.1 | 79.5 ± 7.4 ⁴ | 79.1 ± 7.7 | 80.5 ± 5.7 |
| Sex (% males) | 38.2 | 40.7 | 35.2 | 34.3 | 38.9 |
| Education (y) ² | 9.9 ± 3.8 | 9.1 ± 3.6 | 8.0 ± 2.7 ⁴ | 7.9 ± 2.5 | 8.6 ± 3.3 |
| Clinical characteristics | | | | | |
| Disease duration (mo) | | | 27.2 ± 22.1 | 27.6 ± 22.2 | 25.6 ± 22.1 |
| MMSE score, range 0–30 ⁵ | 27.6 ± 1.8 | 25.5 ± 2.9 ⁴ | 18.6 ± 5.2 ⁴ | 18.6 ± 5.1 | 18.6 ± 5.6 |
| Total cholesterol (mmol/L) | 5.8 ± 1.3 | 6.1 ± 1.3 | 5.7 ± 1.4 | 5.8 ± 1.4 | 5.2 ± 1.0 |
| Systolic blood pressure (mm Hg) | 143 ± 25 | 149 ± 25 | 144 ± 26 | 145 ± 24 | 140 ± 34 |
| Hypertension (%) | 43.6 | 44.4 | 40.7 | 38.4 | 50 |
| Current smokers (%) | 16.4 | 6.2 | 9.9 | 8.2 | 16.7 |
| Diabetes (%) | 27.3 | 19.8 | 18.7 | 20.6 | 11.1 |
| High daily alcohol intake, current, > 5 drinks/d (%) | 1.8 | 1.2 | 0 | 0 | 0 |
| BMI (kg/m ²) | 25.8 ± 5.1 | 25.1 ± 3.9 | 24.7 ± 5.5 | 24.9 ± 5.7 | 24.0 ± 5.1 |
| Minimum medial temporal lobe thickness (mm) ^{5,6} | 14.5 ± 1.9 | 13.7 ± 3.0 | 12.3 ± 2.5 ⁴ | 12.2 ± 2.6 | 12.8 ± 2.1 |
| Biochemical characteristics | | | | | |
| Plasma total homocysteine (μmol/L) ^{2,7} | 14.6 ± 6.1 | 14.6 ± 5.2 | 17.2 ± 7.1 ⁴ | 16.8 ± 7.0 | 18.9 ± 7.9 |
| Plasma total homocysteine >14.6 μmol/L (%) ⁸ | 31 | 42 | 56 ⁹ | 55 | 61 |
| Serum folate (nmol/L) ^{7,10} | 16.9 ± 5.8 | 14.0 ± 5.9 ⁴ | 13.2 ± 5.6 ⁴ | 13.6 ± 5.6 | 11.4 ± 5.2 |
| Serum vitamin B-12 (pmol/L) ⁷ | 278 ± 99 | 275 ± 117 | 276 ± 110 | 281 ± 111 | 254 ± 104 |
| Creatinine (μmol/L) ⁷ | 96.9 ± 24.2 | 90.0 ± 18.6 | 93.8 ± 24.6 | 93.8 ± 26.3 | 93.4 ± 16.3 |

¹ CDR 0.5, Clinical Dementia Rating of 0.5 (indicates mild cognitive impairment); AD, Alzheimer disease; VaD, vascular dementia; MMSE, Mini-Mental State Examination (39).

² $P < 0.006$ (ANOVA).

³ $\bar{x} \pm SD$ (all such values).

⁴ Significantly different from control subjects, $P < 0.05$ (Dunnett's post hoc test).

⁵ $P < 0.0001$ (ANOVA).

⁶ $n = 156$ (32 control subjects, 60 CDR 0.5 subjects, 53 AD subjects, and 11 VaD subjects).

⁷ Values were log transformed before testing.

⁸ $P = 0.012$ (chi-square test).

⁹ Significantly different from control subjects, $P < 0.05$ (after Bonferroni correction for multiple tests).

¹⁰ $P = 0.0006$ (ANOVA).

dementia groups—was not significantly different from that in the control subjects.

The crude (unadjusted) and adjusted ORs for the CDR 0.5, AD, and dementia groups according to tertile concentrations of serum vitamin B-12, serum folate, and plasma total homocysteine in the control subjects are shown in **Table 2**. In the AD group, the chi-square tests conducted before the individual OR comparisons showed nearly significant differences among the 3 folate tertiles in the adjusted model 2 ($P = 0.087$) and among the 3 total homocysteine tertiles in the crude ($P = 0.059$) and in the adjusted model 1 ($P = 0.058$). In the dementia group, all of the comparisons were significant among folate and total homocysteine tertiles based on the chi-square test. The lowest folate tertile, compared with the highest tertile, was associated with both AD (crude OR: 4.2; 95% CI: 1.6, 11.1) and dementia (crude OR: 4.7; 95% CI: 1.9, 11.6). Compared with the lowest tertile, the highest total homocysteine tertile was associated with AD (crude OR: 2.8; 95% CI: 1.1, 6.8) and dementia (crude OR: 3.0; 95% CI: 1.3, 7.0). These ORs did not vary noticeably (Table 2) when adjusted for age, sex, education, and creatinine concentration or when vitamin B-12 and folate or total homocysteine concentrations were included as covariates in the model: subjects in the lowest folate tertile had an adjusted OR of 3.5 (95% CI: 1.1, 11.2;

$P = 0.087$) for AD and of 3.8 (95% CI: 1.3, 11.2) for dementia, and subjects with hyperhomocysteinemia had an adjusted OR for AD of 3.7 (95% CI: 1.1, 13.1) and for dementia of 4.3 (95% CI: 1.3, 14.7).

APOE genotypes were available for 187 of the subjects included in the study: 80% of the elderly control subjects, 83% of the CDR 0.5 subjects, and 83% of the demented patients. When, in these subjects, *APOE* genotype was also entered into the multivariate regression, the ORs for AD and dementia remained almost the same for the highest homocysteine tertile, whereas the associations with the lowest folate tertile were magnified.

Questionable dementia (CDR 0.5) was found to be significantly associated only with the lowest tertile of folate concentration (crude OR: 3.2; 95% CI: 1.3, 7.7) which remained almost unchanged after adjustment for age, sex, education, and creatinine concentration (OR: 3.4; 95% CI: 1.3, 8.7) or when vitamin B-12 and total homocysteine concentrations were added to the model (OR: 3.1; 95% CI: 1.2, 8.1). Here too, in those subjects for whom *APOE* genotype was available, the OR tended to increase slightly after adjustment for this variable.

The mean MMSE score for the CDR 0.5 subjects in the highest total homocysteine tertile was significantly lower than that for subjects in the lowest tertile (**Table 3**). In the same direction, a

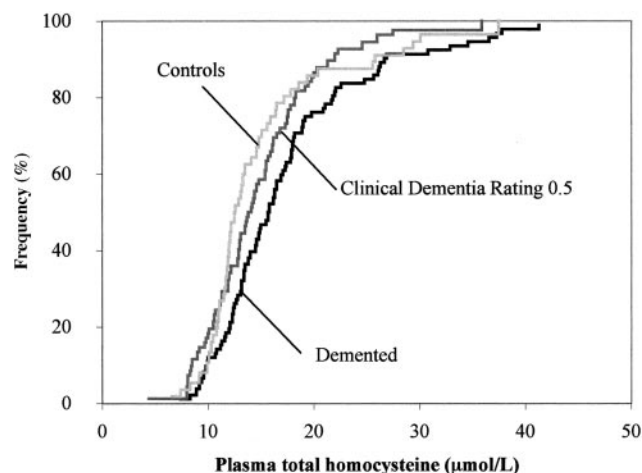


FIGURE 2. Cumulative frequency distribution of plasma total homocysteine concentrations in the control subjects ($n = 55$), the subjects with a Clinical Dementia Rating (CDR) of 0.5 ($n = 81$), and demented subjects ($n = 92$). Kolmogorov-Smirnov test: control subjects compared with CDR 0.5 subjects, $P = 0.32$; control subjects compared with demented subjects, $P = 0.012$.

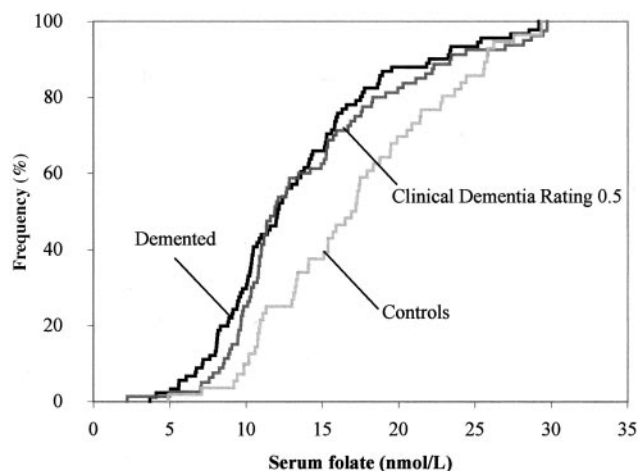


FIGURE 3. Cumulative frequency distribution of serum folate concentrations in the control subjects ($n = 55$), the subjects with a Clinical Dementia Rating (CDR) of 0.5 ($n = 81$), and demented subjects ($n = 92$). Kolmogorov-Smirnov test: control subjects compared with CDR 0.5 subjects, $P = 0.0011$; control subjects compared with demented subjects, $P = 0.0016$.

significant, inverse correlation between MMSE score and total homocysteine was found for CDR 0.5 subjects ($r = -0.35$, $P = 0.001$).

The correlation analyses between plasma or serum concentrations of total homocysteine, folate, vitamin B-12, and creatinine (**Table 4**) not only confirmed the known relation between total homocysteine and creatinine concentrations both in the control ($r = 0.40$, $P = 0.0025$) and AD ($r = 0.39$, $P = 0.0009$) subjects, but they also indicated a significant, inverse association between folate and total homocysteine concentrations in the AD ($r = -0.47$, $P < 0.0001$) and CDR 0.5 ($r = -0.36$, $P = 0.0011$) groups but not in the control group.

On the basis of a covariance analysis to test for the global difference among tertiles of each biochemical variable, no significant age-corrected association was found between the minimum medial temporal lobe thickness and vitamin B-12, folate, or homocysteine concentration in either the AD or dementia group ($P > 0.11$).

The presence of cerebral white matter lesions on computed tomography was not associated with significantly higher total homocysteine or lower folate or vitamin B-12 concentrations in the demented group or in the sample as a whole ($P > 0.13$). Logistic regression analysis showed a significant association of leukoaraiosis only with age ($P = 0.002$) in the dementia group. However, in the whole sample, there was an association with both age ($P = 0.0001$) and with the MMSE score ($P = 0.021$).

DISCUSSION

The findings of the current study suggest a strong association between prevalently mild dementia (AD and VaD) and both high plasma total homocysteine concentrations and relatively low serum folate concentrations: in the highest homocysteine tertile and in the lowest folate tertile, the risk of AD or dementia was >3 times that in subjects in the lowest and highest tertiles, respectively. These associations were independent of known or putative risk factors for dementia or of elevated homocysteine concentrations and were not modified by further adjustment for vitamin B-12 and folate or vitamin B-12 and homocysteine. In

the AD group, the marginally significant difference with the chi-square global test among the 3 folate tertiles was probably due to a multicollinearity problem because of the high correlation between predictors. Irrespective of the findings, it is noteworthy that several published studies failed to adjust for serum creatinine concentration, a well-known factor associated with elevated total plasma homocysteine concentrations. In accordance with the results of Clarke et al (18) and of McIlroy et al (21), the VaD group had both the highest mean total homocysteine concentration and the lowest mean folate concentration.

The results of our study are consistent with those of previous observations (16–19, 21, 27). The mean total homocysteine concentration found in the AD sample ($16.8 \mu\text{mol/L}$; mean age: 79.1 ± 7.7 y) was almost identical to that reported by Clarke et al (18) in their histologically confirmed AD patients ($16.3 \mu\text{mol/L}$; mean age: 76.6 ± 8.0 y). Interestingly, as in Clarke et al's study (18), the cumulative frequency distributions of folate concentrations were more markedly separated than were those of homocysteine concentrations, both in the dementia and the CDR 0.5 groups compared with the control group. In agreement with the findings of the Kungsholmen population-based study (40) and the Bronx Longitudinal Aging Study (41), we found no significant association between serum vitamin B-12 concentrations and dementia or AD.

Subjects with a mild cognitive impairment (ie, CDR 0.5) have a high risk of developing AD in the short term or are already in a preclinical phase of dementia (42). Thus, by comparing the biochemical profile of these subjects cross-sectionally with those of the control group and of the dementia group (the vast majority of whom had dementia of mild severity) we tried to shed light on the possible role of folate, vitamin B-12, and homocysteine in the prodromal phases of the disease. Results of the current study suggest a relative folate deficiency as an early and independent risk factor for mild cognitive decline (OR: 3.1; 95% CI: 1.2, 8.1) and a frequency distribution of this vitamin that was very similar to that seen in the dementia group.

In the current study's control population, both the mean total homocysteine concentration ($14.6 \mu\text{mol/L}$) and the prevalence of a high homocysteine concentration (31%) were slightly higher

TABLE 2

Unadjusted (crude) and adjusted odds ratios (and 95% CIs) for the subjects according to tertiles of serum vitamin B-12, serum folate, and total plasma homocysteine concentrations¹

| | Unadjusted | <i>P</i> ² | Adjusted (model 1) ³ | <i>P</i> ² | Adjusted (model 2) ⁴ | <i>P</i> ² |
|----------------------------|-----------------|-----------------------|---------------------------------|-----------------------|---------------------------------|-----------------------|
| CDR 0.5 | | | | | | |
| Serum vitamin B-12 | | | | | | |
| >303 pmol/L | 1.0 | | 1.0 | | 1.0 | |
| 234–303 pmol/L | 0.8 (0.4, 2.0) | | 0.8 (0.3, 2.0) | | 0.6 (0.2, 1.6) | |
| <234 pmol/L | 1.3 (0.5, 2.9) | 0.65 | 1.0 (0.4, 2.4) | 0.36 | 0.7 (0.3, 1.8) | 0.56 |
| Serum folate | | | | | | |
| >19.5 nmol/L | 1.0 | | 1.0 | | 1.0 | |
| 13.5–19.5 nmol/L | 1.3 (0.5, 3.3) | | 1.0 (0.4, 2.8) | | 0.9 (0.3, 2.6) | |
| <13.5 nmol/L | 3.2 (1.3, 7.7) | 0.011 | 3.4 (1.3, 8.7) | 0.004 | 3.1 (1.2, 8.1) | 0.007 |
| Plasma total homocysteine | | | | | | |
| <11.7 μmol/L | 1.0 | | 1.0 | | 1.0 | |
| 11.7–14.6 μmol/L | 0.8 (0.3, 1.8) | | 0.9 (0.3, 2.3) | | 1.0 (0.4, 2.8) | |
| >14.6 μmol/L | 1.3 (0.5, 2.9) | 0.48 | 1.8 (0.7, 4.7) | 0.28 | 2.0 (0.7, 6.0) | 0.32 |
| AD | | | | | | |
| Serum vitamin B-12 | | | | | | |
| >303 pmol/L | 1.0 | | 1.0 | | 1.0 | |
| 234–303 pmol/L | 0.6 (0.3, 1.6) | | 0.6 (0.2, 1.7) | | 0.3 (0.1, 1.1) | |
| <234 pmol/L | 0.9 (0.4, 2.0) | 0.61 | 0.8 (0.3, 2.0) | 0.66 | 0.4 (0.1, 1.2) | 0.12 |
| Serum folate | | | | | | |
| >19.5 nmol/L | 1.0 | | 1.0 | | 1.0 | |
| 13.5–19.5 nmol/L | 2.6 (0.9, 7.0) | | 2.1 (0.7, 6.4) | | 2.1 (0.6, 6.8) | |
| <13.5 nmol/L | 4.2 (1.6, 11.1) | 0.011 | 3.7 (1.3, 10.7) | 0.048 | 3.5 (1.1, 11.2) | 0.087 |
| Plasma total homocysteine | | | | | | |
| <11.7 μmol/L | 1.0 | | 1.0 | | 1.0 | |
| 11.7–14.6 μmol/L | 1.4 (0.5, 3.6) | | 1.0 (0.3, 3.1) | | 1.1 (0.3, 3.6) | |
| >14.6 μmol/L | 2.8 (1.1, 6.8) | 0.059 | 2.9 (1.0, 8.3) | 0.058 | 3.7 (1.1, 13.1) | 0.044 |
| Dementia (AD + VaD) | | | | | | |
| Serum vitamin B-12 | | | | | | |
| >303 pmol/L | 1.0 | | 1.0 | | 1.0 | |
| 234–303 pmol/L | 0.6 (0.3, 1.4) | | 0.6 (0.2, 1.6) | | 0.3 (0.1, 0.9) | |
| <234 pmol/L | 0.9 (0.4, 2.0) | 0.46 | 0.9 (0.4, 2.1) | 0.59 | 0.4 (0.1, 1.3) | 0.09 |
| Serum folate | | | | | | |
| >19.5 nmol/L | 1.0 | | 1.0 | | 1.0 | |
| 13.5–19.5 nmol/L | 2.3 (0.9, 6.0) | | 1.9 (0.7, 5.5) | | 1.8 (0.6, 5.6) | |
| <13.5 nmol/L | 4.7 (1.9, 11.6) | 0.003 | 4.1 (1.5, 11.2) | 0.014 | 3.8 (1.3, 11.2) | 0.018 |
| Plasma total homocysteine | | | | | | |
| <11.7 μmol/L | 1.0 | | 1.0 | | 1.0 | |
| 11.7–14.6 μmol/L | 1.2 (0.5, 3.1) | | 1.0 (0.4, 2.8) | | 1.1 (0.4, 3.6) | |
| >14.6 μmol/L | 3.0 (1.3, 7.0) | 0.018 | 3.3 (1.2, 9.1) | 0.019 | 4.3 (1.3, 14.7) | 0.018 |

¹ The cutoffs were determined on tertile concentrations in the control group. The risk of being in the CDR 0.5 (Clinical Dementia Rating of 0.5; indicates mild cognitive impairment), Alzheimer disease (AD), or dementia group involved a comparison of each group only against the control group. VaD, vascular dementia.

² Chi-square test.

³ Adjusted for age, sex, education, and creatinine.

⁴ Adjusted for age, sex, education, creatinine, homocysteine, vitamin B-12, and folate.

than were those reported for the Framingham elderly population-based cohort (11.9 μmol/L and 29%, respectively) (32). Although the mean total homocysteine concentration was apparently the same in the control and CDR 0.5 groups, a larger percentage (42%) of subjects with questionable dementia had a plasma total homocysteine concentration that was higher than normal. Moreover, in this mildly cognitively impaired group, the homocysteine concentration appeared to be significantly correlated with global cognitive performance, despite the narrower range of MMSE scores considered. Because the CDR 0.5 group was heterogeneous, ie, made up of subjects who either would or would not develop dementia, we explored post hoc—merely on descriptive grounds—the hypothesis, to be tested in future lon-

gitudinal studies, of the possible presence of 2 different biochemical profiles by splitting the entire CDR 0.5 group according to the level of global cognitive performance by means of the usual MMSE cutoff score of 23/24. The CDR 0.5 subgroup with an MMSE score >23 (*n* = 67) had a biochemical profile that was very similar to that of the entire group, whereas the small subgroup of mildly cognitively impaired subjects with an MMSE score <24 (*n* = 14) seemed to have mean (± SD) concentrations of vitamin B-12 (267 ± 96 pmol/L), folate (11.8 ± 6.0 nmol/L), and total homocysteine (16.9 ± 6.7 μmol/L) resembling those of the demented patients (276 ± 110 pmol/L, 13.2 ± 5.6 nmol/L, 17.2 ± 7.1 μmol/L, respectively). In addition, the percentage of subjects with hyperhomocysteinemia in the CDR 0.5 subgroup

TABLE 3

Mini-Mental State Examination (MMSE) scores of subjects with a Clinical Dementia Rating of 0.5 (mild cognitive impairment) in the 3 plasma total homocysteine tertiles¹

| Plasma total homocysteine tertile | MMSE | <i>P</i> (Dunnett's post hoc test) | <i>P</i> (ANOVA) |
|-----------------------------------|-------------------------|------------------------------------|------------------|
| <11.7 μmol/L (<i>n</i> = 25) | 26.6 ± 0.6 ² | Reference tertile | |
| 11.7–14.6 μmol/L (<i>n</i> = 22) | 26.0 ± 0.6 | NS | |
| >14.6 μmol/L (<i>n</i> = 34) | 24.5 ± 0.5 | <0.05 | 0.013 |

¹ The cutoffs were determined on tertile concentrations in the control group.

² $\bar{x} \pm SE$ (all such values).

with an MMSE score <24 (57%) was almost identical to that in the demented group (56%).

As far as we know, this is the first attempt to study folate, vitamin B-12, and homocysteine concentrations in a group of subjects with a mild cognitive impairment defined according to explicit and reproducible clinical criteria. The results of the current cross-sectional study seem to indicate that relative folate deficiency may already be present before the onset of dementia and, more generally, may represent a risk factor for cognitive decline in the elderly. Although a high total homocysteine concentration also appears to be an early risk factor for cognitive decline, its elevation in dementia could also be interpreted as an epiphenomenon of the disease. Thus, only the longitudinal part of the current study will more properly address the question of whether the risk of developing AD or VaD in subjects with a CDR of 0.5 is associated with hyperhomocysteinemia. In the Framingham longitudinal study (27), an elevated plasma total homocysteine concentration in elderly subjects free of dementia was related to the risk of newly diagnosed dementia or AD over the subsequent 8 y. In contrast, in Rotterdam's community-based study, Kalmijn et al (26) were unable to show an association between an elevated serum concentration of total homocysteine and concurrent cognitive impairment or subsequent cognitive decline after a mean 2.7-y period.

The possibility that the study findings might reflect a spurious association that was due to an overestimation of homocysteine concentrations, because of the nonfasting blood sample collection method used, seems unlikely. First, Thirup and Ekelund (43) showed no significant difference between fasting and postprandial homocysteine values. In any case, because increasing vari-

ability in nonfasting plasma homocysteine concentrations would be random, it is likely that this measurement method would underestimate the true strength of the associations (44).

The current study's apparently more pronounced findings in VaD patients seem to support the hypothesis that hyperhomocysteinemia might play a role in AD pathogenesis through cerebrovascular injury. On the other hand, we were unable to demonstrate an association between total homocysteine concentration and the presence of leukoaraiosis, a neuroimaging abnormality usually considered to be a consequence of chronic cerebral ischemia due to intracranial small vessel pathology. Currently, there is a lack of agreement on the relation between homocysteine and cerebral white matter lesions (45–49) and the presence of silent brain infarcts (47, 50, 51)—another vascular lesion that is associated with an increased risk of dementia. In longitudinal studies, neither vascular variable was significantly associated with an elevated homocysteine concentration (49, 51). The prevalence of cerebrovascular disease by quartiles of homocysteine concentration did not differ in the elderly subjects from the Normative Aging Study (52) nor did the mean homocysteine concentration vary between the AD patients with or without concomitant histologic evidence of cerebrovascular disease (18).

Hyperhomocysteinemia has also been reported to have a neurotoxic action independent of its vascular effects by overstimulation of *N*-methyl-D-aspartate receptors (53) or by an increasing hippocampal neuron vulnerability to excitotoxic insults (54) and amyloid β-peptide toxicity (55).

In agreement with the finding of Clarke et al (18), cross-sectionally we found no significant association between homo-

TABLE 4

Correlations between plasma total homocysteine, serum folate, serum vitamin B-12, and serum creatinine concentrations in the control subjects and in Alzheimer disease (AD) subjects¹

| | Plasma total homocysteine | Serum folate | Serum vitamin B-12 | Serum creatinine |
|---------------------------|---------------------------|--------------|--------------------|-------------------|
| Control subjects | | | | |
| Plasma total homocysteine | | −0.21 | −0.24 | 0.40 ² |
| Serum folate | | | 0.05 | 0.03 |
| Serum vitamin B-12 | | | | 0.14 |
| Serum creatinine | | | | |
| AD subjects | | | | |
| Plasma total homocysteine | | | | |
| Serum folate | −0.47 ³ | | | |
| Serum vitamin B-12 | −0.22 | 0.16 | | |
| Serum creatinine | 0.39 ⁴ | −0.21 | 0.07 | |

¹ Values were log transformed before testing.

² *P* = 0.0025.


³ *P* ≤ 0.0001.

⁴ *P* = 0.0009.

cysteine concentration and minimum medial temporal lobe thickness. In a subsample of AD patients, however, Clarke et al observed a significantly greater change in this neuroradiologic measure over a 3-y period among those patients with higher homocysteine baseline concentrations, although the progression of the atrophy was more rapid in the middle tertile and was not consistent with cognitive decline. In nondemented elderly, a cross-sectional significant association was found between homocysteine concentration and more atrophy of the hippocampus (56, 57), whereas contrasting results were reported with regard to the association of these amino acid values with magnetic resonance imaging measures of cortical atrophy (48, 57).

As for homocysteine, we could not demonstrate a significant age-corrected association between medial temporal lobe atrophy and relatively low folate concentrations in patients with mainly mild dementia and AD. In contrast, in a small sample of older and likely more severe AD patients for whom neuropathologic examination was available, Snowdon et al (58) found a significant correlation between relatively low folate concentrations and severity of postmortem neocortical atrophy. This correlation was even stronger in the subgroups of subjects with minimal atherosclerosis or without brain infarcts, which suggests that central pathologic effects other than vascular effects underlie this association.

It has been reported that a folic acid-deficient diet can promote hippocampal neurodegeneration in amyloid precursor protein mutant transgenic mice (55) and can also increase the vulnerability of dopaminergic neurons to degeneration in a mouse model of Parkinson disease (59). Whether relative folate deficiency may exert an adverse effect on cortical neurons directly, by increasing homocysteine concentration, or by both means needs to be addressed in further longitudinal studies.

It is possible, however, to modify these putative environmental determinants of cognitive deterioration by nutrition: dietary folic acid alone or in combination with vitamin B-12 (60) is in fact a safe, simple, and economic therapeutic strategy to effectively reduce plasma homocysteine concentrations. The adjunct of vitamin B-6 does not seem to have a significant additional effect (60), although each of the B vitamins might have an effect on cognition independent of their potential influence on plasma homocysteine concentrations. A homocysteine-lowering effect in AD patients has also been shown in an open-label trial of folic acid, vitamin B-12, and vitamin B-6 regimen (61). Because associations are not proof of a causal relation, large randomized controlled clinical trials of the effect of B vitamin supplementation on reductions in the risk of incident AD and VaD or on the slowing of the disease progression could provide more decisive answers on how relevant high homocysteine and low folate concentrations are to the onset and course of AD and VaD. 

PQ, CF, MT, and UL were responsible for the study design; PQ conducted the clinical evaluation of the subjects; RP and EZ conducted the neuropsychological evaluation of the subjects; CF performed all of the biochemical analyses; GF was responsible for the *APOE* genotype determinations; MT conducted the data analysis and wrote the statistical analysis section of the article; PQ, MT, and UL interpreted the study results; and UL drafted the manuscript. None of the authors had any conflicts of interest.

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