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Homocysteine and cognitive function in institutionalised elderly

A cross-sectional analysis

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Summary *Background* Several cross-sectional, case-control and prospective studies revealed a relation between homocysteine and cognitive function or dementia. These studies included either patient populations or healthy, community-dwelling elderly people. *Aim of the study* In this study we tested the hypothesis that homocysteine was inversely associated with cognitive function in a population of institutionalised elderly (aged ≥ 60 y; n = 157). *Methods* For testing this hypothesis baseline data of a recently conducted intervention study in institutionalised elderly (median age 83 years) were used. Cognitive function was evaluated by the cognitive subscale of the Alzheimer's disease Assessment Scale (ADAS-cog). The association between fasting plasma homocysteine level and cognitive function was investigated by multiple linear regression analysis. *Results* In the crude model homocysteine concentration was not significantly related to ADAS-cog score ($\beta = 0.061$; $p = 0.45$). Age was found to be related to ADAS-cog score ($\beta = 0.161$; $p < 0.05$). Adjusting for age did however not result in a relation between homocysteine and cognitive function. *Conclusions* In our study no association was found between homocysteine and cognitive function in a population of very old institutionalised subjects.

Key words homocysteine – cognitive function – institutionalised elderly – cross-sectional study

Introduction

Vascular diseases and dementia are common disorders in old age and important predisposing factors of mortality [1, 2]. An elevated plasma homocysteine level has been suggested as one of the possible, modifiable risk factors for cardiovascular diseases [3–5]. An association between vascular diseases and decreased cognitive performance is also suspected [6]. Furthermore, it has been suggested that elevated plasma homocysteine levels as such are associated with neuropsychiatric disorders, such as cognitive impairment [7–9]. Therefore, homocysteine levels and cognitive function might be related either indirectly or directly.

There are some plausible biological mechanisms that might explain the relation between homocysteine and cognitive function. One of the hypotheses is that cognitive impairment is caused by hypomethylation of methyl-acceptors like myelin, neurotransmitters and membrane-phospholipids [10, 11]. Besides that a methyl donor deficiency may disturb the repair of DNA damage by oxidative stress [12]. Another effect of hyperhomocysteinemia on cognitive function is the neurotoxicity of homocysteine [10, 11, 13]. There is evidence showing that elevated homocysteine levels are associated with neuropsychiatric disorders including cognitive decline.

Table 1 presents a summary of earlier studies that investigated the relation between homocysteine and cognitive function or dementia. In several case-control

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Table 1 Earlier studies that investigated the relation between homocysteine and cognitive function or dementia

Case-control studies in groups of patients with a diagnosis of dementia			
Study	Subjects		Conclusion
	Cases	Controls	
Nilsson et al., 1996 [14]	psycho geriatric patients (n = 510) demented and non-demented subgroup	reference population (n = 163)	Plasma homocysteine concentrations were significantly increased in both the demented and the non-demented patients compared to control subjects.
Joosten et al., 1997 [15]	patients with Alzheimer's disease (n = 52)	non-demented hospitalized controls (n = 50) healthy elderly subjects living at home (n = 49)	Mean homocysteine level is significantly higher in patients with Alzheimer's disease as compared to non-demented patients or subjects living at home.
Clarke et al., 1998 [16]	hospital clinic patients, aged 55 years or older, with a clinical diagnosis of dementia of Alzheimer type, including 76 patients with histologically confirmed Alzheimer's disease (n = 164)	elderly volunteer controls without symptoms of memory impairment (n = 108)	The odds ratio of histologically confirmed Alzheimer's disease associated with serum homocysteine concentrations ≥14 μmol/L (top third of the control distribution) compared to individuals with low serum homocysteine (≤11 μmol/L) was 4.5.
McCaddon et al., 1998 [17]	patients of a psycho geriatric assessment centre, aged 65 or over, seen with features compatible with DSM-III-R criteria for primary degenerative dementia of Alzheimer type (n = 30)	cognitively intact age-matched control subjects from a local general practice (n = 30)	Patients had a highly significant elevation of homocysteine compared with controls.
Ravaglia et al. 2000 [18]	demented centenarians with a clinical diagnosis of Alzheimer's disease (n = 34)	cognitively impaired not-demented centenarians (n = 10) cognitively normal centenarians (n = 13)	No significant difference was found for plasma homocysteine levels among the three diagnostic groups.
Cross-sectional studies in community-dwelling, non-demented, healthy elderly			
Study	Subjects		Conclusion
Riggs et al., 1996 [19]	male participants from the Normative Aging Study (n = 70)		Higher concentrations of homocysteine were strongly associated with poorer spatial copying skills, but not with the performance of tests on any of the other cognitive domains (memory, language, perceptual speed, or spatial reasoning).
Morris et al., 2001 [20]	elderly men and women participated in phase 2 of NHANES III (aged ≥60 y, ≥8 years of education, no previous stroke, atp test in one try) (n = 1270 and 1200)		Hyperhomocysteinemia was associated with poorer performance on measures of recall.
Prins et al., 2002 [21]	population-based study of non-demented elderly (n = 1077)		Elevated homocysteine levels are associated with decreased cognitive performance in non-demented elderly people, and the relation was most marked for psychomotor speed.
Budge et al., 2002 [22]	community-dwelling volunteers aged 60 to 91 (n = 158)		Homocysteine was negatively associated with total CAMCOG score. Higher homocysteine levels were associated with poorer performance on the memory and perception sub scores of CAMCOG but not with the other cognitive subscales or MMSE score.

Table 1 *Continued*

Cross-sectional studies in community-dwelling, non-demented, healthy elderly		
Study	Subjects	Conclusion
Stewart et al., 2002 [23]	individuals aged 55 to 75 who were born in a Caribbean nation and living in community accommodation (from registration lists for primary care services) (n = 248)	Raised homocysteine (highest quartile: > 13.85 µmol/L) was significantly associated with cognitive impairment.
Duthie et al., 2002 [24]	survivors of the Scottish Mental Surveys, which surveyed childhood intelligence quotient. Cohort of children born in 1921 or 1936; 183 ABC21 and 148 ABC36, living independently in the local community	In the ABC21 but not the ABC36, homocysteine accounted for approximately 7–8 % of the variance in cognitive performance.
Miller et al., 2003 [25]	community-dwelling elderly Latinos (aged ≥ 60 y) (n = 1789)	Homocysteine is a modest independent predictor of cognitive function in community-dwelling elderly Latinos.
Ravaglia et al., 2003 [26]	healthy, cognitively normal Italian community dwellers aged ≥ 65 y (n = 650)	Elevated plasma homocysteine has an independent, graded association with concurrent cognitive impairment as measured with the MMSE in healthy elderly community dwellers.
Garcia et al., 2004 [27]	cognitively normal, community-dwelling participants aged 65 and older (n = 281)	Significant correlations between levels of homocysteine and the Stroop score and homocysteine and some scores of the California Verbal Learning Test were found.
Prospective community-based studies		
Study	Subjects	Conclusion
Kalmijn et al., 1999 [28]	community-dwelling respondents aged 55 years or over (n = 702)	No inverse association between elevated plasma homocysteine levels and concurrent cognitive impairment or subsequent cognitive decline over a 3-y follow-up was found.
Seshadri et al., 2002 [29]	subjects without dementia (667 women and 425 men) from the Framingham Heart Study (n = 1092)	An increased baseline plasma homocysteine concentration was a strong, independent risk factor for the development of dementia and Alzheimer's disease over an 8-y period.
Teunissen et al., 2003 [30]	normal aging individuals aged 30–80 years (n = 93)	An association between elevated homocysteine concentrations and prolonged lower cognitive performance has been observed after six years of follow-up.

studies patients with a diagnosis of dementia had significantly higher mean total homocysteine levels than controls [14–17]. Ravaglia et al. did however not confirm this difference in homocysteine level [18].

In the non-demented, healthy elderly population, hyperhomocysteinemia has been shown to be associated with poor performance on neuropsychological tests measuring specific cognitive abilities [19–22]. Furthermore, in recent studies elevated homocysteine was significantly associated with poor performance on more general cognitive tests or composite cognitive scores [23–27]. Longitudinal studies on a relation between cognitive performance and homocysteine showed less unequivocal results [28–30].

Most earlier studies found an association between homocysteine and cognitive function or dementia either in a specific patient population or in healthy, community-dwelling elderly. No such association study has been conducted so far in the group of institutionalised elderly. Therefore we decided to test the hypothesis that homocysteine was inversely associated with cognitive function in institutionalised elderly, hereby taking several confounding factors into account.

Material and methods

For testing our hypothesis we used baseline data on cognitive assessment and the biochemical assessment of homocysteine of a recently conducted intervention study. In this trial we investigated the effect of a nutrient dense dairy drink on physical and mental functioning in institutionalised elderly people. The drink was enriched with vitamins, minerals and trace elements added in amounts of approximately 25 to 175 % of US RDA.

Subjects

Elderly who were dependent on professional care because of diminished cognitive function or deteriorated physical health were recruited from nine institutions in the southern part of the Netherlands. In the Netherlands two different kinds of institutionalisation exist; homes for the elderly and nursing homes. For activities of daily living and household management elderly in both types of institution are dependent on professional care, in nursing homes additional medical care is required. In both institutions central meal preparation and distribution of medicines is present. With permission of the board of the (nursing) homes, an invitation letter with information about the study protocol was sent to the residents and to one of their relatives. For all participants written informed consent was obtained from the participants themselves and/or from one of their legal representatives.

Subjects (n = 265) were included in a screening procedure to assess if they met the following inclusion criteria: age ≥ 60 years, Body Mass Index (BMI) ≤ 30 kg/m^2, MMSE (Mini Mental State Examination) score ≥ 10 and institutionalised for at least two months at the start of the study.

Exclusion criteria were: tumours, with unstable body weight at the time of the measurements, terminal care, severe infectious diseases, disorders of the gastro-intestinal tract, use of parenteral food or structural use of tube feeding, intolerant or allergic to one of the ingredients of the intervention product and use of medication or supplements that could influence safe administration of the intervention product. The Medical Ethical Committee of Wageningen University approved the study protocol.

Measurements

In total 176 subjects were eligible and willing to participate. Subjects with both data on ADAS-cog score and homocysteine concentration were included in the analyses described in this article (n = 157). From these subjects we gathered information on general characteristics to describe our population. Furthermore, all subjects underwent anthropometrical and cognitive assessments. Weight and knee-to-floor height [31] were measured and BMI calculated. The cognitive part of the Alzheimer's disease Assessment Scale (ADAS-cog) [32] and Geriatric Depression Scale (GDS) [33] were administered. Fasting blood samples were collected to determine homocysteine levels in plasma.

General characteristics

Information on age, sex, length of stay in (nursing) home, smoking habits (never, former and current), and educational level (low; completed primary education or lower vocational education, medium; intermediate vocational or general education and high; higher vocational training, college or university) was collected from personal files, available at the institution. Information on presence of several chronic diseases, like history of CVD (cardiovascular disease) and diabetes, was collected from medical files, also available at the institution. To assess the presence of chronic renal failure, creatinine levels in blood were measured.

Cognitive assessment

Mental function was measured using the following questionnaires: MMSE for the screening procedure, ADAS-cog score and GDS as baseline and outcome measure of the intervention.

The MMSE is a questionnaire with 12 questions con-

cerning orientation, memory, attention, and the ability to name and to follow verbal and written commands. MMSE is considered to have a high interrater reliability and validity [34].

The ADAS was originally designed as a rating scale for severity of dysfunction in cognitive and non-cognitive behaviour characteristics of persons with Alzheimer's disease (AD). The scale is composed of items with significant interrater and test-retest reliability for Alzheimer patients. Since the symptoms of AD and other dementias overlap to some extent, the ADAS may be applicable to other dementias. The ADAS consists of a non-cognitive part rating emotional and behavioural symptoms with ten items and a cognitive part, used in this study and referred to as ADAS-cog, consisting of 12 items with a total score ranging from 0 (no impairment) to 75 (severe impairment). The target symptoms are supposed to represent several domains of impaired cognitive function. The items rate components of memory and orientation (total score = 40), language (total score = 25), and praxis (total score = 10) [32, 35].

The Geriatric Depression Scale-15 is a short, 15-item instrument specifically developed to assess depression in geriatric populations. It is a reliable and valid self-rating depression screening scale for elderly populations. Questions can be answered with "yes" or "no", with a total score ranging from 0 to 15. Higher scores on GDS indicate the presence of severe depressive symptoms [33, 36].

Biochemical assessment

Blood samples were collected from fasting subjects in a gel tube and an EDTA-containing tube. These tubes were centrifuged (2970 rpm) at a temperature of 4 °C during 10 minutes within 1 hour of blood collection. The serum and plasma samples were stored at –80 °C till further analyses of homocysteine and creatinine.

Total plasma homocysteine concentration was measured using high performance liquid chromatography with fluorescence detection. The lower limit of sensitivity of this method is 0.22 μmol/L in plasma and the method is highly reproducible (intra- and interassay coefficients of variation = 5.0 and 4.5 %, respectively) [37]. Creatinine was determined by measuring absorption of the reaction product of creatinine and picric acid at 520 nm by a Synchron LX20, with a CV below 3 % and a lowest detectable level of 8.84 μmol/L (modified Jaffé method). The laboratory of the Division of Human Nutrition at Wageningen University, the Netherlands performed the homocysteine analyses. The creatinine analyses were performed at Stichting Huisartsenlaboratorium Oost in Velp, the Netherlands.

Confounders

Factors that could potentially influence the relation between plasma homocysteine and cognitive performance were taken into account. In the present analyses age, sex, vascular diseases, diabetes mellitus, chronic renal failure, educational level, smoking habits, BMI and depression were considered as potential confounders [20, 25, 28, 29].

Statistical analyses

Because the variables ADAS-cog score and homocysteine concentration were not normally distributed we performed a log-transformation of those variables and calculated the geometric mean and standard deviation. For the normally distributed confounder BMI, the mean and standard deviation were calculated. For the not normally distributed confounders (age and GDS) the median and 10^{th} and 90^{th} percentile were calculated.

Multiple linear regression was performed to investigate the relation between homocysteine concentration and ADAS-cog score. Age, sex, history of CVD, diabetes, chronic renal failure, educational level, smoking habits, BMI and GDS were tested as potential confounders, but hardly affect the results. The interaction between homocysteine concentration and all potential confounders was not statistically significant.

Statistical significance was defined for all analyses as $p < 0.05$. Data were analysed using the statistical program SPSS, version 11.0 for Windows.

Results

Characteristics of the population

Table 2 presents the characteristics of the study population. The median age of the participants was 83 years. The participants were institutionalised for an average of 21.6 months at the time of the measurements. Eighteen percent of the participants were current smokers. Of the participants, 14 % had diabetes, 20 % had chronic renal failure and 45 % history of CVD. Mean BMI of the participants was 25.3 kg/m^2. Mean plasma homocysteine level was 16.9 μmol/L (Table 2). The educational level of most participants (60 %) was low. The mean score on the ADAS-cog measurement was 14.8 points. The memory and orientation sub score of the ADAS-cog was on average 10.8 points. GDS had a median of 4 points. Differences in general characteristics between men and women were significant for smoking habits and presence of chronic renal failure ($p < 0.001$) (Table 2).

Table 2 General health characteristics of a subgroup of Dutch institutionalised elderly (n = 157)

Variable	Total population	Women (n = 108)	Men (n = 49)
Age (years)[c]	83.0 (72.0; 91.3)	83.0 (72.8; 93.0)	83.0 (67.0; 89.0)
Length of stay in (nursing) home (months)[b]	21.6±2.5	20.3±2.5	24.8±2.5
Smoking habits (%)*			
Never	32	44	4
Former	22	10	49
Current	18	15	24
Missing	28	31	22
History of CVD[d] (%)	45	40	55
Diabetes (%)	14	13	14
Chronic renal failure[e] (%)*	20	12	38
BMI (kg/m^2)[a]	25.3±3.5	25.4±3.6	25.1±3.3
Homocysteine concentration (μmol/L)[b]	16.9±1.5	16.4±1.5	18.0±1.4
Educational level (%)			
Low	60	57	65
Medium	20	19	20
High	7	7	6
Missing	13	16	8
MMSE[c]	21 (12; 27)	21 (12; 27)	22 (14; 28)
ADAS-cog score[b]	14.8±1.8	15.5±1.8	13.5±1.7
Memory and orientation[b]	10.8±1.8	11.3±1.8	9.8±1.7
Language[c]	2 (0; 5)	2 (0; 5)	2 (0; 5)
Praxis[c]	2 (0; 5)	2 (0; 5)	2 (0; 4)
GDS[c]	4 (1; 9)	3 (1; 9)	4 (1; 8)

([a] mean ± SD; [b] geometric mean ± SD; [c] median (p_{10}; p_{90}))
[d] *CVD* cardiovascular disease
[e] serum creatinine > 120 μmol/L
* $p < 0.001$ difference between men and women

Relation between homocysteine and cognitive function

To examine the relation between homocysteine concentration and ADAS-cog score, first a regression model without adjustment for confounders was analysed (Table 3). In this model homocysteine concentration was not significantly related to ADAS-cog score. Of the confounders considered only age turned out to be significantly related to the ADAS-cog score. If age was added as explanatory variable (Model 2 in Table 3), this analysis did not result in a significant relation between homocysteine concentration and ADAS-cog score.

Table 3 Multiple linear regression models for homocysteine concentration (independent variable) versus ADAS-cog score (dependent variable) in a subgroup of Dutch institutionalised elderly (β (p-value))

ADAS-cog score			
Model	Homocysteine concentration	Age	Adj. R^2
1	0.061 (0.448)		–0.003
2	0.057 (0.472)	0.159 (0.047)	0.016

Model 1 homocysteine concentration alone; *Model 2* model 1 + age

Discussion

We hypothesized that there would be a relation between plasma homocysteine level and cognitive function in institutionalised elderly. The cross-sectional analysis in our study did not show such an association in our population.

In the existing literature either specific patient populations or healthy, community-dwelling elderly have been investigated. The reason that in this study no relation was found might be that we investigated a population of institutionalised elderly, which is classified in the literature as "accelerated agers" [38, 39]. This group of subjects may suffer from several different chronic diseases and types of dementia. Therefore, the elderly population in our study was very heterogeneous with respect to diseases and comorbidity. This could have confounded the relation between homocysteine and cognitive function but when we adjusted for the presence of history of CVD, diabetes and impaired renal function in the multiple regression model the relation between homocysteine concentration and ADAS-cog score was still not significant. Residual confounding caused by comorbidity is still possible, although we ad-

justed the analyses for the most important chronic diseases.

In contrast with the heterogeneity in disease, our population might have been too homogenous for two other factors, both the outcome measure (ADAS-cog score) and the main determinant (homocysteine concentration). The reason that we did not find an association between homocysteine concentration and ADAS-cog score might be that a large part of the population had a low ADAS-cog score. Approximately 60 % of the participants had an ADAS-cog score below 20 points (ADAS-cog range 0–75), indicating a homogenous population with a low range of ADAS-cog score. None of the other recent cross-sectional studies on homocysteine and cognitive function used ADAS-cog to assess cognitive function. Therefore, it was not possible to compare the level of and variance in ADAS-cog scores with other studies. The MMSE level (median 21: indicating that the subjects on average had dementia), however, was lower than in four other studies that included MMSE as an outcome measure and did find an association. Prins et al. [21] used in their study participants with a mean MMSE of 27.5 ± 2.1. In the studies of Budge et al. [22], Duthie et al. [24] and Ravaglia et al. [26] the mean MMSE of the participants was around 29 points. Based on MMSE score we could consider that too few people with a good cognitive status were present in our population.

Furthermore, the homocysteine levels that were observed (mean: 16.9 ± 1.5 μmol/L) were relatively high in comparison to other studies. In four other cross-sectional studies fasting homocysteine levels were determined: Riggs et al. [19], Ravaglia et al. [26], Morris et al. [20] and Miller et al. [25] found a homocysteine level of 11.9 μmol/L, 12.3 μmol/L, 10.4 μmol/L and 9.8 μmol/L respectively. Prins et al. [21], Budge et al. [22] and Stewart et al. [23] used non-fasting homocysteine levels of 11.5 μmol/L, 12.6 μmol/L and 12.3 μmol/L respectively. The mean age in these seven studies ranged from 65 to 74 years. For elderly people higher cut-off values for hyperhomocysteinemia have been put forward. In the reference population used by the Dutch Heart Foundation a cut-off value of 17.4 μmol/L for men between 60 and 70 years old and 15.2 μmol/L for women (60–70 years old) is used. For men and women above the age of 70 this cut-off value is respectively 19.1 μmol/L and 18.3 μmol/L. Meaning that the mean level in our population (median age of 83 years) could be considered as normal. Besides the higher level of homocysteine, the variation in homocysteine levels in our population was comparable to other fasting ranges, but was less than in studies using non-fasting levels. Thus the reason that we did not find an association between homocysteine concentration and ADAS-cog score most likely is that there were too few people with low homocysteine levels among the participants.

So in our population relatively low cognitive function (measured as MMSE) and high homocysteine levels were found in rather small ranges. In the literature both factors are related to age. The fact that our population is older (median: 83 years) than in most other cross-sectional studies (mean age between 65 and 74 years) might be an explanation for our findings. In most cross-sectional studies among healthy elderly an inverse association between homocysteine and cognitive function was found (Table 1). Duthie et al. [24] found in a cross-sectional analysis among survivors of the Scottish Mental Surveys (ABC21 and ABC36) that homocysteine was associated with cognitive variation in the older cohort (around 78 years old), but surprisingly not in the younger cohort (around 63 years old). The age of the oldest cohort was however lower than the age of our population. In a case-control study of Ravaglia et al. [18] in Italian centenarians no significant difference was found between homocysteine levels in a demented group and a cognitively normal group. These results suggest that a possible association between homocysteine and cognitive function disappears when getting into the oldest old (above the age of 80 years). One possible explanation could be that selective survival occurs and that only elderly who are less susceptible to elevated levels of homocysteine survive. Furthermore, it is of course also possible that the relation between homocysteine and cognitive function changes with age.

Age was considered as one of the confounding factors in our multiple regression model. Adding age did however not result in a relation between homocysteine and cognitive function. All confounders that were considered in our analyses (age, sex, vascular diseases, diabetes mellitus, chronic renal failure, educational level, smoking habits, BMI and depression) were used in other population-based studies as well [25]. Other factors that could have influenced the relation between homocysteine and cognitive function were levels of blood folate, serum vitamin B_{12} and plasma vitamin B_6. These factors were not adjusted for in our analyses because they are highly related to homocysteine levels and could have over adjusted the relation between homocysteine and cognitive function. Factors like hypertension, genetic factors, alcohol consumption, cholesterol and several other blood values are also potential confounders but unfortunately we did not have information on these variables.

In conclusion, in our study no association was found between homocysteine and cognitive function in a population of elderly institutionalised subjects. Despite possible biological mechanisms and the offered methodological explanations, the hypothesis that homocysteine is related to cognitive function in our population of very old institutionalised elderly is rejected. Studies with more heterogeneous (in respect of homocysteine and cognitive function), very old populations and prospective studies that focus on old age are needed to investigate the possibility that such a relation still exists in the oldest old.

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