Homocysteine and cognitive function in elderly people

Angeles Garcia, Katherine Zanibbi

Abstract

Dementia is characterized by a progressive deterioration of cognitive skills that leads to a decline in the ability to perform daily activities. It affects 8% of people over the age of 65 and results in more than 60,000 new cases in Canada each year. Alzheimer’s disease accounts for more than 50% of cases of dementia in Canada. Projections for the next 50 years show that the number of patients with dementia might triple, mainly because of a large increase in the oldest-old segment of the population. Because of this and the disease’s devastating effects, measures for the prevention and early detection of dementia are crucial. Age and years of education are among the most relevant risk factors for dementia, but in recent years the role of homocysteine has also been investigated. Homocysteine is an amino acid produced in the metabolism of methionine, a process dependent on the B vitamins cobalamin, vitamin B6 and folic acid. There is evidence that increased serum homocysteine levels are associated with declining cognitive function and dementia. We review this evidence in addition to the potential mechanisms through which homocysteine acts on the brain to cause cognitive dysfunction, the metabolism of homocysteine and factors associated with alteration of the normal metabolism.

Population studies

Homocysteine and dementia

An outline of the published research into homocysteine and dementia can be found in Table 1. In brief, elevated serum homocysteine levels have been found to be an independent risk factor for Alzheimer’s disease. Recently, Seshadri and associates, in a population sample of the Framingham cohort, found that elevated levels were a graded independent risk factor for Alzheimer’s disease. Other studies have shown that serum homocysteine levels are significantly higher among patients with dementia than among normal control subjects and that the levels are inversely related to cognitive function in people with dementia. In a recent study, however, elevated homocysteine levels were found to be more common among patients with vascular disease than among those with Alzheimer’s disease. Whether homocysteine contributes to cerebrovascular changes or to the pathological features of Alzheimer’s disease, or both, remains controversial, but regardless of the mechanisms, it appears that elevated homocysteine levels are a risk factor for dementia in older adults.

Homocysteine and cognitive function scores

As can be seen in Table 2, studies involving healthy elderly people have yielded conflicting results. Some have shown significant associations between homocysteine levels and cognitive function, whereas others have not. An early study involving a small sample of a healthy elderly population in Italy showed no significant correlations between the Mini-Mental State Exam (MMSE) scores and homocysteine levels. However, the same authors later found, in a much larger population group, that the risk of lower MMSE scores increased with increasing levels of plasma homocysteine and that homocysteine had an independent graded association with cognitive impairment. Most longitudinal studies have found associations between cognitive function scores and homocysteine levels. In a cohort study, homocysteine levels at baseline and increases in levels over a mean of 2.3 years were found to be related to changes in executive function scores.

The timeframe for changes in cognitive function to occur in relation to homocysteine levels may be long, which

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may leave a substantial window of opportunity for preventive interventions.

In a 35-day trial of B vitamins versus placebo, Bryan and colleagues\(^2\) found an improvement in some memory performance scores in a subgroup of older women. However, contrary to expectations, the overall cognitive and mood changes related to the treatment were felt to be minimal.

### Mechanisms of action of homocysteine in the brain

Homocysteine is metabolized through 2 different pathways: the methionine synthase pathway and the cystathionine pathway (Fig. 1). The effects of homocysteine in the brain are multiple but can be broadly divided into neurotoxic and vascular effects (see Box I).

The evidence for the neurotoxic effects comes largely from in vitro studies.\(^2\) It has been shown in vitro that homocysteine acts as an agonist on the glutamate-binding site and as a partial antagonist on the glycine-binding site of the N-methyl-D-aspartate (NMDA) receptor. Under conditions of elevated glycine concentrations, such as in stroke or head trauma, homocysteine at a concentration of 10 μmol/L had a neurotoxic effect through hyperstimulation of the NMDA receptor that resulted in an excess influx of calcium ions and production of reactive oxygen species.

As in other parts of the body, homocysteine has direct and indirect vascular effects, and an elevation in homocysteine levels has been recognized as an independent cardiovascular risk factor.\(^2\) Elevated levels induce atherogenesis by directly increasing formation of reactive oxygen species and by promoting oxidation of low-density lipoprotein.\(^2\) Reactive oxygen species alter smooth muscle function and promote proliferation of vascular smooth muscle cells. Furthermore, homocysteine has been shown to increase platelet aggregation,\(^2\) which contributes to the occurrence of clinically apparent and silent brain infarcts.

### Table 1: Studies investigating relation between homocysteine and dementia

| Study               | Study population                        | Study design             | Cognitive assessment/diagnostic criteria | Results                                                                 | Comment                                                                 |
|---------------------|-----------------------------------------|--------------------------|-----------------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Stewart, 2002\(^4\) | 238 African-Caribbean adults aged 55–75 yr | Cross-sectional          | CERAD; WHO; MMSE                        | tHcy level > 13.85 μmol/L associated with cognitive impairment (OR 2.86, 95% CI 1.33–6.13) | OR adjusted for age, occupation, hypertension, diabetes, physical activity, and levels of cholesterol, triglycerides and fibrinogen |
| Clarke, 1998\(^5\) | 272 patients aged > 55 yr; control-matched comparison | Case–control             | CERAD, NINDS                            | tHcy >14.0 μmol/l associated with AD (OR 2.0, 95% CI 1.1–3.4)          | OR adjusted for age, sex, smoking, social class and apolipoprotein E    |
| McCaddon, 1998\(^6\) | 60 patients with DSM-III-R criteria for AD | Case–control             | CAMDEX                                  | tHcy level inversely related to scores on cognitive testing            | Results adjusted for age, sex, folate and cobalamin levels, smoking and hypertension. Effect of tHcy and cobalamin found to be interrelated |
| Miller, 2002\(^2\)  | 80 patients with possible or probable AD | Case–control             | NINCDS-ADRDA                            | tHcy level > 12 μmol/L associated with vascular disease (OR 1.0, 95% CI 1.2–82) but not with AD (OR 2.2, 95% CI 0.31–16) | OR adjusted for age, sex, and levels of red blood cell folate, plasma B12, serum creatinine and serum thyroid stimulating hormone |
| Seshadri, 2002\(^9\) | 1092 dementia-free patients in Framingham cohort aged 68–97 yr | Prospective cohort (median follow-up 8 yr) | MMSE, DSM IV criteria, Clinical Dementia Rating Scale, NINCDS-ADRDA | tHcy level associated with increased risk of dementia and AD (OR 1.4, 95% CI 1.1–1.9 per 1 SD increase in log-transformed tHcy level) | RR adjusted for age, sex, apolipoprotein E genotype, education level, history of stroke, smoking, alcohol intake, diabetes, body mass index and systolic blood pressure |
| Vital Trial Collaborative Group, 2003\(^6\) | 149 patients with dementia or mild cognitive impairment | Intervention study of ASA and vitamin supplements on serum biomarkers of dementia | MMSE, TICS-M, DSM IV criteria | At baseline, tHcy level inversely associated with cognitive scores | Results adjusted for age                                                                 |

Note: AD = Alzheimer’s disease, CAMDEX = Cambridge Examination for Mental Disorders of the Elderly, CERAD = Consortium to Establish a Registry for Alzheimer’s Disease, CI = confidence interval, DSM = Diagnostic and Statistical Manual of Mental Disorders, MMSE = Mini-Mental Status Exam, NINCDS-ADRDA = National Institute of Neurological and Communication Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association, NINDS = National Institute of Neurological Disorders, OR = odds ratio, RR = relative risk, SD = standard deviation, tHcy = homocysteine, TICS-M = Telephone Interview of Cognitive Status, WHO = World Health Organization.
An elevation in homocysteine levels has been found to be an independent risk factor for recurrent stroke in patients with pre-existing coronary artery disease and in other populations. It is also related to white matter abnormalities. In the Rotterdam Scan Study, an association between homocysteine levels, silent brain infarcts and white matter lesions was found. The risk of silent brain infarcts, the severity of periventricular white matter lesions and the extent of the subcortical white matter lesions increased with increasing levels of homocysteine, after adjustment for other risk factors, with an overall risk of silent brain infarcts or severe white matter lesions of 1.35 per standard deviation increase in homocysteine level (95% confidence interval [CI] 1.16–1.58). Interestingly, the risk of silent brain infarcts and severe white matter lesions was already significantly increased among subjects whose homocysteine levels were within the upper normal range (between 9.9 and 13.7 µmol/L) compared with subjects whose levels were in the lower normal range (< 8.5 µmol/L). Subjects whose levels were above the normal range had the highest odds ratio for all MRI-detected abnormalities (odds ratio 3.0, 95% CI 1.8–5.2).

In addition to stroke, increased homocysteine levels have been shown to be related to increased cortical and hippocampal atrophy. A recent study showed that subjects with higher homocysteine levels had more cortical and hippocampal atrophy than those with lower levels. Previous results from the Nun study showed that cerebral atrophy was also related to folic acid levels. In a recent review article, Lokk referred to homocysteine as the most sensitive marker of cobalamin/folic acid metabolic function and the factor most strongly correlated with parameters of cognition. In contrast, Sachdev and associates, in a study that included only 36 subjects, found that homocysteine was a significant determinant of the ventricular-brain ratio (an index of brain atrophy) but that it did not contribute to white matter abnormalities or cortical atrophy. Högervorst and associates found that white matter lesions were more prevalent among patients with Alzheimer’s disease than among age- and sex-matched control subjects, and the odds ratio for leukoaraiosis increased significantly for each increase of 5 µmol/L in homocysteine level.

The relative impact of the different mechanisms by which intracellular actions of homocysteine may occur is unknown, and the final in vivo effects might depend on other related intracellular substances, products of the same metabolic pathways, such as S-adenosylhomocysteine.

Factors influencing homocysteine levels

Homocysteine levels are influenced by B vitamins, genetic factors and other factors such as age.

B vitamins

B vitamins are essential nutrients because of their role in the synthesis and repair of DNA. The B vitamins cobalamin, vitamin B6, and folic acid affect homocysteine levels through their roles as cofactors for the enzymes involved in methionine metabolism. Their levels are inversely related, with plasma homocysteine levels increasing as vitamin B concentrations decrease.

Epidemiological studies have shown that the prevalence of cobalamin deficiency increases with age. With a normal cutoff point of 200 pg/mL (150 pmol/L), the prevalence of low serum cobalamin levels among elderly people ranges from 6% to 16%. Cobalamin deficiency may cause multiple abnormalities, including megaloblastic anemia, gastrointestinal changes, peripheral neuropathy, subacute combined degeneration, depression and cognitive impairment. Traditionally, megaloblastic anemia has served as a surrogate marker for clinical investigation of cobalamin deficiency. However, the effects of cobalamin deficiency at the hematological and neurological levels are not parallel; in fact, the concurrent presentation of both hematological and neurological abnormalities is found in only 41% of cases. Moreover, serum cobalamin levels are not the best indicators of cobalamin function at the tissue level. Impaired cellular cobalamin and folic acid function causes an increase in homocysteine, methylmalonic acid and methylicic acid levels. Elevated levels of any of these metabolites can be found in up to 30% of healthy elderly people living in the community and will return to normal after supplementation therapy. In a cohort study, participants who were taking 25 µg or more of cobalamin daily had a significantly lower risk of elevated metabolite levels than participants who did not take multivitamin supplements. Moreover, in a randomized, placebo-controlled pilot study of treatment with monthly injections of cobalamin for 6 months, we found that homocysteine levels were significantly lower in the treatment group than in the placebo group, even though all of the subjects had normal or elevated red blood cell (RBC) folate levels.

Pyridoxine (vitamin B6) is a cofactor in the metabolism of homocysteine through the transsulfuration pathway (Fig. 1). The impact of vitamin B6 treatment on homocysteine levels is controversial. In one study high-dose vitamin B6 therapy was reported to be effective in reducing cardiovascular events in patients with elevated homocysteine levels due to cystathionine B synthase abnormalities; however, such treatment was not effective in reducing elevated homocysteine levels in adults with mild elevations in homocysteine. In a recent case–control study, Kelly and associates found a significantly strong independent association between low levels of vitamin B6 and stroke or transient ischemic attack but not between elevated homocysteine levels and vascular events.

Effects of folic acid fortification of grain products

Folic acid fortification of grain products to prevent neural tube defects in newborns became mandatory in Canada and the United States in 1998. This has resulted in
increased levels of serum folic acid and of RBC folate in the entire population. The effects of these increases on homocysteine levels in the elderly population are largely unknown, but interesting data have started to emerge. A Chilean study recently reported that folic acid fortification of flour had a moderate lowering effect on homocysteine levels in the elderly population (mean age 74.3 years). A retrospective cross-sectional study in Ontario showed that, shortly after folic acid fortification began, RBC folate and serum folate levels were higher than expected before fortification when compared with historical data. However, RBC folate and serum folate levels for that population before fortification were unavailable for comparison. A recent study showed that RBC folate levels in older people living independently in Ontario have continued to increase significantly every year since fortification was started and are currently 2.5 times higher than the levels in 1998. A study of the Framingham Offspring cohort showed that fortification with an average of 140 µg of folic acid per 100 g of cereal grain product resulted in a signifi-

Table 2: Studies investigating relation between homocysteine and cognitive function scores

| Study                  | Study population                  | Study design                         | Cognitive assessment           | Results                                                                 | Comment                                                   |
|------------------------|-----------------------------------|--------------------------------------|--------------------------------|------------------------------------------------------------------------|------------------------------------------------------------|
| Riggs et al, 1996      | 70 people aged 54–81 yr in        | Cross section of prospective cohort  | CERAD, NES2                    | tHcy levels > 12.6 µmol/L associated with poorer spatial copying skills. | Concluded that tHcy may have differential effects on cognitive abilities |
|                        | Normative Aging Study             |                                      |                                | No relation found between tHcy levels and perceptual speed, spatial reasoning or language abilities |                                                            |
| Ravaglia et al, 2000   | 54 people aged > 65 yr in          | Cross section of prospective cohort  | MMSE, clock-drawing test, prose memory test, Corsi block-tapping task, Mental Deterioration Battery | No association found between tHcy levels and cognitive test scores | Results adjusted for age, sex, education level, smoking status, alcohol or coffee consumption, and previous cardiovascular disease |
| Budge et al, 2002      | 158 community-dwelling people aged 60–91 yr | Cross section of prospective cohort  | CAMCOG, MMSE, GDS              | Higher tHcy levels associated with lower memory scores per µmol/L (OR 1.15, 95% CI 1.10–1.27) | OR adjusted for age, sex, serum cystatin C level and systolic blood pressure |
| Duthie et al, 2002     | 334 community-dwelling people who had participated in Scottish Mental Surveys of 1932 and 1947 | Cross section | MMSE, NART, RPM, AVLT, WAIS | tHcy levels negatively associated with scores on RPM, WAIS in older cohort with higher tHcy levels (mean 10.9 µmol/L, 95% CI 10.1–11.5) | Results adjusted for childhood intelligence quotient |
| Prins et al, 2002      | 1077 people aged 60–90 yr in Rotterdam Scan Study | Cross section of prospective cohort  | Abbreviated Stroop test, Letter-Digit Substitution Task, Verbal fluency test, PPMST, Modified Rey's test | Patients with tHcy > 14 µmol/L had lower scores for global cognitive function (difference –0.20, 95% CI –0.30 to –0.11) | Results adjusted for age, sex, education level, depression, serum creatinine level |
| Miller et al, 2003     | 1789 community-dwelling people aged ≥ 60 yr in Sacramento Area Latino Study on Aging | Cross section of prospective cohort  | 3MSE, verbal and visual memory tests, object naming, conceptualization and attention span tests | Inverse relation between tHcy levels and scores on 3MSE (p = 0.02), picture association (p = 0.05), verbal attention span (p = 0.04) and pattern recognition tests (p = 0.001) | Multiple linear regression model included folate, cobalamin, creatinine, age, sex, education and acculturation |
significant increase in RBC folate levels and a decrease in the proportion of people with suboptimal RBC folate levels. To determine the effects of mean increase in folic acid intake that was about twice as large as previously projected. The same authors estimated that fortification resulted in a proportion of people with suboptimal RBC folate levels.60,61 cant increase in RBC folate levels and a decrease in the proportion of people with suboptimal RBC folate levels.60,61

Genetic factors

Remethylation of homocysteine to methionine requires the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the carbon donor in the remethylation reaction. A common MTHFR gene mutation (C-to-T substitution at codon 677, or C677T) results in increased thermolability and decreased activity of the MTHFR enzyme. People

Table 2 continued

| Study | Study population | Study design | Cognitive assessment | Results | Comment |
|-------|------------------|--------------|----------------------|---------|---------|
| Ravaglia et al, 2003 | 650 community-dwelling people aged 65–91 yr (mean 73 yr) with normal cognitive function in Conselice Study | Population-based study | MMSE | Inverse relation between odds of tHcy level > 15 µmol/L and MMSE scores | Results adjusted for age, education level, income, serum creatinine level, serum vitamin B index, active lifestyle, and coffee and meat consumption |
| Garcia et al, 2004 | 281 community-dwelling volunteers aged > 65 yr | Cross section | Stroop, Mattis DRS, CVLT | Subjects with elevated tHcy levels (> 13.9 µmol/L) had lower Stroop scores than those with normal tHcy levels in univariate analysis (p < 0.05) | Strongest association found between methylcitric acid and cognitive scores |
| Kalmijn et al, 1999 | 702 community-dwelling people aged > 55 yr in Rotterdam Study | Prospective cohort; mean follow-up | MMSE | No association between tHcy level and cognitive impairment (highest vs. lowest tertile, OR 0.91, 95% CI 0.52–1.58) | OR adjusted for age, sex, education level and baseline MMSE score |
| McCaddon et al, 2001 | 32 community-dwelling people aged > 65 yr | Prospective cohort; 5-yr follow-up | MMSE, ADAS-Cog | Baseline tHcy predicted MMSE scores (p = 0.001) and ADAS-Cog scores (p = 0.01) at 5-yr follow-up | Results adjusted for age and education. |
| Dufouil et al, 2003 | 1241 people aged > 60 yr in Epidemiology of Vascular Aging Study | Prospective cohort; 4-yr follow-up | MMSE, Trail Making Test Part B, Digit Symbol Substitution Test from the WAIS, Finger Tapping Test | Odds of cognitive decline 2.8 (95% CI 1.2–6.2) in patients with tHcy level ≥ 15 µmol/L | OR adjusted for age, sex, education level, baseline cognition, body mass index, alcohol consumption, smoking, hypertension, hypercholesterolemia, glycemia status, history of vascular disease, and folate and B12 levels |
| Teunissen et al, 2003 | 144 normal aging adults (30–80 yr at baseline) | Prospective cohort; 6-yr follow-up | WLT, LDCT, Stroop | tHcy levels inversely correlated with performance on WLT at baseline but not at follow-up. No relation between tHcy levels and results of other cognitive tests | Results adjusted for age, sex and education level |
| Garcia et al (in press) | 180 normal community-dwelling people aged ≥ 65 yr | Prospective cohort; mean follow-up | Stroop, Mattis DRS, CVLT | Rate of increase in tHcy levels correlated with rate of decline in Stroop scores | Results adjusted for age, education level, time interval between 2 visits, cobalamin and red blood cell folate levels, diabetes and hypertension |

Note: ADAS-Cog = Alzheimer’s Disease Assessment Scale, cognitive component, AVL = Auditory Verbal Learning Test, CERAD = Consortium to Establish a Registry for Alzheimer’s Disease, CAM = Cambridge Examination for Mental Disorders of the Elderly, CVLT = California Verbal Learning Test, CDS = Geriatric Depression Scale, LDCT = Letter-Digit Coding Test, Mattis DRS = Mattis Dementia Rating Scale, MMSE = Mini-Mental Status Exam, NART = National Adult Reading Test, NES = Neurobehavioural Evaluation System, NINCDS-ADRDA = National Institute of Neurological and Communication Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association, PPMST = Paper and Pencil Memory Scanning Task, RPM = Raven’s Progressive Matrices, Stroop = Stroop Colour-Word Test, 3MSE = Modified Mini-Mental Status Exam, TICS-M = Telephone Interview of Cognitive Status, WAIS = Wechsler Adult Intelligence Scale, WLT = Word Learning Test.
homozygous for the C677T variant exhibit significantly reduced MTHFR activity and higher levels of homocysteine than do heterozygous and normal subjects.62 The MTHFR C677T mutation is common and has been described in younger patients with cardiovascular disease.63 Although this mutation might increase the risk of stroke and vascular dementia,64 its influence on homocysteine levels in the elderly population is unclear.65 There is evidence to suggest that the effects of this mutation on homocysteine levels may be most evident when they are combined with low folate levels, and possibly also low riboflavin levels, in the younger population.66–68 Although homozygous patients have a shorter life expectancy than heterozygous patients, it is reasonable to expect that heterozygous subjects might reach older age and have higher homocysteine levels than subjects without the mutation. The prevalence of the heterozygous mutation among Canadians over 55 years old has been estimated to be about 41%.69 The relation of this mutation to dementia is being investigated.

Other factors

Besides genetic factors and those related to B vitamins, age is the single most important determinant of homocysteine levels in the general population. Levels remain relatively stable during the first 4 decades of life and increase steadily thereafter, particularly after the age of 70.70,71 Homocysteine levels in people over 85 are on average double the levels in people less than 40, and the prevalence of elevated homocysteine levels is significantly higher among older people than among younger people.71 Sex has an influence on homocysteine levels among younger adults, with men having slightly higher levels on average than women.71 This sex-specific difference may be due to estrogen levels. One study showed that the mean serum total homocysteine level was significantly lower among postmenopausal women who were taking estrogen replacement therapy (9.5 µmol/L [95% CI 8.9–10.1]) than among postmenopausal women who were not taking estrogen therapy (10.7 µmol/L [95% CI 10.3–11.1]) and men in the same age range (10.4 µmol/L [95% CI 9.8–11.0]).72

Other factors that may have a significant impact on homocysteine levels in specific population groups include renal function, serum albumin levels and the use of diuretics.73

**Conclusion and future directions**

There is evidence that elevated homocysteine levels are related to vitamin B deficiency, cognitive decline and dementia, but there is no proof at present that treatment with B vitamins will reverse cognitive deterioration or dementia, even though it might return homocysteine levels to normal. Malouf and associates74 recently reviewed the results of 2 pilot studies of B vitamins in patients with dementia. No significant cognitive improvements were found with this treatment despite successful reduction of homocysteine levels. More definitive answers should come from an ongoing long-term double-blind, multicentre, randomized placebo-controlled trial involving patients with dementia that will be completed in 2005/06.

Given our knowledge of the mechanisms of action of homocysteine, it is possible to speculate that the effects of elevated homocysteine on the brain may be irreversible, in which case, once the pathological changes have appeared, the
beneficial effects of vitamin supplementation therapy in patients with dementia would be limited. However, as with risk factors for other medical conditions, detection of elevated homocysteine levels in older adults and treatment with B vitamins at an early stage, before cognitive decline is clinically apparent and pathological changes have appeared, may be an effective intervention. Such primary prevention trials are worth investigating.

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