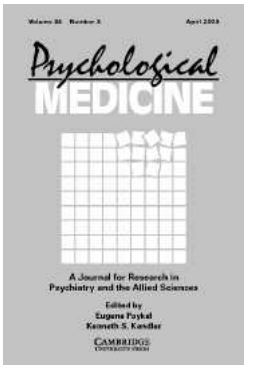

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Relationship of homocysteine, folic acid and vitamin B_{12} with depression in a middle-aged community sample

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ABSTRACT

Background. Case control studies have supported a relationship between low folic acid and vitamin B_{12} and high homocysteine levels as possible predictors of depression. The results from epidemiological studies are mixed and largely from elderly populations.

Method. A random subsample of 412 persons aged 60–64 years from a larger community sample underwent psychiatric and physical assessments, and brain MRI scans. Subjects were assessed using the PRIME-MD Patient Health Questionnaire for syndromal depression and severity of depressive symptoms. Blood measures included serum folic acid, vitamin B_{12}, homocysteine and creatinine levels, and total antioxidant capacity. MRI scans were quantified for brain atrophy, subcortical atrophy, and periventricular and deep white-matter hyperintensity on T2-weighted imaging.

Results. Being in the lowest quartile of homocysteine was associated with fewer depressive symptoms, after adjusting for sex, physical health, smoking, creatinine, folic acid and B_{12} levels. Being in the lowest quartile of folic acid was associated with increased depressive symptoms, after adjusting for confounding factors, but adjustment for homocysteine reduced the incidence rate ratio for folic acid to a marginal level. Vitamin B_{12} levels did not have a significant association with depressive symptoms. While white-matter hyperintensities had significant correlations with both homocysteine and depressive symptoms, the brain measures and total antioxidant capacity did not emerge as significant mediating variables.

Conclusions. Low folic acid and high homocysteine, but not low vitamin B_{12} levels, are correlates of depressive symptoms in community-dwelling middle-aged individuals. The effects of folic acid and homocysteine are overlapping but distinct.

INTRODUCTION

Patients with depression have been noted in several case control studies to have a high prevalence of folic acid and vitamin B_{12} deficiency (Crellin *et al.* 1993; Alpert *et al.* 2000). The functional significance of these findings is controversial, as the deficiencies could conceivably be a consequence of depression rather than its cause. In support of the relevance of folic acid deficiency for depression are the findings that folic acid replacement assists recovery from depression (Coppen *et al.* 1986; Godfrey *et al.* 1990). The biological mechanism for this has been suggested to be the involvement of folic acid and B_{12} in one-carbon metabolism which is directly relevant to the production of

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monoamine neurotransmitters and other important methylation reactions in the brain (Reynolds *et al*. 1984).

The role of folic acid and vitamin B_{12} in one-carbon metabolism is intimately related to their involvement in homocysteine metabolism, such that total plasma homocysteine level is considered to be a sensitive marker of the functional deficiency of these vitamins (Reutens & Sachdev, 2002). Homocysteine is a sulphur-containing amino acid that is proatherogenic, prothrombotic and cytotoxic through its tendency to increase oxidative stress and induce DNA strand breakage and apoptosis (Mattson & Shea, 2003). Homocysteine has been demonstrated to be a risk factor for stroke and has been associated with brain atrophy and leukoaraiosis (Sachdev *et al*. 2002, 2004). Since both large and small vessel diseases of the cerebral vasculature have been associated with depression (Krishnan *et al*. 1997), homocysteine is arguably a risk factor for depression through this mechanism. Patients with severe depression have been noted to have raised homocysteine levels (Bottiglieri *et al*. 2000), and it has been suggested that this may be due to higher rates of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) enzyme gene (Hickie *et al*. 2001). Hyperhomocysteinaemia was, however, not associated with depressive subtype or treatment response in one study (Fava *et al*. 1997).

The above findings are from case control studies in patient populations. The results of population-based studies, particularly in the elderly, have been mixed. In the Women's Health and Aging Study (Penninx *et al*. 2000), serum homocysteine levels, folic acid levels and the prevalences of folic acid deficiency and anaemia were not associated with depression status, but those with vitamin B_{12} deficiency had a two-fold increased risk of severe depression. In the Rotterdam Study (Tiemeier *et al*. 2002), high homocysteine and low folic acid and B_{12} levels were all associated with depression, but the underlying mechanisms were considered to be different. More recently, Almeida *et al*. (2004) reported increased levels of homocysteine in older women with higher levels of depression.

In view of the above findings, we measured homocysteine, folic acid and B_{12} levels in a community sample of middle-aged individuals, and examined such variables as physical health, total antioxidant capacity, brain atrophy and white-matter hyperintensities (WMHs) as possible mediating variables in any association of these with depression.

METHOD

Sample

The sample was drawn from the Personality and Total Health (PATH) Through Life Project designed to study the risk and protection factors for normal ageing, dementia and other neuropsychiatric disorders (Sachdev *et al*. 2004). The study cohort comprised 2551 individuals who were residents of the city of Canberra and the adjacent town of Queanbeyan, Australia. They had a mean of 13·77 years of education and were recruited randomly through the electoral roll. Enrolment to vote is compulsory for Australian citizens. The response rate was 58·3% for the total sample. About one subject in five was selected at random for participation in this substudy, which involved providing a blood sample and undergoing a MRI scan. Of 622 participants so approached, 412 (66·2%) [men = 211 (51·2%)] had data on the blood chemistry variables, brain MRI and psychiatric variables to comprise the study sample. In order to assess for any systematic selection biases, we compared these 412 subjects with the 210 not included. The included subjects did not differ in age, gender, level of education and any brain measures examined, and performed similarly on the Spot-the-Word test (Baddeley *et al*. 1992) as a measure of 'pre-morbid' intelligence. However, they were more likely to have English as their first language, had better physical health, and performed better on immediate and delayed recall than those who refused participation. Approval for the study was obtained from the ethics committees of the Australian National University, Canberra, and the University of New South Wales, Sydney, Australia.

Psychiatric and physical health

Most participants were interviewed at home by a professional survey interviewer, but some chose to be interviewed at the Centre for Mental Health Research, Australian National University. Participants were asked to complete a questionnaire on a Hewlett-Packard 620LX

palmtop personal computer using the Surveycraft software (SPSS Inc., Chicago, IL, USA) for computer-assisted personal interviewing. The interview covered sociodemographic characteristics, anxiety and depression, substance abuse, cognitive function, well-being, physical health, health habits, use of health services, personality, coping, early life psychosocial risk factors, current psychosocial risk factors and nutrition.

As part of the community survey, participants completed the depression section of the Primary Care Evaluation of Mental Disorders (PRIME-MD) Patient Health Questionnaire (PHQ) which asks about DSM-IV Major Depression symptoms in the last 2 weeks (Spitzer *et al.* 1999). This questionnaire had nine symptoms, each scored on a scale of 1–4. A depressive symptoms score indicating severity is derived by summing these items (range 0–27). The PHQ office coding algorithm was used to classify participants as having a depressive syndrome (major or other). Participants were also asked 'In the last month have you taken or used any medications (including herbal remedies) for depression?' In some of the analyses reported below, those who responded 'yes' were grouped together with those having a depressive syndrome on the PHQ.

Physical disability was assessed using the Physical Component Summary of the 12-item Short Form Health Survey (SF-12) (Ware *et al.* 1996). This is a self-report questionnaire that rates the subject's limitation in various activities (vigorous, e.g. running; moderate, e.g. vacuum cleaning or bowling; lifting groceries; climbing stairs; bending or kneeling; walking from 100 m to >1 km; bathing or dressing; performing regular work).

Blood measurements

Blood samples were obtained after overnight fasting for biochemical measurements. For total homocysteine, blood was centrifuged within minutes of collection and the plasma stored at −70 °C for later measurement. Total homocysteine was measured by reverse phase high performance liquid chromatography (HPLC) with fluorescence detection after derivatization with 4-(aminosulphonyl)-7-fluorobenzo-2-oxa-1,3-diazole (reference range: women <12 μmol/l; men <15 μmol/l). Bio-Rad Homocysteine by HPLC reagent kits were used (Bio-Rad Laboratories, Sydney, Australia) on a Shimadzu HPLC system (Shimadzu Scientific Instruments, Sydney, Australia). Serum folic acid (reference range: 5·5–33·3 nmol/l) and vitamin B_{12} (97–394 pmol/l) were determined by immunoassay on an Immulite 2000 analyzer (Bio-Mediq DPC, Melbourne, Australia). Serum creatinine (60–110 μmol/l) was measured by the Beckman LX20 analyser (Beckman Coulter, Sydney, Australia). Total antioxidant capacity was measured by chemiluminescence detection in a BMG Fluostar Optima plate reader, using ABEL wide-range antioxidant kit (Knight Scientific Ltd, Plymouth, UK).

MRI scans

All subjects were imaged with a 1·5 T Philips Gyroscan ACS-NT scanner (Philips Medical Systems, Best, The Netherlands) for T1-weighted 3D structural and T2-weighted fluid attenuated inversion recovery (FLAIR) sequence MRI. A scout mid-sagittal image was first acquired to locate anterior to posterior commissure (AC-PC) plane. The T1-weighted MRI was acquired in coronal orientation using a T1-FFE sequence with the following parameters: repetition time (TR)/echo time (TE) = 28·05/2·64 ms, flip angle = 30°, matrix size = 256 × 256, field of view (FOV) = 260 × 260 mm, slice thickness = 2·0 mm and mid-slice to mid-slice distance = 1·0 mm, yielding over-contiguous coronal slices. The FLAIR sequence was acquired in coronal orientation with TR/TE/TI = 11000/140/2600 ms, matrix size = 256 × 256, FOV = 230 × 230 mm, slice thickness = 4·0 mm with no gap between slices.

Image analysis

MRI scans were transferred to an independent Windows NT workstation and analysed using the software packages ANALYZE (Mayo Foundation, Rochester MI, USA) and SPM99 (Cognitive Neuroscience Group, National Hospital for Nervous Diseases, London, UK). The intracranial and total brain volumes were computed automatically using an algorithm within SPM99. The difference of the two was divided by the intracranial volume (ICV) to yield the brain atrophy index. The anterior- and mid-ventricular ventricle-to-brain ratios (VBRs) were measured using the method of Victoroff *et al.* (1994). High intra-rater and inter-rater reliability

[intra-class correlation coefficients (ICC) $>0{\cdot}9$] were established for each of the above measures. WMHs were identified on FLAIR sequences. A special computer program was written by one of us (W.W.) to automatically delineate WMHs in both the periventricular and deep white-matter regions (Wen & Sachdev, 2004). The absolute volume of total white matter and WMHs were determined, and the percentage of white matter with a hyperintense signal was calculated for each subject. Both absolute and relative volumes were used in the analyses. Twenty scans were processed twice to determine the re-test reliability of the procedure, and 100% correspondence was noted. For concurrent validity, the scans were visually rated by two independent clinicians experienced in examining MRI scans on a modified Fazekas scale (Fazekas *et al.* 1987). In total, 100 scans were visually rated and the ICC test between the automated measures and the visual ratings showed a modest correspondence, with ICC = 0·43 ($F=1{\cdot}76$, df = 99, $p=0{\cdot}003$) for the whole brain WMH, ICC = 0·63 ($F=2{\cdot}74$, df = 99, $p=0{\cdot}000$) for WMH in the deep white-matter areas and ICC = 0·59 ($F=2{\cdot}44$, df = 99, $p=0{\cdot}000$) for periventricular WMH. Pearson correlations were also carried out and the results were: whole brain, $r=0{\cdot}791$ ($p=0{\cdot}000$); deep white matter, $r=0{\cdot}724$ ($n=100$, $p=0{\cdot}000$); periventricular, $r=0{\cdot}717$ ($n=100$, $p=0{\cdot}000$).

Analysis of data

The measure of depressive symptoms endorsed on the PRIME-MD was the main dependent variable of interest. In addition, syndromal depression was diagnosed if the subject met DSM-IV criteria for major or other depression or had taken antidepressant medication in the previous month. The predictors of interest were homocysteine, folic acid and vitamin B_{12} levels and these were divided into quartiles for analyses, which is consistent with previous publications in this field (Bottiglieri *et al.* 2000; Almeida *et al.* 2004). Predictors of depression symptom scores were evaluated using negative binomial regression, which was used in preference to linear regression because of the extreme skew of the score distribution. For the negative binomial regressions, effect size was measured by incidence rate ratios (IRRs), which give the rate of increase in depression symptoms for each unit increase in the predictor variable (Kelsey *et al.* 1996). Adjustment was made for sex, physical health index on the SF-12, smoking and serum creatinine levels. Predictors of depression caseness were evaluated using logistic regression, with the odds ratio used as the index of effect size. The analysis for folic acid and vitamin B_{12} were performed with and without an adjustment for homocysteine. Finally, path analyses were performed to determine which mediating variables could explain the association between predictor variables and the dependent variable (depressive symptoms score).

RESULTS

Of the 412 participants, 211 (51·2%) were men, and the mean age was 62·54 years. The characteristics of the sample are described in Table 1. The mean depressive symptoms score on the PHQ was 2·10 (S.D. = 2·82) in men and 3·07 (S.D. = 3·07) in women, the difference being non-significant. The overall depressive symptoms score was 2·30 (S.D. = 2·95). Depression was diagnosed in 16 (3·9%) cases, and an additional 20 (4·9%) had taken an antidepressant medication and did not have a full depressive syndrome at the time of interview. When cases were compared with non-cases, the only variables they significantly differed on were folic acid levels and physical health. A similar result was obtained when depressive symptoms rather than depressive syndrome were used for determination of the associations.

Analyses were performed using depressive symptom score as the dependent variable. Homocysteine levels had a significant linear relationship with depressive symptoms score in men but not in women (Fig. 1). Being in the lowest quartile of total homocysteine was associated with lower depressive symptoms score (IRR = 0·72, $p<0{\cdot}05$), and this was significant after adjusting for folic acid and vitamin B_{12} levels (Table 2). Being in the lowest quartile of folic acid, on the other hand, was associated with increased number of depressive symptoms (IRR = 1·35, $p<0{\cdot}05$). Adjustment for total homocysteine levels reduced the IRR for folic acid to a marginal level. Vitamin B_{12} levels did not have a significant association with depressive symptoms.

Table 1. *Sociodemographic attributes, health, blood and brain measures by depressive symptoms score on PRIME-MD Patient Health Questionnaire*

Attribute	All participants ($n=412$)	Depressive symptoms score: None ($n=147$)	One ($n=75$)	Two ($n=47$)	Three ($n=44$)	Four or five ($n=52$)	Six or more ($n=47$)	
Number (%)	412 (100)	147 (35·4)	75 (17·1)	47 (12·0)	44 (9·8)	52 (12·2)	47 (13·2)	*p*
% Female	48·79	41·50	53·33	55·32	47·73	53·85	53·19	0·364
Mean age (S.D.), years	62·54 (1·45)	62·47 (1·51)	62·44 (1·28)	62·57 (1·60)	62·98 (1·39)	62·54 (1·51)	62·49 (1·33)	0·444
% currently smoking	7·52	6·12	5·33	4·26	6·82	7·69	19·15	0·055
% ever smoked	35·19	34·69	36·00	34·04	47·73	30·77	29·79	0·532
Number of life events in past 6 months (S.D.)	0·84 (1·06)	0·69 (0·90)	0·88 (1·22)	0·91 (0·93)	0·86 (1·09)	0·81 (1·07)	1·18 (1·32)	0·158
SF-12 Physical health (S.D.)	49·85 (8·70)	52·63 (6·04)	51·67 (7·76)	46·50 (10·01)	47·18 (8·73)	47·99 (9·89)	46·00 (10·88)	0·001
Blood measures								
Folate (nmol/l)	24·23 (10·49)	24·22 (10·65)	25·26 (9·47)	25·09 (10·19)	26·28 (11·91)	24·99 (11·10)	18·92 (8·29)	0·011
Vitamin B_{12} (pmol/l)	305·16 (136·92)	288·39 (132·02)	311·67 (147·26)	312·48 (116·38)	339·79 (165·83)	302·50 (137·17)	310·26 (122·52)	0·382
Total antioxidant capacity (μmol/l)	352·36 (73·72)	352·72 (74·03)	356·64 (81·96)	345·87 (60·01)	351·00 (77·59)	353·12 (71·10)	351·32 (73·81)	0·986
Creatinine (μmol/l)	80·42 (17·26)	81·13 (18·21)	82·03 (14·22)	78·09 (18·02)	81·09 (16·19)	79·25 (19·49)	78·66 (16·63)	0·771
Homocysteine (μmol/l)	12·24 (3·99)	12·56 (4·10)	11·17 (2·74)	11·74 (4·08)	12·65 (3·73)	12·65 (4·85)	12·65 (4·28)	0·128
Brain measures								
Brain atrophy index	0·210 (0·022)	0·210 (0·022)	0·206 (0·020)	0·214 (0·022)	0·214 (0·027)	0·209 (0·021)	0·213 (0·023)	0·385
Mid-ventricular VBR	0·184 (0·036)	0·186 (0·039)	0·179 (0·030)	0·179 (0·027)	0·190 (0·037)	0·181 (0·040)	0·187 (0·035)	0·535
Periventricular WMH/Total WM	0·0064 (0·0045)	0·0063 (0·0042)	0·0055 (0·0028)	0·0070 (0·0049)	0·0050 (0·0031)	0·0072 (0·0057)	0·0083 (0·0060)	0·003
Deep WMH/Total WM	0·0041 (0·0083)	0·0037 (0·0060)	0·0028 (0·0033)	0·0041 (0·0057)	0·0030 (0·0039)	0·0068 (0·0149)	0·0060 (0·0134)	0·057

VBR, ventricle-to-brain ratio; WMH, white-matter hyperintensity; WM, white matter.

Table 2. *Associations between depressive symptoms score as dependent variable and select blood measures*

	Incidence rate ratio (95% CI)*	*p*
Adjusting for sex, physical health and smoking		
Lowest quartile homocysteine	0·72 (0·54–0·98)	0·037
Lowest quartile folate	1·35 (1·03–1·79)	0·033
Lowest quartile vitamin B_{12}	0·83 (0·62–1·11)	0·214
Adjusting for sex, physical health, smoking, highest quartile creatinine, and lowest quartiles folate and vitamin B_{12}		
Lowest quartile homocysteine	0·71 (0·52–0·96)	0·027
Adjusting for sex, physical health, smoking, highest quartile creatinine		
Lowest quartile folate	1·36 (1·03–1·79)	0·032
Lowest quartile vitamin B_{12}	0·83 (0·62–1·11)	0·205
Adjusting for sex, physical health, smoking, highest quartile creatinine and lowest quartile homocysteine		
Lowest quartile folate	1·32 (0·99–1·74)	0·054
Lowest quartile vitamin B_{12}	0·82 (0·61–1·10)	0·182

* Using negative binomial regression.

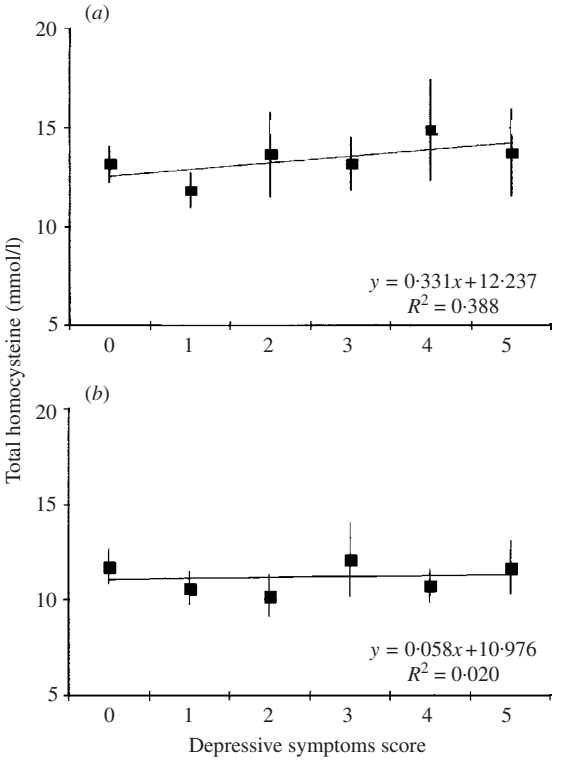

FIG. 1. Mean homocysteine levels by depressive symptoms score on the PRIME-MD Patient Health Questionnaire for (*a*) men and (*b*) women.

Regression analyses were performed using depressive syndrome as the dependent variable and homocysteine, folic acid and vitamin B_{12} levels as the predictor variables. The analyses controlled for sex, physical health, smoking and creatinine levels, and in the case of homocysteine, for folic acid and vitamin B_{12} levels as well. None of the predictor variables emerged as being significant.

Path analyses were carried out to determine if the association of folic acid to depressive symptoms score was possibly mediated through homocysteine and vice versa, and whether the association of homocysteine with severity of depressive symptoms was mediated through biologically plausible factors such as WMHs and brain atrophy. A number of models were examined, with the predictor, mediating and dependent variables as follows: (i) total antioxidant capacity as mediating between homocysteine and depressive symptoms; (ii) homocysteine as mediating between folic acid and depressive symptoms; (iii) homocysteine as mediating between vitamin B_{12} and depressive symptoms; (iv) brain atrophy as mediating between homocysteine and depressive symptoms; (v) subcortical atrophy (as measured by VBR) as mediating between homocysteine and depressive symptoms; (vi) deep WMHs as mediating between homocysteine and depressive symptoms; and (vii) periventricular WMHs as mediating between homocysteine and depressive symptoms. A mediating variable must be significantly associated with both the dependent and independent variables (i.e. the dependent variable regressed on the independent variable must yield a significant regression coefficient or β weight), and the product of the β weights must

be significantly different from zero (MacKinnon *et al*. 2002). This criterion was not met by any of the potential mediating variables examined. Deep WMHs score, a possible marker of small vessel disease in the brain, was significantly correlated with depression ($r=0{\cdot}144$, $p=0{\cdot}003$) as well as homocysteine levels ($r=0{\cdot}133$, $p=0{\cdot}007$), but it was not significant in the path analyses.

DISCUSSION

This study showed an association of high homocysteine and low folic acid levels with an increased severity of depressive symptoms in an epidemiological sample of middle-aged individuals. The same result was not seen with syndromal depression as the dependent variable. This discrepancy is explained by the fact that the prevalence of depression in our study was low, thereby reducing the power to detect a significant association. Indeed, the odds ratio for being a case of depression when in the lowest quartile for homocysteine was 0·58, but the 95% confidence interval was 0·12–2·67. In an epidemiological sample such as in this study, the low point-prevalence of depression is not surprising. In the Rotterdam Study (Tiemeier *et al*. 2002), the prevalence of depressive syndrome using a screening instrument was 7·0%. We therefore used the rating of depressive symptoms as an index of depression severity.

Our findings are partially consistent with some other epidemiological evidence in this field. In a recent study of older women with a mean age of 74·7 years (Almeida *et al*. 2004), graded homocysteine levels were associated with depression scores on a self-report measure, after adjusting for confounding variables. The Rotterdam Study (Tiemeier *et al*. 2002) found high homocysteine, low vitamin B_{12} and, to a lesser extent, low folic acid levels to be associated with syndromal depression in individuals with a mean age of 71·2 years. The association was significant only for vitamin B_{12} after adjustment was made for functional disability and cardiovascular risk factors. In a study of community-dwelling, physically disabled women with a mean age of 77·3 years (Penninx *et al*. 2000), serum homocysteine and folic acid levels were not associated with syndromal depression, but metabolically significant vitamin B_{12} deficiency was associated with a twofold increased risk of severe depression.

A noteworthy finding of our study was the gender difference in the relationship of homocysteine with depression, which was significant for men but not for women. We considered the following possible explanations for this finding: (i) since men had higher homocysteine levels than women (mean levels 13·14 *v*. 11·12 μmol/l, $p<0{\cdot}001$), it is possible that the relationship is apparent at the higher levels; (ii) the aetiology of depressive symptoms is different in men and women, with there being greater heterogeneity in women; (iii) our study also found gender differences in the aetiology of WMHs (Sachdev *et al*. 2004), indicating that the mediating variables may be different in men and women, leading to different patterns of relationship.

All the above studies, including the present one, are cross-sectional, and any causal inferences should therefore be drawn with great caution. The differences between our study and two others that examined vitamin B_{12} are noteworthy. Methodological differences must be pointed out. Our study had a younger sample and depressive symptoms rather than depressive syndrome was the main variable of interest. It is possible that vitamin B_{12} deficiency is a significant factor in depression of old age, especially for severe depression which was the case in the Women's Health and Aging Study (Penninx *et al*. 2000). The possibility that it is a consequence of depression rather than its cause cannot be ruled out, as depression reduces appetite and food intake and may limit the varieties of food consumed (Bottiglieri, 1996). If vitamin B_{12} deficiency indeed causes depression, then its dissociation from folic acid deficiency suggests that their mechanisms of relatedness to depression are different. In addition to its role in the one-carbon cycle, vitamin B_{12} plays a role in the methylmalonic acid pathway, and its deficiency leads to a rise in levels of methylmalonic acid. Depressed subjects have been reported to have higher methylmalonic acid levels (Penninx *et al*. 2000). While we did not measure this metabolite in our study, our finding of a lack of significance of low vitamin B_{12} levels suggests that it is probably the one-carbon cycle, with its stronger relationship to folic acid levels, that is of greater salience in depression. Another function of vitamin B_{12} that may be

relevant for depression is its role in myelination (Hall, 1990).

Our finding of the relationship of folic acid levels to depressive symptoms is consistent with previous work in this field (Crellin *et al.* 1993; Alpert *et al.* 2000). Indeed, folic acid has been shown to enhance the therapeutic effect of antidepressants (Godfrey *et al.* 1990) and the prophylactic effects of lithium (Coppen *et al.* 1986). The relationship of folic acid and depressive symptoms in our study was significant after adjusting for confounding factors such as sex and physical health. Adjusting for homocysteine levels marginally affected this relationship, but homocysteine did not emerge as a mediating factor. On the other hand, homocysteine was an independent predictor of depressive symptoms after accounting for folic acid and vitamin B_{12} levels, even though homocysteine had significant correlations with folic acid ($r = -0{\cdot}279$) and vitamin B_{12} ($r = -0{\cdot}315$) levels. One limitation of our data is that we did not measure red cell and cerebrospinal fluid folic acid levels which are considered to be more reliable indicators of cerebral folic acid status (Bottiglieri *et al.* 2000). Nevertheless, the independence of folic acid and homocysteine in their relationship to depression is a noteworthy finding.

The basis of the link between folic acid deficiency and depression has been considered to be a failure of methylation, due to a shortage of the supply of methyl groups from 5-methyltetrahydrofolate. Since this metabolic pathway is closely linked to the conversion of homocysteine to methionine, folic acid and homocysteine are functionally related in this process, and high homocysteine is a sensitive marker of folic acid deficiency. Methionine is the precursor of *S*-adenosylmethionine (SAM) which reportedly has effects on mood (Bottiglieri *et al.* 1994). It is also the methyl donor in many reactions in the brain involving neurotransmitters, nucleoproteins and membrane phospholipids (Reynolds *et al.* 1984). Folic acid and homocysteine are closely linked in this metabolic pathway, and supplementation with folic acid is an effective method of reducing homocysteine levels (Stein & McBride, 1998). It has also been suggested that folate deficiency may impair the synthesis of tetrahydrobiopterin, a cofactor essential for the synthesis of 5-HT and other monoamines involved in the pathogenesis of depression (Coppen *et al.* 1989).

There are other mechanisms by which homocysteine may cause depression. High homocysteine is a risk factor for atherosclerosis and stroke (Boushey *et al.* 1995) as well as small vessel disease (Sachdev *et al.* 2003), and may, thereby, increase the risk of vascular depression. It also is a risk factor for atrophy because of its ability to induce NMDA receptor-mediated excitotoxicity (Kim & Pae, 1996), produce mitochondrial dysfunction and thereby apoptosis (Kruman *et al.* 2000), and act as an oxidant (Ho *et al.* 2001). We examined brain atrophy and WMHs as possible mediating factors in the relationship between homocysteine and depression. While the correlations of WMHs with homocysteine and depression were suggestive, the path analysis did not confirm white-matter disease as a mediating variable. Total antioxidant capacity also did not emerge as a possible mediating risk or protective factor.

This study had some limitations. First, it was cross-sectional. Although we hypothesized about the mechanisms driving the associations among our variables, we recognize that relationships will need to be demonstrated at the individual level through longitudinal follow-up. Second, although the narrow age range makes the sample more homogeneous, it had the disadvantage that we were unable to examine the effects of the predictor variables in older individuals. It is possible that the brain effects of low vitamins do not manifest until much later in life, which may explain the lack of association seen with vitamin B_{12} in our study, in contrast with two other studies of somewhat older subjects (Penninx *et al.* 2000; Tiemeier *et al.* 2002). A third limitation was the non-inclusion of a number of potential participants although we did not consider this to have systematically biased our sample by excluding those who were depressed or had vitamin deficiencies. Fourth, we acknowledge the limitation of the PHQ in identifying syndromal depression. The PHQ reportedly performs well in comparison with the *Structured Clinical Interview for DSM-IV* (SCID; First *et al.* 1995) as the criterion standard for the diagnosis of DSM-IV 'major depressive disorder', but not so for 'any depressive disorder' (Lowe *et al.* 2004). However, it performs as well if not better than other self-report instruments,

and the lower operating characteristics for 'any depressive disorder' may be partially accounted for by the heterogeneous nature of 'other depressive disorders' (Lowe *et al*. 2004). Since the salient findings of our study relate to the severity of depressive symptoms, this limitation of the PHQ does not have a major impact on the results.

In conclusion, this study showed that low folic acid and high homocysteine, but not low vitamin B_{12} levels, are predictors of depressive symptoms in community-dwelling middle-aged individuals. Since their effects are to some extent independent of each other, the mechanisms of their effects are likely to be overlapping but distinct. More work is necessary in understanding these mechanisms. Our findings in subsyndromal depression suggest that there may be a role for folic acid *per se* in the prophylaxis of depression and the beneficial effect may not be restricted to the elderly population. Furthermore, attention should be paid to homocysteine levels in depressive illness and its prophylaxis. Systematic intervention studies are necessary to substantiate or refute these suggestions.

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DECLARATION OF INTEREST

None.

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