Association between depressive symptoms and serum concentrations of homocysteine in men: a population study[1–3]

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ABSTRACT

Background: Results of studies of the association between blood concentrations of homocysteine and depression in general populations and among psychiatric patients are inconsistent.

Objective: The objective was to study the association between depression and serum concentrations of total homocysteine (tHcy).

Design: A cross-sectional study of a sample of 924 men aged 46–64 y was conducted as a part of the Kuopio Ischaemic Heart Disease Risk Factor Study. Those who had a history of psychiatric disorder (6.0%) were excluded. Depressive symptoms were assessed with the 18-item Human Population Laboratory Depression Scale. Those who scored ≥5 at baseline or at the 4-y follow-up were considered to have a tendency toward depression.

Results: The participants were ranked according to their blood tHcy concentration and divided into tertiles. Those in the upper tertile for serum tHcy had a more than twofold (odds ratio: 2.30; 95% CI: 1.35, 3.90; $P = 0.002$) higher risk of being depressed than did those in the lowest tertile for serum tHcy. The results remained significant after adjustment for the month of study, history of ischemic heart disease, smoking habits, alcohol consumption, marital status, education, and socioeconomic status in adulthood (odds ratio: 2.23; 95% CI: 1.30, 3.83; $P = 0.004$).

Conclusion: High serum concentrations of tHcy may be associated with depression in middle-aged men. *Am J Clin Nutr* 2004;80:1574–8.

KEY WORDS Depression, homocysteine, folate, Kuopio Ischaemic Heart Disease Risk Factor Study

INTRODUCTION

Low blood folate and cobalamin (vitamin B-12) concentrations have been found in patients with major depression in a number of studies (1–4). Furthermore, low blood folate concentrations have been associated with a poor response to antidepressant treatment (5, 6), and in some studies there has been an inverse correlation between blood folate concentrations and the severity of depression (2, 5).

A connection between B-vitamin status and depression could be explained by direct effects of the vitamins on monoamine metabolism in the central nervous system (CNS). However, low B-vitamin status leads to hyperhomocysteinemia, which has been linked to other neuropsychiatric disorders, including Alzheimer disease. Thus, it has been suggested that hyperhomocysteinemia itself could cause or aggravate depression (7, 8). In addition to poor diet, lifestyle factors such as smoking (9) and high alcohol consumption (10) have been found to increase total homocysteine (tHcy) concentrations. Depression is also known to be associated with several lifestyle factors (11, 12). Thus, several lifestyle-connected confounders may bias the results of studies on the association between tHcy and medical conditions.

Bell et al (13) suggested that homocysteine may play a role in depression among the elderly. However, studies of the relation between tHcy and depression have been contradictory. For example, Bottiglieri et al (14) found that high concentrations of tHcy in psychiatric inpatients were associated with more severe depression as measured with the use of the Hamilton Depression Rating Scale (15), and Bjelland and coworkers (16) found an increased risk of depression in association with high plasma tHcy concentrations in the general population. Furthermore, results of a randomized placebo-controlled trial suggested that enhancement of the effectiveness of the antidepressant fluoxetine by the coadministration of folic acid (500 fg/d) was due to the reduction in tHcy. Specifically, tHcy was related to Hamilton Depression Rating Scale scores in the group treated with both folic acid and fluoxetine, but not to those in the placebo group or the group treated with fluoxetine alone (17). Several studies failed to support an independent role for elevated tHcy concentrations in depression: 3 studies found no association between tHcy and depression (6, 18, 19), and a population-based study showed the disappearance of an association between tHcy and depression after correction for cardiovascular disease risk factors and functional disability (20). In this study, we used data collected in the

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Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD Study) to examine the relation between tHcy concentrations and depression among middle-aged Finnish men.

SUBJECTS AND METHODS

Subjects

The KIHD Study is an ongoing, population-based study designed to investigate risk factors for cardiovascular disease and other outcomes in middle-aged men from eastern Finland (21). A total of 2682 participants (82.9% of those eligible), aged 42, 48, 54, or 60 y, were enrolled in the baseline study between 1984 and 1989. The study protocol was approved by the Research Ethics Committee of the University of Kuopio and Kuopio University Hospital, and the subjects gave written informed consent.

Out of a total of 1229 men invited for the 4-y follow-up of the KIHD Study, 52 had died, had severe illness, or migrated from the region, and 139 either could not be contacted or refused to participate. Of the remaining 1038 men, data were incomplete for 110 men. Those who reported having been previously diagnosed as having any psychiatric disorder were excluded ($n = 57$, 6.0% of the cohort), which left 871 men for analysis.

Measurements

The subjects came to the Research Institute of Public Health to provide venous blood samples between 0800 and 1000. They had been instructed to abstain from ingesting alcohol for the previous 3 d and from smoking and eating for 12 h before presenting. After the subjects had rested in a supine position for 30 min, blood samples were obtained by venipuncture and collected into vacuum tubes (Venoject; Terumo, Leuven, Belgium). No tourniquet was used. Blood for tHcy measurements was drawn into serum tubes. Serum was separated within 60 min and stored at −20 °C before analysis. The CV between batches ($n = 30$) for 2 pooled plasma samples was 4.3% and 5.4%. Analysis of tHcy by using HPLC was conducted in 2001 at the National Public Health Institute, Helsinki, as described by Schwab et al (22).

The serum tHcy samples were kept in storage for ≈7 y at −20 °C before analysis. The distribution of values from assays of stored samples is similar to that of values from assays of freshly drawn blood. Alfthan et al (23) tested the stability of tHcy samples that had been kept in storage for 7 y at −20 °C and thawed twice without significant deterioration. The mean serum tHcy concentration had been 9.1 μmol/L (range: 6.9–13.2 μmol/L) at baseline, and that 7 y later was 9.3 μmol/L (range: 7.5–13.2 μmol/L) (23). This suggests that the deterioration of tHcy samples was unlikely in our study.

Measurements of serum α-tocopherol, lycopene, and cholesterol concentrations were presented elsewhere (24). Serum for α-tocopherol, lycopene, and β-carotene measurements was stored at −80 °C until extracted with ethanol and hexane and measured by using an HPLC method in which α-tocopherol acetate was an internal standard (25). Lipoproteins were separated from fresh serum samples by combined ultracentrifugation and precipitation (26). The serum total cholesterol (Kone Instruments, Espoo, Finland) concentrations were measured enzymatically with the use of an autoanalyzer (Kone Specific, Kone Instruments).

Assessment of depressive symptoms

Depressive symptoms were assessed with the use of the self-administered 18-item Human Population Laboratory Depression Scale (HPL Scale), which was specifically developed for screening general population samples (27, 28) and which is highly correlated with the 21-item Beck Depression Inventory score (29, 30). The HPL Scale consists of items dealing with mood disturbance, a negative self-concept, loss of energy, problems with eating and sleeping, difficulty in concentrating, and psychomotor retardation or agitation. The HPL Scale score is generated by assigning one point for each true or false answer that is indicative of depression. After exclusion of the subjects with previous psychiatric disorder, Cronbach's α for the HPL Scale was 0.69 at baseline and 0.65 on follow-up. A cutoff of ≥5 was used earlier to define clinically significant depression (29), and we therefore applied the same cutoff in this study. Those who scored ≥5 either at baseline or on 4-y follow-up were considered to have a tendency toward depression ($n = 109$; 12.2% of participants). Poor appetite as a covariate was derived from the HPL Scale.

Other characteristics

Participants also completed questionnaires at baseline that evaluated their sociodemographic background, current smoking habits (yes or no), alcohol consumption (g/wk), marital status, and education. Marital status was converted to the variable "living alone" (living in marriage or in common-law marriage or living alone). A variety of indicators of adult socioeconomic status were available, including current income, current and previous occupations, the highest level of education, the perception of financial security, and housing tenure. In addition, an index of material living conditions was created by summing the number of material possessions from a list of 12 (eg, dishwasher, car, and telephone). The variable "adult socioeconomic status" was formed from these indicators. The weight and height of the participants were measured by a nurse, and the body mass index (in kg/m^2) was computed. Subjects were defined as smokers if they had ever smoked on a regular basis and had smoked any cigarettes or cigars or a pipe within the previous 30 d.

Statistical analysis

Differences in the assessed characteristics between depressed participants and the rest of the cohort were examined by using Student's *t* test, the Mann-Whitney *U* test, and the chi-square test. Participants were also ranked and divided into tertiles according to their serum tHcy concentrations. Differences in the assessed characteristics between subjects according to tertiles of serum tHcy concentrations were examined by using analysis of variance, the chi-square test, and the Kruskal-Wallis test. The cutoff of >11.9 μmol/L that we used to indicate an elevated serum tHcy concentration is the same as was used in an earlier study (14). Correlations were examined with the use of Pearson's correlation test.

Odds ratios relating depression to serum concentrations of tHcy were calculated by using a logistic regression model after adjustment for age and the year of the examination (model 1) and further adjustment for history of ischemic heart disease, smoking habits, alcohol consumption, living alone, education, and adulthood socioeconomic status (model 2). We chose these covariates because they are known to associate with depression or tHcy

TABLE 1
Characteristics of the study population of middle-aged men with (HPL Scale ≥5) and without (HPL Scale <5) depressive symptoms[1]

Characteristics	Subjects with depressive symptoms (n = 109)	Subjects without depressive symptoms (n = 762)	P for difference
Age (y)	57.0 ± 6.2[2]	55.9 ± 6.8	0.128[3]
Alcohol intake (g/wk)	91.7 ± 133.4	74.6 ± 123.5	0.351[4]
BMI (kg/m^2)	27.5 ± 3.5	27.6 ± 3.7	0.703[3]
Graduated from high school (%)	15.6	11.8	0.260[5]
Living alone (%)	10.1	11.2	0.740[5]
Smoker (%)	34.9	30.4	0.351[5]
Symptomatic IHD or IHD history (%)	31.2	19.2	0.004[5]
Poor appetite (%)	13.3	3.6	<0.001[5]
Serum homocysteine (μmol/L)	11.9 ± 5.0	10.8 ± 3.1	0.003[3]

[1] HPL Scale, Human Population Laboratory Depression Scale; IHD, ischemic heart disease.
[2] $\bar{x}$ ± SD (all such values).
[3] Student's t test.
[4] Mann- Whitney U test.
[5] Chi-square test.

concentrations or both (9, 10, 11, 12, 31). Model 2 was also adjusted further for poor appetite. Serum tocopherol, lycopene, and total cholesterol were used as additional covariates to form model 3 from model 2. High concentrations of serum tocopherol and lycopene and low concentrations of total cholesterol may reflect a healthy diet. Model 3 was created to investigate the possibility that a healthier diet among the nondepressed participants could bias the results or that the other healthy characteristics of food could explain the association between depression and tHcy concentrations.

RESULTS

The characteristics of the study subjects with and without depression are presented in **Table 1**. Depressed participants were more likely than were the other participants to have a history of ischemic heart disease and poor appetite, and they also had higher serum tHcy concentrations.

The characteristics of the study population according to the tertiles of serum tHcy are summarized in **Table 2**. Higher concentrations of tHcy were associated with older age, living alone, and a poor appetite.

The serum tHcy concentration was elevated (cutoff > 11.9 μmol/L) in 36.7% of the depressed participants and in 26.4% of the nondepressed participants (P = 0.024). The mean HPL Scale scores were significantly higher among those with an elevated serum tHcy concentration than among other study subjects ($\bar{x}$ ± SD HPL Scale score: 1.6 ± 1.9 compared with 1.3 ± 1.7; P = 0.005).

Depression was most common among participants whose homocysteine concentrations were in the upper tertile. Furthermore, there was a linear trend in the proportions of depressed participants (Table 2).

In model 1, depression was more than twice as common among men whose serum tHcy concentrations were in the highest tertile than among men whose serum tHcy concentrations were in the lowest tertile (OR: 2.30; 95% CI: 1.35, 3.90; P = 0.002). In multivariate analysis adjusted for ischemic heart disease history, smoking habits, alcohol consumption, living alone, education, and adulthood socioeconomic status (model 2), the risk was at the

TABLE 2
Characteristics of the study population in tertiles of serum total homocysteine (tHcy)

Characteristics	Lower tertile (n = 284)	Middle tertile (n = 296)	Upper tertile (n = 291)	P
Serum tHcy (μmol/L)	2.8–9.6[1]	9.6–11.4	11.4–51.2	—
Depressed cases [n (%)]	23 (7.9)	37 (12.7)	49 (17.1)	<0.001[2]
Serum homocysteine (μmol/L)	8.8 ± 1.0[3]	10.4 ± 0.5	14.0 ± 4.3	<0.001[4]
Age (y)	54.9 ± 6.6	55.9 ± 6.8	57.6 ± 6.5	<0.001[4]
Alcohol intake (g/wk)	80.2 ± 113.3	70.7 ± 101.9	78.9 ± 156.1	0.198[2]
BMI (kg/m^2)	27.7 ± 3.6	27.5 ± 3.5	27.5 ± 3.5	0.584[4]
Graduated from high school (%)	10.7	11.4	15.1	0.104[5]
Living alone (%)	9.0	9.4	14.4	0.035[5]
Smoker (%)	28.8	28.6	25.1	0.314[5]
Poor appetite (%)	2.0	3.1	6.5	0.005[5]

[1] Range (all such values).
[2] Kruskal-Wallis test.
[3] $\bar{x}$ ± SD (all such values).
[4] ANOVA, P for trend.
[5] Chi-square test, P for linear trend.

same level (OR: 2.23; 95% CI: 1.30, 3.83; $P = 0.004$). The risk of depression in participants in the middle tertile was also almost significantly elevated in model 1 (OR: 1.65; 95% CI: 0.95, 2.86; $P = 0.059$) and in model 2 (OR: 1.67; 95% CI: 0.96, 2.91; $P = 0.068$). Further adjustment of model 2 for poor appetite slightly weakened the association (OR: 1.97; 95% CI: 1.14, 3.41; $P = 0.016$). Adjustment for serum tocopherol, lycopene, and total cholesterol did not significantly change the results of the analysis (OR: 2.21; 95% CI: 1.29, 3.79; $P = 0.004$).

We also repeated the analyses after the exclusion of all those participants who had a history of any cancer or any cardiovascular disease except hypertension. This left 658 participants to be analyzed, of whom 52 had a tendency toward depression. The results were essentially the same in model 1 (OR: 2.20; 95% CI: 1.05, 4.58; $P = 0.037$) and in model 2 (OR: 2.24; 95% CI: 1.06, 4.74; $P = 0.035$).

DISCUSSION

In the current study, participants whose serum tHcy concentrations were in the highest tertile had a risk of depression that was more than twice that of participants whose serum tHcy concentrations were in the lowest tertile. These results are consistent with those of 2 previous analyses of data from the same study. Specifically, using the baseline data, we found a cross-sectional association between low folate intake and depression (32). Furthermore, we showed prospectively that having a low-folate diet at baseline increased one's risk of receiving a hospital discharge diagnosis of depression during 11–16 y of follow-up (33). Findings from all 3 of these studies support the hypothesis that folate-homocysteine metabolism is associated with depression in middle-aged men from eastern Finland. It is not known whether these findings can be generalized to Finnish women, Finnish men of different ages, or to subjects in other countries.

Morris et al (19) found that blood folate concentrations, but not plasma tHcy concentrations, were associated with depression in the general US population. However, the mean age of the subjects in that study was only 26.2 y, whereas it was 56.1 y among the subjects in the current study. As might be predicted from the age difference, mean tHcy concentrations also differed markedly. Whereas the mean tHcy concentration in the depressed young Americans in that study was ≈8.5 mmol/L, that of the depressed older subjects in the current study was nearly 12 mmol/L.

More than one-third of the depressed participants in the current study had high tHcy concentrations (cutoff: >11.9 μmol/L). Bottiglieri et al (14) recorded increased plasma tHcy concentrations in 52% of depressed inpatients by using the same cutoff as we did, whereas Fava et al (6) observed increased tHcy concentrations in 20% of depressed outpatients (cutoff: >13.1 μmol/L). A comparison between the findings of Bottiglieri et al and those of Fava et al might ascertain that patients with more severe depression who require hospitalization also have higher concentrations of tHcy than do outpatients. However, a high concentration of tHcy was also a common finding in our general population sample. It may be connected with a low intake of folate, because previous studies on this sample showed that only 25% of the subjects reached the recommended daily intake of 300 μg folate (32–35).

The mechanisms of homocysteine's action in the CNS and its link with depression are unclear. It has been suggested that homocysteic acid and cysteine sulfinic acid, as metabolites of homocysteine, may have an excitotoxic effect on the *N*-methyl-D-aspartate receptors in the CNS. They may also inhibit the *S*-adenosylmethionine–dependent methylation of biogenic amines and phospholipids (8, 36). One way to metabolize homocysteine is to methylate it back into methionine, the immediate precursor of *S*-adenosylmethionine, which in turn serves as the methyl donor in many methylation reactions in the synthesis of the monoamines. Thus, although tHcy could theoretically cause depression via direct neurotoxicity, an elevated plasma tHcy concentration may merely be a marker of impaired monoamine metabolism, which causes depression through reduced CNS methylation.

An elevated serum tHcy concentration, rather than being an independent risk factor, may be an indicator of an unhealthy lifestyle, which in turn leads to an increased risk of depression. However, in our sample, many other well-known risk factors for depression were not associated with an increased serum tHcy concentration, and adjustment for those risk factors did not change the results. Poor appetite as a symptom of depression could lead to a decreased intake of B vitamins, which could then lead to elevated concentrations of tHcy, but adjustment for poor appetite did not change the results. Deterioration of the samples during storage could also bias our results. However, Alfthan et al (23) did not find deterioration during a similar storage period.

Several serious, long-term diseases have been linked to hyperhomocysteinemia, and having such a disease may be another cause of depression. Connections of this kind that are currently unknown may bias our findings. To avoid this as far as possible, we also performed the analysis by excluding all those participants who had any history of cancer or chronic heart disease, and that exclusion did not change the results.

We did not use a structured interview to identify psychiatric disorders according to specific diagnostic criteria, as some other investigators have done (19, 20). Thus, some of the study participants whom we classified as depressed might not have qualified for a psychiatric diagnosis such as major depressive episode or dysthymia. However, the similarity between the prevalence of depressive symptoms in our study (ie, 10.4% at baseline and 7.3% on 4-y follow-up, if those with a psychiatric history were included) and the estimate by Lindeman et al (37) of the prevalence of major depressive episode (7.2%) among Finnish men aged 15–75 y may suggest that subjects who scored ≥5 on the HPL Scale had experienced a clinically important depressive episode. Nevertheless, we do not claim that an HPL Scale score ≥5 is exactly the same as a clinical diagnosis of depression. We included in our study sample all men who had a HPL Scale score ≥5 either at baseline or on follow-up, because there is some evidence that depressive symptoms are persistent and relatively stable over time. The subthreshold depressive symptoms also seem to be stable (38).

Conclusion

This study indicates that homocysteine metabolism a plays role with respect to depression, at least in middle-aged men from eastern Finland. Together, this study and our previous studies on this material support the link between folate-homocysteine metabolism and depression. Further research is needed to determine

whether these associations are linked to age, sex, or diet or to other, underlying biologic factors.

TT was responsible for data analysis and manuscript preparation, SV analyzed and interpreted data, AR revised the manuscript, GA revised the manuscript and interpreted laboratory data, JH supervised the study and revised the manuscript, KN revised the manuscript and interpreted laboratory data, HV revised the manuscript, GAK planned the study, and JTS planned the study and edited the manuscript. None of the authors had any financial or personal conflict of interests.

REFERENCES

1. Ghadirian A, Ananth J, Engelsmann F. Folic acid deficiency and depression. Psychosomatics 1980;21:926–9.
2. Carney M, Chary T, Laundy M, et al. Red cell folate concentrations in psychiatric patients. J Affect Disord 1990;19:207–13.
3. Wolfersdorf M, Konig F. Serum folic acid and vitamin B-12 in depressed inpatients. Psychiatr Prax 1995;22:162–4 (in German).
4. Abou-Saleh M, Coppen A. Serum and red blood cell folate in depression. Acta Psychiatr Scand 1989;80:78–82.
5. Wesson VA, Levitt AJ, Joffe RT. Change in folate status with antidepressant treatment. Psychiatry Res 1994;53:313–22.
6. Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B_{12}, and homocysteine in major depressive disorder. Am J Psychiatry 1997;154:426–8.
7. Stabler SP, Allen RH, Savage DG, Lindebaum J. Clinical spectrum and diagnosis of cobalamin deficiency. Blood 1990;76:871–81.
8. Parnetti L, Bottiglieri T, Lowenthal D. Role of homocysteine in age-related vascular and non-vascular diseases. Aging (Milano) 1997;9:241–57.
9. Jaques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613–21.
10. Koehler KM, Baumgartner RN, Garry PJ, Allen RH, Stabler SP, Rimm EB. Association of folate intake and serum homocysteine in elderly persons according to vitamin supplementation and alcohol use. Am J Clin Nutr 2001;73:628–37.
11. Salin-Pascual RJ, Alcocer-Castillejos NV, Alejo-Galarza G. Nicotine dependence and psychiatric disorders. Rev Invest Clin 2003;55:677–93.
12. John U, Meyer C, Rumph HJ, Hapke U. Depressive disorders are related to nicotine dependence in the population but do not necessarily hamper smoking cessation. J Clin Psychiatry 2004;65:169–76.
13. Bell IR, Edman JS, Selhub J, et al. Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. Acta Psychiatr Scand 1992;86:386–90.
14. Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MWP, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry 2000;69:228–32.
15. Hamilton MA. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;23:56–62.
16. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B_{12}, homocysteine and the MTHFR 677C→T polymorphism in anxiety and depression. Arch Gen Psychiatry 2003;60:618–26.
17. Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. J Affect Disord 2000;60:121–30.
18. Penninx BW, Guralnik JM, Ferucci L, Fried LP, Allen RH, Stabler SP. Vitamin B_{12} deficiency and depression in physically disabled older women: epidemiologic evidence from the Women′s Health and Aging Study. Am J Psychiatry 2000;157:715–21.
19. Morris SM, Fava M, Jacques PF, Selhub J, Rosenberg IW. Depression and folate status in the US population. Psychother Psychosom 2003;72:80–7.
20. Tiemeier H, van Tuijl HR, Hofman A, Meijer J, Kiliaan AJ, Breteler MM. Vitamin B_{12}, folate, and homocysteine in depression: the Rotterdam Study. Am J Psychiatry 2002;159:2099–101.
21. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 1988;20:46–50.
22. Schwab U, Törrönen A, Toppinen L, et al. Betaine supplementation with a low calorie diet decreases plasma homocysteine concentrations but does not affect body weight, body composition, resting energy expenditure or serum lipid concentrations. Am J Clin Nutr 2002;76:961–7.
23. Alfthan G, Pekkanen J, Jauhiainen M, et al. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis 1994;196:9–19.
24. Porkkala-Sarataho E, Nyyssönen K, Salonen JT. Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in non-vitamin E supplemented men. Atherosclerosis 1996;124:83–94.
25. Porkkala-Sarataho E, Nyyssönen K, Kaikkonen J, et al. A randomized, single-blind, placebo-controlled trial of the effects of α-tocopherol on the oxidation resistance of atherogenic lipoproteins. Am J Clin Nutr 1998;68:1034–41.
26. Salonen JT, Nyyssönen K, Tuomainen T-P, et al. Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentration: a four year follow-up study in men. BMJ 1995;311:1124–7.
27. Roberts RE, O'Keefe SJ. Sex differences in depression reexamined. J Health Soc Behav 1981;22:394–400.
28. Roberts RE. Prevalence of depressive symptoms among Mexican Americans. J Nerv Ment Dis 1981;169:213–9.
29. Kaplan GA, Roberts RE, Camacho TC, Coyne JC. Psychosocial predictors of depression: prospective evidence from the Human Population Laboratory Studies. Am J Epidemiol 1987;125:206–20.
30. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring daepression. Arch Gen Psychiatry 1961;4:561–71.
31. Lespérance F, Frasure-Smith N, Talajic M. Major depression before and after myocardial infarction: its nature and consequences. Psychosom Med 1996;58:99–110.
32. Tolmunen T, Voutilainen S, Hintikka J et al. Dietary folate and depressive symptoms are associated in middle-aged Finnish men. J Nutr 2003;133:3233–6.
33. Tolmunen T, Hintikka J, Saarela A et al. Dietary folate and the risk of depression: a prospective follow-up study. Psychother Psychosom (in press).
34. National Committee of Nutrition: Finnish recommendations for nutrition. Helsinki: Oy Edita Ab, 1998.
35. Voutilainen S, Rissanen T, Virtanen J, Lakka TA, Salonen JT. Low dietary folate intake is associated with an excess incidence of acute coronary events. Circulation 2001;103:2674–80.
36. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademetionine (S-adenosylmethionine) in neurological disorders. Drugs 1994;48:137–52.
37. Lindeman S, Hämäläinen J, Isometsä E, et al. The 12-month prevalence and risk factors for major depressive episode in Finland: a representative sample of 5993 adults. Acta Psychiatr Scand 2000;102:178–84.
38. Merikangas KR, Zhang H, Avenoli S, Acharyya S, Neuenschwander M, Angst J. Longitudinal trajectories of depression and anxiety in a prospective community study. Arch Gen Psychiatry 2003;60:993–1000.