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TOMMI TOLMUNEN

Depression, B vitamins and Homocysteine

Doctoral dissertation

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Abstract

Numerous studies have been published on the connection between folate, cobalamin and depression. Most of these have been carried out on psychiatric patients in cross-sectional settings. In a few studies, augmentation of anti-depressive medication with folic acid derivates, methylfolate or folinic acid has enhanced the treatment outcome. There have also been a few population-based studies concerning the connection between depression and blood levels of folate, cobalamin and total homocysteine (tHcy). However, the results have been inconsistent. The aims of this work on a population sample (KIHD, n = 2700) and a sample of psychiatric patients (KUDEP, n = 115) were to examine the association between:

1) dietary intake of B vitamins and depression,
2) elevated blood levels of tHcy and depression, and
3) blood levels of folate and cobalamin and the treatment outcome among psychiatric outpatients with major depressive disorder.

The risk of both current depression and of having a discharge diagnosis of depression during the follow-up period was increased among those participants who had a low dietary intake of folate. There was no association between the intake of cobalamin and depression. A low intake of folate was common among the participants, whereas the level of cobalamin was adequate in practically all of them. Furthermore, depression was more common among those participants who had elevated serum levels of tHcy. Finally, among the psychiatric patients, those who had high levels of blood cobalamin had a better treatment outcome on six-month follow-up. There was also a weak association between blood folate and the treatment outcome, but this association was not significant after adjustments.

It can be concluded that dietary factors may have importance in both the prevention of and recovery from depression. These findings may also be of importance to public health. These associations could be explained either by the effect of these vitamins on blood levels of tHcy or by their role in the synthesis of monoamines. Elevated levels of tHcy may be a cause of depression or instead an indicator of poor folate or cobalamin status.

National Library of Medical Classification: WM 171, QU 145, QU 187
Medical Subject Headings: depression; depressive disorder; homocysteine/blood; folic acid/blood; vitamin B12/blood; vitamin B12 deficiency; diet; treatment outcome; humans; Finland/epidemiology; epidemiologic studies

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Yhteenveto

Masennuksen ja B-vitamiinien yhteyksistä on julkaistu lukuisia poikkileikkaustutkimuksia erityisesti psykiatrisissa potilasaineistoissa. Psykiatrisilla potilailla on todettu olevan veressään matalampia pitoisuuksia B-vitamiineja ja korkeampia pitoisuuksia homokysteiiniä kuin terveillä verrokeilla. Tämä ero on erityisen selvä masentuneilla potilailla. Muutamissa tutkimuksissa folaatin, metyylifolaatin tai muun vastaavan yhdisteen käyttö masennuslääkkeen rinnalla on lisännyt hoidon tehoa. Tähän mennessä on julkaistu myös viisi väestötutkimusta masennuksen, folaatin, kobalamiinin ja homokysteiinin välisistä yhteyksistä. Tulokset eivät kuitenkaan ole olleet yhteneväisiä.

Tämän työn tarkoituksena oli tutkia yleisväestöotoksessa (KIHD, 2700 tutkittavaa) ja psykiatrisessa potilasaineistossa (KUDEP, 115 tutkittavaa):
1) ruokavalion sisältämän folaatin ja kobalamiinin yhteyksiä masennukseen yleisväestössä,
2) homokysteiinin ja masennuksen yhteyksiä yleisväestössä ja
3) veren folaatti- ja kobalamiinipitoisuuksien yhteyttä vakavasta masennuksesta toipumiseen psykiatrisilla avohoitopotilailla.

Vähäinen folaatin saanti oli yhteydessä sekä ajankohtaiseen masennukseen että seuranta-aikana ilmaantuvaan masennukseen. Korkeat veren homokysteiini-pitoisuudet olivat yhteydessä ajankohtaiseen masennukseen. Korkeat veren kobalamiinipitoisuudet puolestaan olivat positiivisesti yhteydessä vakavasta masennuksesta toipumiseen. Alhainen folaatin saanti voi olla masennukseen sairastumisen riskitekijä. Yhteys voi selittyä joko matalien B-vitamiinipitoisuuksien aiheuttamalla kohonneella veren homokysteiinipitoisuudella tai sillä että B-vitamiineja tarvitaan monoaminien synteesissä. Kohonneet veren homokysteiinipitoisuudet saattavat joko aiheuttaa suoraan masennusta tai olla merkki elimistön huonosta B-vitamiinitilanteesta. Myös B_{12} pitoisuuden ja masennuksesta toipumisen mahdollinen yhteys selittyy samoilla mekanismeilla. Havainnoilla saattaa olla kansanterveydellistä merkitystä sekä masennuksen ehkäisyn että mahdollisesti myös hoidon kannalta.

Yleinen suomalainen asiasanasto: depressio; folaatti; B_{12}-vitamiini; homokysteiini
National Library of Medical Classification: WM 171, QU 145, QU 187
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Dedicated to my Beloved Mother
who has nourished me with the vitamins
of her wisdom.

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I wish to thank Veli-Pekka Valkonen, MD, who guided me to the Research Institute of Public Health. Originally I only wanted to find some control patients for our study. However, this led me to many nice people and to extensive collaboration with them in a truly fruitful way. Probably the most important of these was Docent Sari Voutilainen, RD, who supervised this work. I also thank Tiina Rissanen, PhD, RD, and Anu Ruusunen, MSc, RD, from the same field. Our collaboration was really pleasant and fluent when everybody could share their special knowledge in their own field. I am thankful to Docent Kristiina Nyyssönen, PhD, for her help in the laboratory data, Docent Georg Alfthan, PhD, of the National Public Health Institute, Helsinki, for good comments and Professor George A. Kaplan for his support.

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ABBREVIATIONS

ANOVA	Analysis of variance
BH_4	Tetrahydrobiopterin
BHMT	Betaine homocysteine methyl transferase
BMI	Body mass index
CBS	Cystathione beta-synthase
DNA	Deoxyribonucleic acid
CNS	Central nervous system
CI	Confidence interval
CL	Cystathionine γ-lyase
CRH	Corticotropin-releasing hormone
CS	Cystathione-β-synthase
CSF	Cerebrospinal fluid
CV	Coefficient of variation
CVD	Cardiovascular diseases
DHF	Dihydrofolic acid
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Third Revised Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Revised Edition
dTMP	Deoxythymidine mono-phosphate
dUMP	Deoxyuridine monophosphate

ECA	Epidemiological Catchment Area Study
FAD	Flavin adenine dinucleotide
Hb	Haemoglobin
HCR	Hematocrit
HDRS	Hamilton Depression Rating Scale
5-HIAA	5-hydroxyindoleacetic acid
HPL	Human Population Laboratory
HPLC	High-performance liquid cromatography
5-HT	5-hydroxytryptamine (serotonin)
HVA	Homovanillic acid
ICD	International Classification of Diseases
IHD	Ischaemic heart disease
IMP	Inosine monophosphate
KIHD	Kuopio Ischaemic Heart Disease Risk Factor Study
KUDEP	Kuopio Depression Study
MAT	Methionine-adenosyl transferase
MCV	Mean corpuscular volume
MHPG	3-methoxy-4-hydroxyphenyl glycol
MRI	Magnetic resonance imaging
MS	Methionine synthase
MSR	Methione synthase reductase
MTHFR	Methylenetetrahydrofolate reductace

NMDA	N-methyl-D-aspartate
OR	Odds ratio
PLP	Pyridoxal phosphate
r	Pearson's correlation coefficient
RBC	Red blood cell
RCH_3	Methylated product
RDA	Recommended dietary allowance
RH	Methyl acceptor molecule
RR	Risk ratio
SAD	Seasonal affective disorder
SAM	S-adenosylmethionine
SD	Standard deviation
SERT	Serotonin transporter
SSRI	Selective serotonin re-uptake inhibitor
tHcy	Total homocysteine
THF	Tetrahydrofolate
UM-CIDI	University of Michigan version of the Composite International Diagnostic Interview
US	United States
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Tolmunen T, Voutilainen S, Hintikka J, Rissanen T, Tanskanen A, Viinamäki H, Kaplan GA, Salonen JT. Dietary folate and depressive symptoms are associated in middle-aged Finnish men. Journal of Nutrition 2003;133:3233-3236.

II Tolmunen T, Hintikka J, Ruusunen A, Voutilainen S, Tanskanen A, Valkonen VP, Viinamäki H, Kaplan GA, Salonen JT. Dietary folate and the risk of depression in Finnish middle-aged men. A prospective follow-up study. Psychotherapy and Psychosomatics 2004;6:334-339.

III Tolmunen T, Hintikka J, Voutilainen S, Ruusunen A, Alfthan G, Nyyssonen K, Viinamäki H,. Kaplan GA, Salonen JT. Association beween depressive symptoms and serum homocysteine concentrations in men: A population study. American Journal of Clinical Nutrition 2004;80:1574-1578.

IV Hintikka J, Tolmunen T, Tanskanen A, Viinamäki H. High vitamin B_{12} level and good treatment outcome may be associated in major depressive disorder. BMC Psychiatry 2003;3:17.

CONTENTS:

1. INTRODUCTION

Depression is a major public health problem in Finland. A large number of factors have been shown to have connections with depression. The determining factors for the condition cannot be identified in most cases, and it is known to be a multi-factorial disease. The relationship between the causes and consequences is not always obvious.

It is probable that genetic and psychological factors and their interactions are the most important aetiological factors in the pathogenesis of depression. However, there is a growing body of evidence that lifestyle habits, such as physical exercise and diet, also affect the mood.

The origin of research into the connection between folate and depression dates back to Victor Herbert, who treated his self-induced folate deficiency including depressive symptoms by folate supplementation (Herbert 1961). Since then a number of studies have been published concerning the decreased folate levels of depressed patients (Ghadirian et al. 1980; Abou-Saleh and Coppen 1989; Carney et al. 1990; Wolfersdorf and Konig 1995). Anti-depressive medication has been supplemented successfully with methyltetrahydrofolate (Godfrey et al. 1990; Passeri et al. 1993), folic acid (Coppen and Bailey 2000), and folinic acid (Alpert et al. 2002).

Seven population-based studies have been published concerning the connection between depression and blood levels of folate, cobalamin and total homocysteine (tHcy). However, the results have been inconsistent (Ebly et al. 1998; Penninx et al. 2000; Lindeman et al. 2000a; Tiemeier et al. 2002; Bjelland et al. 2003; Morris et al. 2003; Hakkarainen et al. 2004)

The aim of this work was to examine the association between dietary B vitamins and depression in population-based samples. Furthermore, we studied the connection between tHcy and depression, as the results of previous studies on this topic have been mixed. Finally, we investigated the association between vitamin levels in the blood and the treatment outcome of major depression.

2. REVIEW OF THE LITERATURE

2.1 Depression

Major depression is considered a major public health problem. In Finland depression is the most common reason for early disability pensions (Isohanni 2002). It is also a cause of enormous individual suffering.

2.1.1 Prevalence of depression

The prevalence of major depression has varied between studies, possibly partly because of the different methods used. The diagnostic criteria of major depression are shown in Appendices 1 and 2 according to DSM-III-R and DSM-IV (American Psychiatric Association 1987; 1994). Prevalence figures also vary depending on the time period investigated, which makes these numbers more difficult to interpretate. In the study of Lindeman et al. (2000b), the 12-month prevalence of a major depressive episode was 10.9% for females and 7.2% for males after adjustment for age. Blazer et al. (1994) reported the one-month prevalence of depression to be 4.9% and the life-time prevanlence 17.1% in the US population. Regier et al. (1993) recorded a 3.7% one-month prevalence of major depression in the US Epidemiological Catchment Area Study (ECA). In the study of Patten (1997), the 12-month prevalence of major depression was 6.3% in American subjects aged 15-64 years. In the Mini-Finland Health Survey, the one-month prevalence of neurotic depression was 4.6% (Lehtinen et al. 1990). A telephone survey utilizing the UM-CIDI Short Form (University of Michigan version of the composite International Diagnostic Interview) found the 6-month prevalence of a major depressive episode to be 4.1% in Finland (Isometsä et

al. 1997). In a large study by Ayuso-Matheos et al. (2001), the lifetime prevalence of depressive disorders in five European countries was found to be 8.6%.

Results concerning the duration of major depressive episodes and depression are commonly also somewhat inconsistent. In the study of Spijker and co-workers (2002), the median duration of a major depressive episode in the general population of the Netherlands was 3 months, although 20% of the participants had not recovered at 24 months. The severity of depression and comorbid dysthymia were the determinants of persistance. This means that there is a high risk of chronicity in depression. Melartin et al. (2004) found that the median length of depression with full criteria was 1.5 months after entry to treatment. The median time to full remission was 8.1 months after entry. In clinical samples the duration of depression seems to be even longer (Solomon et al. 1997).

The risk of new episodes in major depression has been estimated to be 80% (Solomon et al. 1997). Melartin and co-workers (2004) found that the most important predictors of a longer episode duration and recurrence were the severity of depression and current co-morbidity. During the follow-up time of 18 months the prevalence of recurrency was 38%.

Faedda et al. (1993) found that 10% of all mood disorders had seasonal patters. Almost all of their patients with seasonal affective disorder (SAD) had either fall-winter depression with or without spring-summer mania or spring-summer depression with or without fall-winter mania. Some patients have atypical features (hypersomnia, hyperphagia, leaden paralysis or rejection sensitivity) instead of classical symptoms of depression such as insomnia and poor appetite. Angst et al. (2002) found that the life-time prevalence of atypical major depression episodes was 4.8% and 7.3% for the broader syndromal definition. The prevalence of atypical depression among the patients with major depression has varied from 11 to 36%

(Posternak and Zimmerman 2002; Matza et al. 2003). Atypically depressed patients may have more suicidal tendencies and impairment of function than patients with typical depression (Matza et al. 2003).

In a study by Merikangas et al. (2003), the persistance of subclinical depressive symptoms was also considerable. Especially comorbid anxiety and depression tended to be more persistant than either syndrome alone. Subjects with anxiety also tended to develop futher depression. The patterns of stability were found to be similar for both subthreshold-level and obvious disorders. It may also be that depression is a life-long, chronic disease with phases of full-blown symptoms, partial remission, and with no symptoms. Kennedy et al. (2004) studied 61 patients with major depression for 10 years. During the follow-up time 52% had no symptoms, 15% had mild symptoms, and 20% were in partial remission. One-sixth of the participants had no time without depressive symptoms. They used medication 60% of the follow-up time.

The acute onset of a major depressive episode may predict early recovery (Keller et al. 1982). In some studies female sex has also predicted good recovery (Meyers et al. 2002). Being married may also enhance the recovery from depression (Hoencamp et al. 2001).

In general populations, female sex (Jenkins et al. 1997), low socioeconomic status, unemployment (Blazer et al. 1994) and smoking (Kendler et al. 1993) have been associated with major depressive disorders. Depression may also be associated with an urban environment, but the results are inconsistent (Jenkins et al. 1997; Lindeman et al. 2000b). In the study of Lindeman et al. (2000b), factors associated with a major depressive episode were urban residency, smoking, alcohol intoxication, and chronic medical conditions. Being single and obese was also found to be risk factors, but only for males.

2.1.2 Late-life depression

Van den Berg et al. (2001) studied 132 depressed persons aged 57 years or more from the general population in Belgium. He suggested that the late-life depression can be divided into three subgroups according to the different aetiological pathways:

1. Early onset depression with long-standing psychobiological vulnerability.
2. Late onset depression as a reaction to severe life stress.
3. Late onset depression with vascular risk factors.

In the study of Pahkala et al. (1995) the point prevalence of depression among elderly Finns (65 or over) was 16.5%. It often appears to be long-standing and recurring (Kivelä et al. 1988, Pahkala et al. 1995). In the prospective study of Schoevers et al. (2003) the point prevalence of depression at baseline was 12.0% in a sample of people aged 65-84.

Pulska et al. (1999) reported that depression was not associated with sex but with older age, widowhood and lower education in men. A high risk of depression was associated with being in long-term institutional care in both women and men. Interestingly, dysthymic disorder was mostly explained by the high occurrence of somatic diseases and disabilities. Dysthymia was not found to be a precursor of somatic diseases (Pulska et al. 1998).

Steffens et al. (2002) found that mortality was associated with an older age of depression onset. In the study of Schoevers et al. (2003), men with neurotic depression had a 2.7 times higher mortality during six years of follow-up and this risk was significant after adjustment for all possible explanatory factors. In women the increase in mortality was not significant. Depression was also a predictor of mortality in elderly people in the study of Pulska et al. (1999). However, dysthymic disorder

was not an independent risk factor for mortality in the Finnish population study (Pulska et al. 1998). The hypothesis for vascular depression will be discussed later.

2.1.3 Etiology and pathophysiology of depression

The etiology of depression has been suggested to be multifactorial. Despite the inevitable significance of psychological factors connected to the family environment and psycholocal traumas, only a few biological hypotheses for the etiology of depression are presented here, as these are suggested to explain the possible associations between B vitamins and depression.

Genes have been suggested to have 40-50% contribution to depression. A large number of studies have been published on the serotonin transporter gene and depression (Ewald 1998; Caspi et al. 2003; van Dyck et al. 2004). There have also been studies on other genes affecting monoamine metabolism such as the 5-HT_{1A} receptor gene (Lemonde et al. 2003; Strobel et al. 2003) and genes coding monoamine oxidation (Du et al. 2002; Nagatsu 2004).

The role of serotonin has been considered as central in the pathogenesis of depression. Brain-imaging studies have detected altered serotonin transporter (SERT) densities in depressed patients (Ichimiya et al. 2002) and their recovery has been associated with clinical improvement (Laasonen-Balk et al. 2004). Depletion of the serotonin precursor tryptophan or inhibition of serotonin synthesis creates depressive symptoms in both healthy participants and in depressed participants in remission (Neumeister 2003). Most antidepressive medications also act through increased serotonin transmission (Leonard 2003).

It appears that the calecholamines also have some role in the pathogenesis of depression. Depressed patients who were in remission after noradrenergic antidepressive treatment experienced depressive symptoms when their noradrenergic transmission was inhibited (Brunello et al. 2002). There have also been a few studies on the association between dopaminergic transmission and depression, although the results are still inconsistent (Laasonen-Balk et al. 1999; Brunswick et al. 2003; Laasonen-Balk et al. 2004).

It has also been postulated that there could be sub-groups of depressed patients with different kinds of imbalance in their nervous system. There is already some evidence that some patients may have preferentially noradrenergic and others serotonergic dysfunctions (Brunello et al. 2002).

Recently the monoamine hypothesis of depression has been criticized. It has been claimed that neurotrophic mechanisms are more important for the pathogenesis of depression (Castrén 2004). However, the body of evidence supporting the monoamine hypothesis is still larger than that for the other hypothesis.

There is also considerable evidence that corticotropin-releasing hormone (CRH) has a role in the mediation of anxiety and depressive mood. It has been shown that CRH levels in cerebro-spinal fluid are elevated in depression. It appears that there are functional interactions between the noradrenergic system and CRH. CRH seems to increase the firing rate of the locus ceruleus, which is the major brain noradreline-containing nucleus (Ur and Grossman 1992; Claes 2004).

It seems possible that cardiovascular diseases (CVD) and depression are not only associated, but that there could even exist even a distinct subtype of depression with a vascular etiology. This so called "vascular depression" is suggested to be characterised by reduced depressive ideation compared to depression in general,

subcortical neurological dysfunction, apathy and psychomotor changes (Baldwin and O'Brien 2002).

The pathogenesis of vascular depression is suggested to be based on the acute or chronic damage of the cerebral vascular system. This hypothesis is partly based on studies examining depression after a stroke (Aben et al. 2001). Taragano et al. (2001) augmented anti-depressive treatment with the calcium-channel blocker nimodipine. Augmentation led to a greater overall improvement and less recurrence of depression. The findings of this clinical study support the hypothesis that cerebro-vascular diseases may be involved in the pathogenesis and recurrence of depression in patients with late-life depression (Taragano et al. 2001). In the post-mortem study of Thomas et al. (2001), subjects with at least one episode of major depression had a significant increase in atheromatous disease. However, no differences were found in micro-vascular disease either generally in the brain or locally in the frontal lobes. In the MRI study of Simpson et al. (2000), psychomotor retardation was independently related to the total white-matter score. According to their finding, late-life depression may also be associated with specific neurological changes. However, no studies have been published on the association between the vascular depression and tHcy or folate.

2.2 Folate

Folate belongs to the group of B vitamins and is also termed vitamin B_9, though it is usually referred to as folate or folic acid. Folates are a group of water-soluble B vitamins with a heterocyclic structure. The diet includes about 100 different folates. Folates are essential for humans and other mammals, as they lack the ability to syntethize folate. Fully oxidized folic acid is found in a synthetized form in pharmaceutical use or as a fortification in foodstuffs. Dietary folates mostly occur as

long-chained folate polyglutamates (National Committee of Nutrition 1998). In this dissertation the singular form "folate" is used to refer to all dietary folates.

2.2.1 Folate metabolism

Dietary folate is in a polyglutamate form and is first enzymically deconjugated to the monoglutamate form in the gut before absorption. Synthetic folic acid can be absorbed directly. After deconjugation folate is sequestered by membrane-associated folate-binding proteins and transported across the brushborder membrane (Chandler et al. 1986; Halsted 1990).

Folate is converted to the biologically active form tetrahydrofolate (THF) in the liver. Folate in the body folate is mainly in the form of methylfolate. The conversion reaction back to the active THF form requires cobalamine. Folate is stored in the liver; the storage may be sufficient to last as long as 40-80 days before deficiency symptoms develop. Folate levels in red blood cells are assumed to reflect the amount of folate stored in the liver and tissues, whereas serum folate reflects its dietary intake (Wu et al. 1975).

2.2.2 Functions of folate

Folate coenzymes act as acceptors or donators of one-carbon units in many metabolic pathways in the body (Figure 1). The enzyme methylenetetrahydrofolate reductace (MTHFR) has a key role in single-carbon metabolism as it converts methylene-THF to 5-methyl-THF, which is needed in the synthesis of tHcy. Folate participates in more than 100 transmethylation reactions (Wagner 1995).

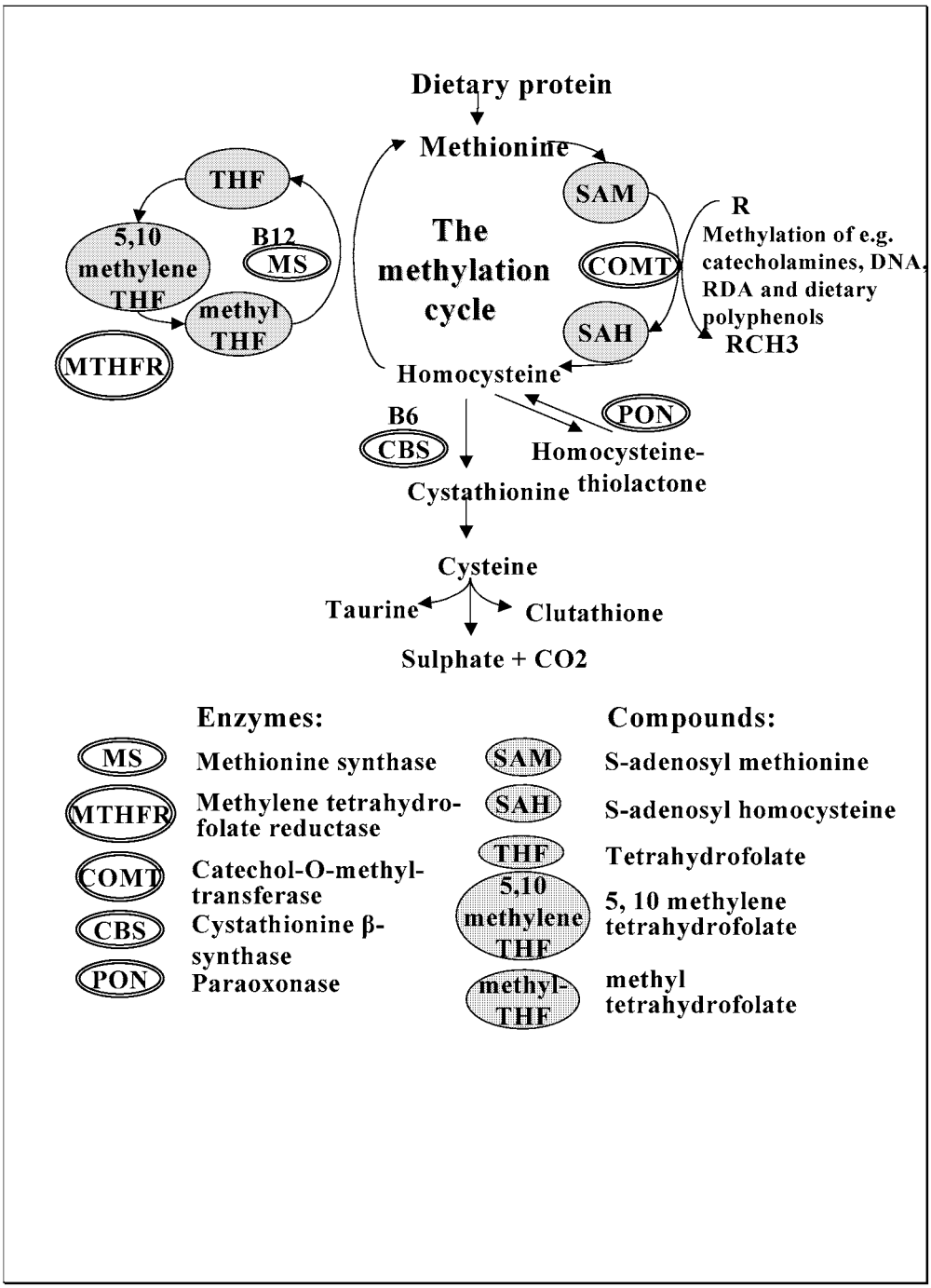

Figure 1. Overview of one-carbon metabolism. (Modified from Bolander-Gouaille and Bottiglieri 2003, with permission from Sari Voutilainen)

2.2.3 Recommendations for folate intake

Recommendations for folate intake have varied between countries, and have generally been increased with the growing number of studies concerning the connections between folate and many diseases. In Finland the recommended dietary allowance (RDA) for folate is 300 μg/d, whereas in the United States it is 400 μg/d. The energy-adjusted recommendation is 36 μg/MJ (National Committee of Nutrition 1998). According to recent Finnish studies the intake of folate in both men and women is commonly inadequate (Alfthan et al. 2003). According to the Finravinto 2002 study the daily intake of folate is approximately 90% of the RDA in men and 80% of the RDA in women (Männistö et al. 2003). However, the energy-adjusted folate intake is higher in women as the caloric intake of the men is commonly greater and women favour the good sources of folate in their diet (Männistö et al. 2003). In our sample, which consisted of men from Eastern Finland, an adequate daily folate intake was even rarer than generally seen in Finland (83% of the RDA; Voutilainen 2000).

2.2.4 Sources of folate in the Finnish diet

Folate occurs especially in full-grain products, in fresh vegetables, citrus fruits and in liver. In Finnish diet the most important sources of folate are whole grain products, vegetables and fruits (National Committee of Nutrition 1998).

2.2.5 Clinical manifestations of folate deficiency

Folate deficiency is considered one of the most common nutritional deficiencies. The elderly, women who use hormonal contraceptives or are pregnant, smokers and those

with alcohol dependence are especially at risk of folate deficiency (Selhub et al. 1993, Cravo et al. 1996). Megaloblastic anemia is a classical manifestation of folate deficiency. Folate deficiency is also associated with many neurological disorders that may even result in mental and physical retardation (Bottiglieri et al. 1995).

Folic acid supplementation is considered to offer many potential benefits and is usually considered safe. Doses as high as 5 mg/d (Vermeulen et al. 2000) and even 10 mg/d (Brattström et al. 1988) have been used in trials concerning the lowering of blood tHcy concentrations without reported side-effects. However, the usefulness and safety of such high-dose supplementation have also been questioned. The absorption and biotransformation process is saturated at doses in the region of 400 μg of folic acid (Lucock 2004). So far it is not known which groups of people really require folic acid supplementation and what a reasonable amount of folic acid is.

2.3 Cobalamin

Cobalamin belongs to the groups of B vitamins. It is also called vitamin B_{12}. Cobalamin was originally discovered in 1926 when pernisious anemia was treated by feeding patients with liver. Its chemical structure, which was finally discovered twenty years later, includes a cobalt-carbon bond (Zubay et al. 1995).

2.3.1 Cobalamin metabolism

Parietal cells of the gastric glands secrete an intrinsic factor that is essential for the absorption of cobalamine by the ileal mucosa. Failure of this absorption system is the most common reason for cobalamin deficiency (Guyton 1986). In Finland most people with megaloblastic anemia I carry a cubilin P1297L mutation in the disease-

specific intrinsic factor-cobalamin receptor. These patients have normal production of intrinsic factor in contrast to patients with classical pernicious anemia (Kristiansen et al. 2000). Previously in Finland, intestinal infection with a broad tapeworm was a common reason for cobalamin defiency (Pelkonen 1993).

2.3.2 Functions of cobalamin

Both folate and cobalamin are required for the synthesis of purines and pyrimidines in the human body. Therefore, they have an important role in nucleic acid and nucleoprotein synthesis. Probably the most important fuction of cobalamin is to serve as a coenzyme in the conversion of ribonucleatides to deoxyribonucleatides, which is important in the replication of genes. Through its involvement in gene expression cobalamin promotes growth and the formation and maturation of red blood cells. Therefore, a deficiency of cobalamin leads to pernicious anemia, which is caused by the failure of red blood cell maturation (Guyton 1986; Zubay et al. 1995). Similar mechanisms could also explain the neuropsychiatric complications of deficiencies of folate and cobalamin. However, there is a remarkably high cellular turnover in the blood compared to the nervous system, and this may imply that such pathways are relatively less important in the central nervous system (CNS). This could also explain the lack of correlation between hematological and neurological manifestations of the deficiency of these vitamins (Bottiglieri et al. 1995).

In the CNS, cobalamin is connected with several metabolic functions in which it acts as a hydrogen acceptor coenzyme. Its metabolism is illustrated in Figure 1. It is needed for two different types of metabolic reactions. Methylcobalamin is needed as a co-enzyme in reactions in which tHcy and methylenetetrahydrofolate are metabolised to methionine and tetrahydrofolic acid. In the mutase reaction, in which methylmalonic acid co-enzyme-A created in the metabolism of propione acid is

metabolised to succinyl co-enzyme-A, 5'-deoxyadenosylcobalamin serves as a co-enzyme. Cobalamin deficiency leads to elevated levels of both tHcy and methylmalonic acid (Guyton 1986; Zubay et al. 1995).

2.3.3 Recommendations for cobalamin intake

The RDA for cobalamine in Finland is 3 µg/d. In healthy adults cobalamin deficiency is a rare condition. In some cases malabsorption can occur (National Committee of Nutrition 1998).

2.3.4 Sources of cobalamin in the Finnish diet

The sources of cobalamin in the Finnish diet are animal products such as meat, fish and dairy products. The most common reason for cobalamin deficiency is impaired absorption in the gastrointestinal tract. A low dietary intake of cobalamin is uncommon in Finland (National Committee of Nutrition 1998; Voutilainen 2000).

2.3.5 Clinical manifestations of cobalamin deficiency

Among cobalamin deficient patients with megaloblastic anemia depressive symptoms, dementia and peripheral neuropathy have been described as the most common neuropsychiatric symptoms. However, in the study of Shorvon and co-workers (1980) one-third of the patients with a deficiency of either cobalamin or folate severe enough to produce a megaloblastic anemia had no neurological or psychiatric complications. Furthermore, in many studies a sub-threshold deficiency of cobalamin has been associated with depression (Abou-Saleh and Coppen, 1989).

Another interesting finding of Shorvon and co-workers (1980) was that cobalamin deficiency was especially associated with spinal cord and peripheral nerve disorders, whereas folate deficiency was associated more with psychiatric disorders, mainly depression. The pathogenesis of these neurological complications is unclear. Studies with animal models have led to the hypothesis that the reason may be the neurotoxic effects of the accumulated S-adenosylhomocysteine as a result of the impaired metabolism of methionine (Bottiglieri et al. 1994).

2.4 Homocysteine

Homocysteine is a sulphur-containing amino acid that is not known to have any useful role in the human body. It is formed from the essential amino acid methionine. Folate is needed in the methylation process converting tHcy back to methionine. The circulating tHcy levels can be increased by defects in intracellular tHcy metabolism. Studies on MTHFR (Rozen 1996) and methionine synthase (MS) enzymes (Leclerc et al. 1996) have shown that tHcy metabolism can be impaired by mutations in the genes encoding the enzymes involved in this process.

2.4.1 Homocysteine metabolism

A strong association has been found between the dietary intake of folate and levels of tHcy in the blood (Stabler et al. 1990; Homocysteine lowering Trialists' Collaboration 1998). The metabolism of homocysteine is illustrated in Figures 1 and 2. Furthermore, low levels of cobalamin may lead to elevated levels of tHcy (Zubay et al. 1995). Low levels of riboflavin (B_2) may also lead to the accumulation of tHcy in people who have a low folate status (Jacques et al. 2002). Methylfolate supplies methyl groups in the synthesis of methionine from tHcy, while cobalamin and

pyridoxine (B_6) serve as cofactors or substrates for enzymes involved in tHcy metabolism. Methione is a precursor of S-adenosylmethione (SAM), which serves as a methyl donor in many methylation reactions in the brain, and is thought to have antidepressant properties (Bottigglieri 1996; Bottiglieri et al. 2000).

Few years ago Silaste and co-workers (2003) studied the effects of diet on serum tHcy in 37 healthy females. During a five-week high-folate diet the mean decrease in tHcy was 13%. The participants ate at least seven servings of vegetables, berries and citrus fruit per day.

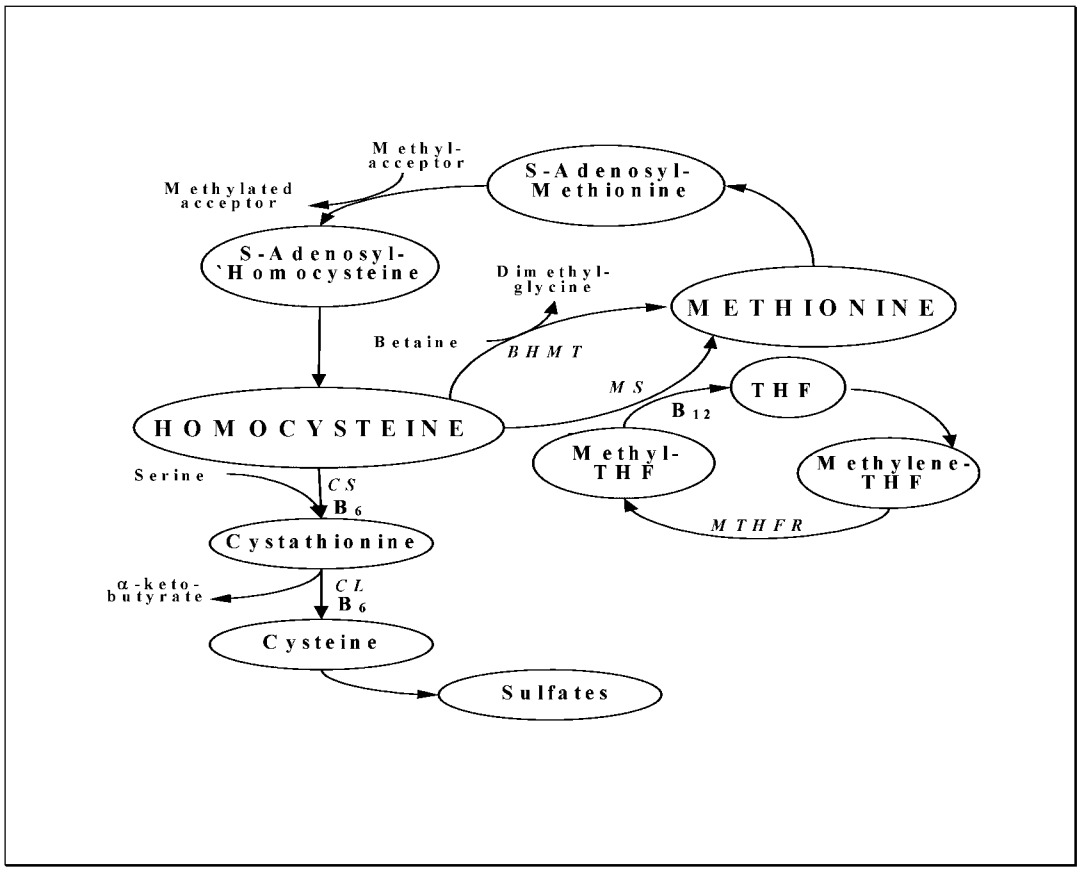

Figure 2. Homocysteine metabolism. Abbreviations: BHMT = Betaine homocysteine methyl transferase, CL = cystathionine γ-lyase, CS = cystathione-β-synthase, MS = methionine synthase, MTHFR = methylenetetrahydrofolate reducase, THF = tetrahydrofolate. (With permission from Sari Voutilainen.)

2.4.2 Clinical manifestations of high homocysteine levels

Interest in tHcy has been most intensive in the field of heart diseases. In a meta-analysis of 30 prospective or retrospective studies a total of 5073 ischaemic heart disease (IHD) events and 1113 stroke events were evaluated (Homocysteine Studies Collaboration 2002). The conclusion was that elevated tHcy is at most a modest independent predictor of IHD and stroke risk in healthy populations. Further studies were suggested to evaluate the impact of disease risk of the genetic variance that affects blood tHcy concentrations. Taken together, the causality between elevated tHcy levels and cardiovascular diseases is still unclear.

2.5 Gene polymorphisms of folate-mediated one-carbon metabolism

Single nucleotide polymorphisms in genes that encode folate-dependent enzymes can result in apparent folate deficiency, which can become symptomatic even in the absence of overt folate deficiency (Bailey and Gregory 1999). They can also worsen the metabolic phenotypes resulting from folate deficiency (Rozen 2001). The most important of the polymorphisms are in MTHFR, methionine synthase (MS), methione synthase reductase (MSR), cystathione-β-synthase (CBS), and methionine-adenosyl transferase (MAT; Rozen 2001). Some polymorphisms and some medical conditions whose risk is increased by them are shown in Table 1.

The metabolic impact of the polymorphisms is modulated by the vitamin status of the individual. Thus, some individuals with harmful polymorphisms may be more vulnerable to the effects of the vitamin intake deficiency. A sub-discipline within human nutrition based on these interactions is sometimes referred to as "nutrigenomics" (Lucoc 2004).

Some of these above-mentioned polymorphisms are quite common. MTHFR and MS play an important role in the re-methylation pathway. A study by Bjelland et al. (2003) on the Norwegian general population found the prevalence of MTHFR677C→T to be 8.4% for the T/T genotype. In this study the participants with the T/T genotype had higher levels of plasma tHcy than those with the C/C genotype (13.66 μmol/l vs. 10.62 μmol/l) and lower plasma folate levels (7.49 nmol/l vs. 8.46 nmol/l). Similar findings have been reported in many other studies (Jaques et al. 1996; Silaste et al. 2001). In the study of Tsai and co-workers more than 50% of 1085 US participants carried polymorphic traits influencing their Hcy metabolism (Tsai et al.1996).

CBS is the key-enzyme in the transsulphuration pathway of Hcy metabolism (Tsai et al.1996). The prevalence of the 86-bp insertion (844ins68) was 20% in the US population. It has been associated with low plasma tHcy concentrations (Tsai et al. 1999). Severe mutations of the CBS gene are rare. Their prevalence in the general population has been less than 1% (Kluijtmans et al. 1996; Folsom et al. 1998).

Silaste et al. (2003) studied polymorphisms of key enzymes in Hcy metabolism and their effects on the increase in folate and decrease in tHcy levels. The subjects with the T/T genotype in the MTHFR C677T gene had the most extensive reduction in the plasma tHcy concentration during the high-folate diet. Malinow et al. (1997) reported a similar finding with folic acid supplementation. Silaste and co-workers (2001), however, found no effect of the CBS gene and the A2756G transition of the MS gene on the responsiveness of tHcy levels to a high folate intake.

Table 1. Single nucleotide polymorphisms of B vitamin genes associated with clinical conditions.

- MTHFR677C→T
  - Depression (Bjelland et al. 2003).
  - Elevated levels of tHcy leading to increased risk for cardiovascular disease (Frosst et al. 1995).
  - Down's syndrome (James et al. 1999).
- MTHFR1298A→C
  - Spina bifida (van der Put et al. 1998).
- MS2756A→G
  - Vascular disease with thromboembolic events (Yates and Lucock 2002).
- MS66 MS66A→G
  - Down's syndrome (Hobbs et al. 2000).

2.6 Depression and B vitamins

In fact, it is not known whether the association between low levels of folate and depression is caused by a low intake, poor absorption or a greater need for folate, or whether low blood levels of folate are the result of a poor appetite due to depression.

2.6.1 Theories of the connection between depression and B vitamins

Several theories have been postulated about the connection between depression and B vitamins. The monoamine hypothesis and the homocysteine hypothesis are clearly the most important and they have been supported by many studies.

2.6.1.1 Monoamine hypothesis

Reynolds and co-workers (1984) presented a hypothesis that the relationship between folate deficiency and depression might be mediated through monoamines. Pteridine cofactor is necessary for the hydroxylation of tryptophan and tyrosine (Kaufmann1981). This means that folate deficiency could interfere with this rate-limiting step in the synthesis of serotonin and catecholamines. This hypothesis has been supported by many other studies (Bottiglieri et al. 1992, Bottiglieri et al. 2000). Bottiglieri and co-workers (2000) detected low levels of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) of depressed patients with folate deficiency, which indicates that the association between depression and folate may be mediated through monoamine synthesis.

Bottiglieri et al. (2000) found that over half of their severely depressed inpatients had raised plasma tHcy levels. Patients with raised plasma tHcy had significant lowering of serum, red cell, and CSF folate, CSF SAM and CSF monoamine metabolites. According to Bottiglieri and co-workers, this might indicate that there exists a biological subgroup of depression with folate deficiency, impaired methylation, and monoamine neurotransmitter metabolism among patients with severe depression.

Botez and co-workers (1982) reported low levels of 5-HIIA in CSF in psychiatric patients with folate deficiency. Folate deficiency has been associated with low 5-HIAA levels of CSF in children with neurological impairment (Hyland et al. 1988).

The hypothesis that B vitamins are connected to brain monoamine levels is also supported by some animal models (Deana et al. 1977; Gospe et al. 1995). There is also some evidence that folate influences dopamine metabolism. In the study of Bottiglieri and co-workers (1992), red-cell folate levels correlated with CSF homovanillic acid (HVA), a metabolite of dopamine. In some studies serum and CSF

folate have correlated, which indicates that there is an active transport mechanism from the serum across the blood-brain barrier (Spector 1979).

2.6.1.2 Homocysteine hypothesis

A connection between B vitamin status and depression could be explained by direct effects of the vitamins on monoamine and catecholamine metabolism in the CNS. However, a low B vitamin status leads to hyperhomocysteimia, which has been linked to other neuropsychiatric disorders, including Alzheimer's disease. Thus, it has been suggested that hyperhomocysteinemia, *per se*, could cause or aggravate depression (Stabler et al. 1990; Parnetti et al. 1997). Also, Bell et al. (1992b) suggested that tHcy may play a role in depression, especially among the elderly.

The mechanisms of action of tHcy in the CNS and its link with depression are unclear. It has been suggested that homocysteic acid and cysteine sulphinic acid, as metabolites of tHcy, may have an exitotoxic effect on the N-methyl-D-aspartate (NMDA) receptors in the CNS. They may also inhibit SAM dependent methylation of biogenic amines and phospholipids (Parnetti et al. 1997; Bottiglieri et al. 1994). One way to metabolize tHcy is to methylate it back into methionine, the immediate precursor of SAM, which in turn serves as the methyl donor in many methylation reactions in the synthesis of the monoamines. An increased level of blood tHcy may indicate that the methylation processes in the CNS are generally low. According to this theory, the rise in the circulation levels of tHcy might be an indicator of the impairment of monoamine metabolism leading to depression, rather than an independent neurotoxic factor behind the depression.

Studies on the relationship between tHcy and depression, however, have been contradictory (Table 2). For example, while some studies have found no association

between tHcy and depression (Fava et al. 1997; Penninx et al. 2000; Morris et al. 2003), Bjelland and co-workers (2003) observed an increased risk of depression in general population subjects with high plasma levels of tHcy. In one population-based study, elevated tHcy was associated with depression, but this association disappeared after correction for cardiovascular factors and functional disability (Tiemeier et al. 2002). Furthermore, Bottiglieri and co-workers (2000) found that high concentrations of tHcy in psychiatric inpatients were associated with more severe depression measured with the Hamilton Depression Rating Scale (HDRS; Hamilton 1960). Finally, in a randomized placebo-controlled trial of co-administration of folic acid (500 μg/d) with antidepressant medication fluoxetine, the effect of fluoxetine was enhanced in the folate supplemented group, and only among those whose tHcy was related to HDRS scores after ten weeks of treatment (Coppen and Bailey 2000). Thus, successful tHcy lowering may have been responsible for the improvement in depressive symptoms.

Table 2. Case-control studies of tHcy and depression

Study	Subjects	Results
Fava et al. (1997)	213 drug-free outpatients with major depression mean age 39.9 y	Relationships between serum folate, cobalamin and tHcy and depressive subtype and treatment response were studied. Low levels of folate, but not tHcy or cobalamin, were associated with melancholia and a poor response to fluoxetine.
Bottiglieri et al. (2000)	46 inpatients with major depression, 18 healthy controls and 20 with a neurological disorder	Depressed patients (52%) with elevated tHcy had lower levels of cerebrospinal fluid (CSF), 5-hydrocyindoleacetic acid (5-HIIA), homovanillic acid (HVA) and 3-methydoxy-4-hydroxyphenylglycol (MHPG), which indicates impaired monoamine metabolism. There was negative correlation between tHcy and red-cell folate in depressed patients but not controls.
Coppen and Bailey (2000)	127 (major depression) > 18 years, mean 42 vs 44	An improved treatment response with folic acid was associated with a lowering of tHcy levels rather than with an increase in folate levels.

2.6.1.3 S-adenosylmethionine

SAM serves as the methyl donor in the CNS in numerous methylation reactions, such as the synthesis of nucleoproteins, proteins, membrane phospholipids, and monoamines (Reynolds et al. 1984). The synthesis of SAM is a multi-step pathway, which involves the vitamins folate and cobalamin (Figure 1). SAM has been shown to have antidepressant properties in a number of studies (Bottiglieri et al. 1994; Mischoulon and Fava 2002). Essentially, SAM and folic acid – at least in folate-deficient patients - may have similar clinical effects on mental function. This is one of the reasons that has led to the suggestion that failure of the methylation processes may underlie some affective disorders (Reynolds et al. 1984). The administration of SAM to depressed patients has been associated with a rise in both CSF 5HIAA and HVA (Bottiglieri et al. 1984).

2.6.2 Folate and depression

The origin of research into the connection between folate and depression dates back to four decades ago. Victor Herbert published many studies on folic acid and cobalamin (Herbert and Zalusky 1962). In one these he used himself as a study subject. Herbert followed a folate deficient diet for four months and he developed depression, insomnia, irritability, fatigue and forgetfulness. Then he treated his self-induced folate deficiency, including depressive symptoms, by folate supplementation. When he began to eat folate he recovered from all these symptoms (Herbert 1961).

2.6.2.1 Case-control studies with psychiatric patients

Patients with major depression have had lower serum or erythrocyte levels of folate in a number of case-control studies (Ghadirian et al. 1980; Abou-Saleh and Coppen 1989; Carney et al. 1990; Wolfersdof and Konig 1995). Furthermore, low serum (≤ 2,5 ng/mL) or erythrocyte (≤ 200 ng/mL) folate levels have been found in 15-38% of depressed participants (Abou-Saleh and Coppen 1989; Fava et al. 1997). However, anaemia has been rare among these patients, which indicates that the haematological indices are not good screening tests for significant folate deficiency in psychiatric patients (Carney et al. 1990; Godfrey et al. 1990; Alpert and Fava 1997).

Carney and co-workers (1990) divided 285 patients into five diagnostic groups. Folate deficiency was significantly more common in patients with depression, mania or schizophrenia or euthymic patients. Furthermore, folate deficiency was twice as common in the sub-group of endogenous compared to the neurotically depressed subjects.

Contrary to these findings, which imply an association between a low folate level and depression, Lee et al. (1998) recorded higher erythrocyte folate levels in depressed Chinese inpatients than in controls. They suggested that culturally patterned dietary practices can influence the relationship between the folate status and depression in different populations.

In a few studies low levels of folate have been associated with poor treatment outcome. In study of Papakostas et al. (2004a) low serum folate levels were found to be associated with further treatment resistance among patients with fluoxetine-resistant depressive disorder. Papakostas and co-workers (2004b) found also that low serum folate levels predicted higher risk for depressive relapse during 28 weeks continuation phase of treatment with fluoxetine.

2.6.2.2 Severity of depression

In some studies there has also been an inverse relationship between blood folate levels and the severity of depression (Coppen and Abou-Saleh 1982; Abou-Saleh and Coppen 1989; Carney et al. 1990; Wesson et al. 1994). In the study of Wilkinson and colleagues (1994), depressed subjects had lower serum levels of 5-methyltetrahydrofolate than controls and the levels correlated inversely with the severity of depression before treatment. Not only the severity but also the duration of depressive symptoms has correlated inversely to blood folate levels. Levitt and Joffe (1989) reported a negative correlation between serum folate and the duration of the current episode of depression in 44 depressed patients. Respectively, Coppen and Abou-Saleh (1982) found in a two-year follow-up study of 26 patients with bipolar disorder and 81 with unipolar mood disorder that those who had the highest plasma folate levels had less "affective mobility" measured in terms of the severity and duration of depressive disorder symptoms.

2.6.2.3 Augmentation of anti-depressive medication with folate

Low blood levels of folate have been linked with a poor response to antidepressant treatment (Wesson et al. 1994; Fava et al. 1997). In line with this, the augmentation of antidepressant medication with methylfolate or folinic acid has resulted in better clinical and social recovery (Table 3; Godfrey et al. 1990; Passeri et al. 1993; Coppen and Bailey 2000; Alpert et al. 2002). Nevertheless, there is a need for more randomized, double-blind studies on this topic.

Godfrey et al. (1990) had 132 patients with major depression or schizophrenia with a sub-threshold or definite folate deficiency. They used 200 μg/l as the threshold level

of red-cell folate. The study setting was double-blind. Patients were given 500 mg/d methylfolate for six months in addition to standard psychotrophic treatment with a tricyclic antidepressant, monoamine oxidase inhibitor, lithium or oral or depot neuroleptics. Both depressed participants and those with schizophrenia improved better clinically and socially in the group receiving active treatment.

The study of Passeri et al. (1993) included 96 patients aged at least 65. The patients had had both dementia and depression with HDRS scores exceeding 18. HDRS is widely used in assessment of the severity of depression, with scores ranging between 0 and 52. It consists of items concerning mood, guilt, insomnia, appetite, sexual desire, and somatic symptoms of depression. Scores exceeding 18 are considered to indicate severe depression (Hamilton 1960). The patients had normal levels of red-cell folate. Those patients who responded to two weeks of placebo treatment prior to trial were excluded. The patients were treated with either 50 mg/d methyltetrahydrofolate or 100 mg/d trazodone. However, there were no significant differences in the treatment results between the groups.

Coppen and Bailey (2000) studied 127 patients with folate levels within the normal range and HDRS scores of 20 or more. The patients were randomly divided into groups receiving 20 mg/d fluoxetine with or without 500 μg/d folic acid. Patients in the folic acid group had a significantly greater improvement over a 10-week follow-up. Women seemed to benefit more from folic acid augmentation.

Alpert and co-workers (2002) enrolled 22 adults with an inadequate response to selective serotonin re-uptake inhibitors (SSRI). They used folinic acid, which was metabolized to an equivalent dose of 15-30 mg/d methyl folate. After four weeks of treatment the HDRS scores of the patients declined by an average of 6.3 points, which can be considered a modest improvement. However, the sample size was small and there was no placebo group in this study.

The results of the above-mentioned studies were reviewed and meta-analyzed using Cochrane method for systematic analysis (Taylor et al. 2003). It was concluded that augmentation of anti-depressive medication with folate may be useful, but more evidence is needed. There is no evidence that B vitamins without anti-depressants were effective in the treatment of depression.

Table 3. Clinical studies with folate derivates

Study	Subjects	Dosage	Results
Godfrey et al. (1990)	24 with major depression, 17 with schizophrenia 20-70 years	Methylfolate 15 mg/d	Red-cell folate < 200 μg/l at baseline. Augmentation of standard medication led to better results than the placebo.
Passeri et al. (1993)	96 (depression and dementia) > 65 years	Methylfolate 50 mg/d	Normal levels of folate at baseline. Methylfolate alone was equally effective than 100 μg/d trazodone.
Coppen and Bailey (2000)	127 (major depression) > 18 years, mean 42 vs 44	Folic acid 500 μg/d	Normal levels of folate at baseline. Fluoxetine 20 mg together with folic acid was more effective than fluoxetine alone.
Alpert et al. (2002)	22 (with SSRI-refractory depression) 26-68 years	Folinic acid 15-30 mg/d	Normal levels of folate at baseline. Agumentation of the current medication improved the treatment response. No placebo group.

SSRI: Serotonin selective re-uptake inhibitor

2.6.2.4 Population-based studies

A few studies have been published in which the association between blood folate levels has been examined in general populations. However, the results have been inconsistent (Table 4). Ebly et al. (1998) noted that elderly participants with low levels of serum folate were more likely to be depressed. Morris and co-workers (2003) recently reported that depressed members of the general US population had lower serum and red blood cell folate concentrations. This was especially apparent among those subjects who had recently had an episode of depression. However, Penninx et al. (2000) found no association between blood folate and depression among disabled, older women. Furthermore, Lindeman et al. (2000a) detected no such association in a general population of elderly males and females. A study by Tiemeier and co-workers (2002) suggested that folate deficiency was not independently related to depressive disorders. Finally, a Norwegian study (n = 5948) suggested that low plasma folate levels are not significantly related to depression without comorbid anxiety disorder in the general population (Bjelland et al. 2003). In a recent study by Hakkarainen et al. (2004) among 27 000 smoking Finnish men, the intake of folate did not associate with self-reported feelings of depression, anxiety or insomnia.

Table 4. Population studies on depression and single-carbon metabolism

Study	Subjects	Results
Ebly et al. (1998)	1771 Canadians > 65 years	Low serum folate was associated with depression and dementia.
Lindeman et al. (2000a)	1130 whites and hispanics from New Mexico > 65 years	Low serum folate associated with cognitive dysfunction. Serum folate and cobalamin did not associate with mood. tHcy was not measured.
Penninx et al. (2000)	700 disabled, nondemented women > 65 years	Serum folate, folate deficiency and tHcy did not associate with depression. Depressed subjects had higher serum methylmalonic acid levels and nonsignificantly lower serum cobalamin levels.
Tiemeier et al. (2002)	From 3884 subjects, 278 depressed and 416 randomly-selected reference subjects > 55 years	Serum folate, cobalamin and tHcy associated with depression. After adjustment for functional disability and cardiovascular diseases only cobalamin associated independently.
Morris et al. (2003)	2948 healthy subjects from US aged 15-39 years	Low red-cell folate, but not cobalamin or tHcy, associated with a lifetime diagnosis of major depression. This was found to be most characteristic of recently recovered subjects.
Bjelland et al. (2003)	5948 Norwegian subjects aged 46 – 49 or 70 – 74 years	High tHcy and MTHFR 677C→T polymorphism but not cobalamin associated with depression. Plasma folate associated inversely with depression in a subgroup of middle-aged women.
Hakkarainen et al. (2004)	27 000 smoking Finnish males aged 50-69 years	Dietary folate intake did not associate with self-reported feelings of depression, anxiety or insomnia.

tHcy: total homocysteine, MTHFR: methyltetrahydrofolate reductase

2.6.3 Depression and the other B vitamins

Most of the current literature concerns the folate and depression. Nevertheless, some studies have also been published on the connection between depression and other B vitamins, especially on associations between cobalamin (vitamin B_{12}) and depression.

2.6.3.1 Cobalamin

Low levels of cobalamin have been found in the serum and red blood cells of patients with depressive disorders (Bottiglieri 1996; Fava et al. 1997; Engström and Träskman-Bendz 1999). Older, physically disabled women with metabolically significant cobalamin deficiency have been found to have a two-fold higher risk of depression than women with normal plasma levels of cobalamin (Penninx et al. 2000). The association between depression and cobalamin is suggested to be mediated through tHcy metabolism or the synthesis of monoamines.

Another possible explanation for the association between cobalamin and depression is the effect of cobalamin on human circadian rhythm of melatonin and body temperature. Cobalamin has been reported to modulate human melatonin secretion (Yamazaki et al. 1991). Intravenous administration of cobalamin increased the rectal temperature in the later hours of the daytime (Uchiyama et al. 1995). In the study of Hashimoto et al. (1996), light exposure phase-advanced the melatonin rhythm in a cobalamin-receiving group but not in the placebo group. Thus, cobalamin seems to enhance the light-induced phase-shift in the human circadian rhythm. As circadian rhythms are disturbed in depression, similar mechanisms could also take place in the association between cobalamin and depression. However, as these connections have been studied in healthy volunteers, this is just a hypothesis.

The findings from three recent large population-based studies have, however, been contradictory (Tiemeier et al. 2002; Morris et al. 2003; Bjelland et al. 2003; Table 4). A Dutch study suggested that cobalamin deficiency is independently related to depressive disorders (Tiemeier et al. 2002). Morris et al. (2003) reported that cobalamin levels did not associate with depression in a sample of the general US population. A study by Bjelland et al. (2003) suggested that cobalamin levels are not related to depression without comorbid anxiety disorder in the general population.

No studies have reported on associations between circulating cobalamin levels and the treatment response in patients with depressive disorders.

2.6.6.2 Riboflavin, pyridoxine and thiamine

Studies have also been published on the association between depression and riboflavin (B_2; Nobbs 1974, Carney et al. 1982) and pyridoxine (B_6; Carney et al. 1979, 1982, Hvas et al. 2004). Thiamine (B_1) deficiency has been found to be common among psychiatric patients (Carney et al. 1979) and to have an association with schizophrenia and alcoholism (Carney et al. 1982), even though thiamine deficiency was suggested to be secondary to anorexia rather than a causal factor. Thiamine is used during alcohol withdrawal in an intramuscular form in order to prevent Wernicke-Korsakoff syndrome as a result of thiamine deficiency caused by alcohol-induced malnutrition. Thiamine deficiency can also be caused by a decreased absorption of thiamine from the gastro-intestrinal tract or impaired thiamine utilization in the cells (Martin et al. 2003). Thiamine serves as a cofactor in carbohydrate metabolism.

Nevertheless, the augmentation of antidepressive treatment with the vitamins thiamine, riboflavin, and pyridoxine increased cobalamin levels in elderly patients

with major depression without specific supplementation, and there were trends towards greater improvements in scores for depression ratings (Bell et al. 1992a).

2.7 Single carbon metabolism, cognitive impairment and dementia

Depression is associated with different neuropsychological impairments. The most consistent findings concern attention deficits and executive function (Austin et al. 2001). Most of the cognitive impairments associated with depression are reversible, but some of them may be irreversible (Beblo et al. 1999). The number of depressive episodes is associated with the cognitive imparament in euthymic depression patients (Kessing 1998). Depression also seems to be a risk factor for dementia (Mejia et al. 2003; Leinonen et al. 2004). Depression and dementia may also have some common risk factors, such as CVD (Taragano et al. 2001). A poor B-vitamin status and elevated levels of tHcy may also be common risk factors, especially in late-life depression and dementia.

Concentrations of SAM have been reported to be reduced in several different brain regions of patients with Alzheimer's disease (Morrison et al. 1996). An association has also been found between dysfunction of the monoamine neurotransmitter and Alzheimer's disease (Palmer and DeKosky 1993). Low levels of cobalamin (Selley et al. 2002) and folate have been recorded in demented patients (Bottiglieri et al. 2001; Selley et al. 2002). Elevated levels of tHcy have been associated with an increased risk of dementia. In the Framingham study, subjects (n = 1092, mean age 76 y) with tHcy levels exceeding 14 μmol/l had nearly a doubled risk of developing Alzheimer's disease (Seshadri et al. (2002). In some studies, supplementation with B vitamins has led to improved cognitive functions in elderly patients (Bell et al. 1992a; Abalan 1999).

Increased levels of tHcy have been found to associate with poor cognitive functioning in the elderly. This finding does not seem to be connected to blood levels of folate or cobalamin (Nilsson et al. 1994). However, it can be speculated that mild or subclinical vitamin deficiencies may be a reason for both elevated levels of tHcy and poor performance in neuropsychological tests. The following hypotheses have been postulated to explain the direct influence of tHcy on cognitive functions:

1) tHcy may have an effect on the vascular endothelium, leading to a change in the homeostatic condition from antithrombotic to thrombogenic, in the same way as is thought to occur in cerebrovascular diseases (Malinow 1995).
2) Hypomethylation secondary to reduced synthesis of methionine and SAM may explain the connection between tHcy and cognitive functions. Reduced levels of SAM have been associated with both depression and dementia (Bottiglieri and Hyland 1994).
3) Elevated levels of tHcy may lead to increased production of homocysteic acid and cysteine sulphinic acid, the exitotoxic sulphur-containing aminoacids. This can lead to neuroinjury and cell death via excessive glutamate receptor activation, as these amino acids act as endogenous agonists of NMDA receptors (Lipton and Rosenberg 1994).

2.8 Conclusion on the connection between B vitamins and depression

In conclusion, low circulating levels of folate have been found from depressed patients in many studies. Furthermore, some studies have recorded elevated levels of tHcy, but the results have been inconsistent. Augmentation of anti-depressive medication with B vitamins, especially folic acid, has been reported to result in a better treatment outcome. Studies have also been published on the association between cobalamin and depression, and a few on the association between riboflavin and pyridoxine and mood disorders. Here again, the findings have been inconsistent.

Only one study has been published on the association between the dietary intake of folate and depression.

Because of the lack of prospective studies it cannot be concluded whether the low B vitamin status in depressed patients is a cause or a consequence of depression. Furthermore, it is not known whether the B vitamin status affects the treatment outcome.

3. AIMS OF THE STUDY

The aim of the present study was to evaluate the associations between the dietary intake of B vitamins, the blood levels of B vitamins and tHcy, and depression in both cross-sectional and prospective study designs. The specific aims were:

1) To determine whether a low folate intake is associated with the presence of depression in middle-aged men from a general population. It was also investigated whether there were associations between the intake of cobalamin, riboflavin and pyridoxine, and the risk of depression. (I)

2) To assess whether a low folate intake among subjects initially free of depression is associated with an increased incidence of severe depression in a prospective study design. It was also examined whether there were associations between cobalamin intake and the incidence of depression. (II)

3) To study whether high circulating levels of tHcy are associated with an increased risk of depression in a general population. (III)

4) To determine whether there are associations between serum cobalamin and red-cell folate levels and the six-month treatment outcome in patients with major depressive disorder. (IV)

4. SUBJECTS AND METHODS

In this work two separate samples of study subjects were used.

4.1 Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study

The Kuopio Ischaemic Heart Disease (KIHD) Study is a population-based study of risk factors for ischaemic heart disease and other outcomes among middle-aged men in the Kuopio region in Eastern Finland (Salonen 1988).

4.1.2 Participants

A total of 2682 participants aged 42–60 years (82.9% of those eligible) were recruited for the baseline examination, which occurred between March 1984 and December 1989. The study protocol was approved by the Research Ethics Committee of University of Kuopio and all participants provided written informed concent.

In the study of the association between depression and the dietary intake of folate in a cross sectional setting we excluded those participants who reported having previously been diagnosed with a psychiatric disorder (n = 146, 5.6% of the cohort). The data were incomplete for 93 participants and complete data were thus available for 2443 men. (I)

In the prospective study of the association between the dietary folate intake and the risk of receiving a discharge diagnose of depression during the follow-up time, the data were incomplete for 82 participants, 287 partcipants were excluded due to

having depression at baseline and six due to other exclusion criteria, leaving 2307 men to be analyzed. We also reported the results after several exclusions. (II)

Out of a total of 1229 men invited for the 4-year follow-up of the KIHD Study, 52 had died, suffered severe illness, or had migrated from the region, and 139 could not be contacted or refused to participate. Of the remaining 1038 men, data were incomplete for 110. 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 to be analyzed. (III)

4.1.3 Assessment of depressive symptoms

Depressive symptoms were assessed with the self-report 18-item Human Population Laboratory (HPL) depression scale (Kaplan et al. 1987; Appendix 3.). The HPL depression scale has been especially developed for screening general population samples (Roberts 1981; Roberts and O'Keefe 1981) and is highly correlated with the 21-item Beck Depression Inventory score (Beck et al. 1961; Kaplan et al. 1987). The scale consists of items dealing with mood disturbance, a negative self-concept, loss of energy, problems with eating and sleeping, trouble with concentration, and psychomotor retardation or agitation. The HPL score is generated by assigning one point for each true or false answer that is indicative of depression. For some items, the response "often" or "never", whichever was appropriate, was assigned one point. The range of the HPL depression scale is 0 – 18. After exclusion of the subjects with a previous psychiatric disorder, Cronbach's alpha for the HPL depression scale was 0.69 at baseline and 0.65 on 4-year follow-up. A cut-off score of 5 or more has earlier been used to define clinically significant depression, and we therefore applied the same cut-off point in this study (Kaplan et al. 1987). Those who scored 5 or more either at baseline or on 4-year follow-up were all considered to have a tendency

towards depression (n = 109, 12.2% of participants; III). Poor appetite as a covariant was derived from the HPL depression scale.

4.1.4 Outcomes of prospective follow-up

Data on receiving a discharge diagnosis of depressive disorder during the follow-up period were obtained by computer linkage to the National Hospital Discharge Register in 2000. The average follow-up time for the cohort was 13 years. Diagnoses were made according the ICD-8 (years 1985-1986), ICD-9 (years 1987-1995, criteria for mental disorders were equal to DSM-III-R) and ICD-10 (years 1996-2000). Altogether, 76 participants had a depressive disorder as a discharge diagnosis during the follow-up period, of whom 23 were excluded because of significant depressive symptoms at baseline and 6 because of later receiving a discharge diagnosis of schizophrenia, delusional psychosis or bipolar disorder. The remaining participants who were hospitalised and had been diagnosed as having major depression (ICD-9: 2961-, ICD-10: F32.1-3, F33.1-3; n = 31), a depressive disorder not otherwise specified (NOS) (ICD-9: 2968A, ICD-10: F32.9, F33.9; n = 15), chronic depression (dysthymia; ICD-8: 300.41, ICD-9: 3004A, ICD-10: F34.1; n = 4) or adjustment disorder with depressive symptoms (ICD-9: 3090A; n = 3) during the follow-up period were categorised as having suffered severe depression. As six participants had had several hospitalisations and diagnoses, the total number of diagnoses was 53, although the number of hospitalised participants was 47. (II)

4.1.5 Assessment of food consumption

The dietary intake of nutrients was quantitatively assessed by means of a four-day food recording at the KIHD study baseline examination. Nutrient intake was

calculated using Nutrica® software, which is mainly compiled using Finnish values for the nutrient composition of foods and takes into account the loss of vitamins during food preparation. The software has been developed at the Research Centre of the Social Insurance Institution of Finland. Intake levels of vitamins were adjusted for dietary energy intake using the residual method (Willet and Stampfer 1998). Energy adjustment is based on the notion that a larger, more physically active person requires a higher calorific intake, which is associated with a higher absolute intake of all nutrients. The participants were classified into tertiles in the cross-sectional analysis and two categories according to the median energy-adjusted intake of folate and cobalamin (above and below median) in prospective analysis.

In this sample only 13% of subjects regularly used antioxidant or vitamin supplements. Because commonly used supplements in the 1980s did not include folic acid, vitamin users were not excluded from the present analysis.

4.1.6 Assessment of other characteristics

Participants also completed questionnaires at baseline relating to their background, current smoking habits (yes/no), alcohol consumption (grams per week), marital status, education and adulthood socioeconomic status. 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 twelve (television, dishwasher, car, telephone, etc). The variable was formed from these indicators. The weight and height of the participants were measured by a nurse and the body mass index (BMI) was calculated.

4.1.7 Laboratory data

The serum tHcy in the KIHD study was analyzed by high-performance liquid cromatography (HPLC) in 2001 at the National Public Health Institute, Helsinki, Finland as described by Schwab and co-workers (2002). The subjects came to provide venous blood samples between 8 am and 10 am. They were instructed to abstain from ingesting alcohol for three days and from smoking and eating for 12 hours. After the subjects had rested in a supine position for 30 minutes, 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 prior to analysis. The coefficient of variation (CV) between batches (n = 30) for two pooled plasma samples was 4.3% and 5.4%.

Measurements of serum α-tocopherol, lycopene and cholesterol concentrations have previously been presented elsewhere (Porkkala-Sarataho et al. 1998). Serum for α-tocopherol, lycopene and β-carotene determinations was stored at –80 °C until extracted with ethanol and hexane and measured by an HPLC method by using alpha-tocopherol acetate as an internal standard. Lipoproteins were separated from fresh serum samples by combined ultracentrifugation and precipitation (Salonen et al. 1995). The serum total cholesterol concentration was determined enzymatically with an autoanalyzer (Kone Specific, Kone Instruments, Finland). We used these variables as covariants to disclose the possibility of a commonly healthier diet and life-style among the non-depressed participants biasing the results, or of the other healthy characteristics of food explaining the association between depression and the tHcy.

4.2 Kuopio Depression Study (KUDEP)

The Kuopio Depression Study (KUDEP) consisted of two separate samples. The general population survey included a stratified sample of adults between the ages of 25 and 64 years. The other part of the study was a naturalistic follow-up study with outpatients that we used in this work (Honkalampi 2001; Haatainen 2004).

4.2.1 Participants

The original patient sample included 203 outpatients with depressive disorders in the Department of Psychiatry, Kuopio University Hospital, Kuopio, Finland. They were invited to an interview between February 1996 and January 1998. Among these, 115 patients with major depressive disorder participated both at baseline and on six-month follow-up, and this subsample was used in this study. Approval for the study was obtained from the Ethics Committee of Kuopio University Hospital and the University of Kuopio. All patients provided written informed consent before entering the study.

There were 70 females (61%) and 45 males (39%) in the sample, with a mean age of 43.9 (SD 10.9, range 21 - 69) years. Thirty-one of the patients (27%) had a positive family history of depressive disorders. The first episode of depressive symptoms had occurred on average 9.6 (SD 10.2) years before. Twenty-four percent of the patients were current smokers (n = 27) and twenty-six percent used alcohol weekly (n = 30). None of these variables associated with the treatment outcome.

Patients were interviewed at baseline and on 6-month follow-up. During the study period they were treated by their regular outpatient psychiatrists and therapists. Information on the treatment received during the study period was collected from

case notes and follow-up interviews. The antidepressive medication used during the study period was considered to be adequate if the length of the treatment exceeded 3 months and if the daily dose used was within the range deemed efficient (tricyclics ≥ 150 mg, citalopram ≥ 20 mg, fluoxetine ≥ 20 mg and paroxetine ≥ 20 mg) (Sorvanniemi et al. 1998; Tollefson 1998). Patients were asked whether they had had an adjunct therapeutic relationship during the study period. The frequency and length of the therapeutic relationship and the presence of inpatient care received during the follow-up were also recorded.

4.2.2 Assessment of depressive symptoms and diagnosis of depression

At entry, the diagnosis of a current episode of major depressive disorder was confirmed by means of the Structured Clinical Interview for DSM-III-R, conducted by a trained interviewer (Spitzer et al. 1989). This study started shortly after the publication of the fourth version of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), but this newer version could not be used because of the lack of a Finnish translation at that time. Nevertheless, in diagnosing a major depressive episode the DSM-III-R and DSM-IV have only small differences in criteria B-E (American Psychiatric Association 1987; 1994). Most of these differences concern the wording rather than the basic principles. In DSM-III-R two weeks of delusions or hallucinations in the absence of prominent mood symptoms is an exclusion criterion of major depression (Spizer et al. 1989; American Psychiatric Association 1987).

The level of depression was assessed using the 17-item HDRS both at baseline and after six months (Hamilton 1960). Cronbach's alpha for HDRS ratings was 0.74 at baseline and 0.81 on follow-up. The mean HDRS score at baseline was 18.8 (SD 6.5), and the mean decline in the HDRS score during the study period was 6.8 (SD 7.4). The treatment outcome was defined in the following manner: a full response was

defined as a reduction of more than 50% in the HDRS score between baseline and follow-up, a partial response as a reduction of between 25 and 50%, and nonresponse as a reduction of less than 25% (Fava and Davidson 1996). We did not use any HDRS cut-off point for remission in our analyses because our aim was to study associations between vitamin levels and treatment responses and not those between vitamin levels and remission. Symptoms of weight loss and gastrointestinal symptoms, including poor appetite, were derived from HDRS ratings for statistical analyses.

4.2.3 Assessment of other characteristics

Patients completed questionnaires relating to their sociodemographic background, current smoking habits (yes/no), patterns of alcohol use (used at least once a week/other), family history of depression (one or both parents have been treated for depression; yes/no) and the duration of depressive illness (years from the first episode of depressive symptoms). Weight and height were also measured, and BMI was calculated.

4.2.4 Laboratory data

At entry to the KUDEP study, the serum cobalamin and erythrocyte folate level as well as haemoglobin (Hb), mean corpuscular volume (MCV), red blood cell count (RBC) and hematocrit (HCR) were determined for each patient. After preliminary baseline analyses, it was decided to determine serum cobalamin from serum samples that had been stored at -20 °C during the six-month follow-up. Erythrocyte folate could not be determined on follow-up because we had no frozen samples of erythrocytes.

Cobalamin and erythrocyte folate levels were determined using time-resolved fluoroimmunoassays in the laboratory of Kuopio University Hospital. Reference ranges, which were provided by the laboratory, were 140 - 540 pmol/l for a normal serum cobalamin level and 315 - 850 nmol/l for a normal erythrocyte folate level. Levels below these ranges were defined as low, and above these ranges as high.

4.3 Statistical analysis

In the cross-sectional study differences between the characteristics of depressed participants and the rest of the cohort were examined using the Student's t-test, Mann-Whitney U-test and chi-squared test. Participants were divided into tertiles according the energy adjusted intakes of the vitamins. We used Cronbach's alpha as a measure of the internal consistency of the HPL depression scale. The odds ratio (OR) for depression was examined using a logistic regression model adjusted for age and examination years (Model 1) and for smoking habits, consumption of alcohol, appetite, BMI, living alone, education, adulthood socioeconomic status and total fat consumption (Model 2). Values in the text are means ± SD. (I)

In the prospective study the relative risk of depression was examined using the Cox's proportional hazards regression model adjusted for age and examination years (Model 1) and for socioeconomic status, the HPL depression score, the energy-adjusted daily intake of fibre and vitamin C, and the total fat intake (Model 2). We used these variables as covariants to exclude the possible bias of a commonly healthier life-style and diet associating with depression. We used the backward stepwise method of the Cox's proportional hazards regression model to assess those covariates that had the strongest associations with depression. Because of the relatively small number of cases, the number of covariates was restricted and we

chose the six covariates that had the strongest associations. The model originally included all the variables presented in Table 8 except for the absolute intake of the vitamins. Folate intake was allowed to compete freely with the other candidate covariates in the baseline analysis, and it had the strongest association with depression. Differences in baseline characteristics between those who received a discharge diagnosis of depression during the follow-up period and the rest of the cohort were examined using the Student's t-test, Mann-Whitney U-test and chi-squared test. In prospective analysis we used this division of two categories because of the small number of outcome events. (II)

In the study of the association between the serum tHcy levels and depression, differences in the assessed characteristics between depressed participants and the rest of the cohort were examined using the Student's t-test, Mann-Whitney U-test and chi-squared test. Participants were also ranked and divided to tertiles according to the serum levels of tHcy. Differences in the assessed characteristics between the tertiles of serum tHcy were examined using analysis of variance (ANOVA), the chi-squared test and the Kruskal-Wallis test. We used a cut-off level of >11.9 μmol/l to indicate an elevated serum tHcy concentration, because this cut-off level has been used in some earlier studies (Bottiglieri et al. 2000). The median level of tHcy in our material was 10.4. Correlations were examined with Pearson's correlation test. ORs relating depression to serum levels of tHcy were calculated using a logistic regression model adjusted for age and examination years (Model 1), history of ischaemic heart disease, smoking habits, alcohol consumption, living alone, education and adulthood socioeconomic status (Model 2). We also adjusted Model 2 further for poor appetite, serum α-tocopherol, lycopene and total cholesterol concentration. (III)

In the study of the association between the treatment outcome and blood levels of folate and cobalamin, comparisons were made between patients in full response, partial response and nonresponse groups, which are defined in detail in chapter 4.2.2.

The statistical methods used were the chi-squared test, Pearson's correlation coefficient, the Student's t-test, ANOVA, and univariate and multivariate linear regression analysis. (IV) As these studies were observational in nature the power calculations were not used.

5. RESULTS

The results of the original studies are presented separately here.

5.1 Association between dietary folate intake and depressive symptoms (I)

The characteristics of the study subjects with current depressive symptoms and the rest of the cohort are presented in Table 5. Depressed men were older and living alone more often than the others. They also had a lower daily energy intake, higher waist to hip ratio, and they reported a poor appetite more frequently. They had a higher alcohol consumption than the rest of the cohort.

Table 5. Characteristics of the study population of the middle aged men stratified by the presence (HPL ≥5) or absence (HPL< 5) of current depressive symptoms[1,2]

	(HPL ≥5)	(HPL< 5)	p value
N	228	2215	
Age, y	53.8 ± 4.5	53.0 ± 5.2	0.014[3]
Body mass index, kg/m^2	26.9 ± 4.0	26.8 ± 3.5	0.659[3]
Waist to hip ratio	0.96 ± 0.06	0.95 ± 0.06	0.049[3]
Alcohol intake, g/wk	96.6 ± 134.9	72.4 ± 134.6	0.003[4]
Energy intake, MJ/d	9.53 ± 2.54	9.95 ± 2.62	0.020[3]
Fat intake, g/d	100.7 ± 35.2	101.8 ± 33.7	0.639[3]
Education: graduated from high school, %	18.7	18.3	0.870[5]
Marital status: living alone, %	17.8	12.1	0.012[5]
Poor appetite, %	18.3	3.6	< 0.001[5]
Smoking, %	33.8	31.2	0.426[5]

[1]Values are means ± SD or %, [2]HPL is Human Population Laboratory Depression Scale, [3] Student's t –test, [4] Mann-Whitney U-test, [5] Chi-squared test

Depressed men had a lower absolute daily intake of folate and pyridoxine than the others. Only 23.6% of the men consumed the RDA of 300 µg/d of folate, while 100% of the cohort reached the RDA of the other B vitamins (Table 6). The vitamin intakes of the whole cohort were: 253.9 µg/d for folate, 9.5 µg/d for cobalamin, 1.9 mg/d for pyridoxine and 2.2 mg/d for riboflavin.

Table 6. Daily intake of B vitamins of the middle aged men stratified by the presence (HPL ≥5) or absence (HPL< 5) of current depressive symptoms[1,2]

	HPL ≥ 5 (n = 228)	HPL ≤ 5 (n = 2215)	Finnish RDA[3]	Subjects consuming the RDA, %	p-value[4]
Folate, µg/d	236.0 ± 69.1	256.2 ± 74.7	300	23.6	< 0.001
Cobalamin, µg/d	9.3 ± 8.4	9.6 ± 9.6	2.0	100.0	0.666
Pyridoxine, mg/d	1.8 ± 0.5	1.9 ± 0.5	1.5	100.0	0.025
Riboflavin, mg/d	2.1 ± 0.8	2.2 ± 0.8	1.6	100.0	0.110

[1]Values are means ± SD or %, [2]HPL is Human Population Laboratory Depression Scale, [3]Reference: National Committee of Nutrition 1998. [4]Significance of the difference between groups; Student's t-test.

The men were divided into tertiles according to their daily intakes of folate, cobalamin, pyridoxine and riboflavin. In the lowest tertile of folate intake 11.5% of the men were depressed and in the highest third 7.2%. Respective figures for the other vitamins were 8.8% and 10.0% for cobalamin, 8.4% and 9.4% for pyridoxine, and 9.1% and 8.8% for riboflavin (Table 7).

Table 7. Numbers and proportions of depressed middle aged men in the tertiles of folate, cobalamin, pyridoxine and riboflavin intakes[1]

Vitamin intake	Lowest tertile	Middle tertile	Highest tertile	p-value for linear trend[2]
Folate, n (%)	93 (11.5)	76 (9.1)	59 (7.2)	0.002
Range, µg/d	45.4 – 226.0	226.8 – 269.1	269.3 – 587.5	
Cobalamin, n (%)	73 (8.8)	74 (9.0)	81 (10.0)	0.442
Range, µg/d	2.2 – 5.9	5.9 – 8.7	8.7 – 136.0	
Pyridoxine, n (%)	68 (8.4)	82 (10.0)	78 (9.4)	0.503
Range, mg/d	0.3 – 1.7	1.7 – 2.1	2.1 – 4.4	
Riboflavin, n (%)	75 (9.1)	81 (9.9)	72 (8.8)	0.866
Range, mg/d	0.4 – 1.9	1.9 – 2.4	2.4 – 5.3	

[1]Values are n (%) of men in the tertile who have current depressive symptoms, and the range of vitamin intakes in tertiles of 2443 middle-aged Finnish men, [2]Chi-squared test

Participants in the lowest tertile of folate intake had a 67% higher risk of being depressed than the men in highest tertile (Table 8, Model 1). When adjusted for age, examination years, smoking habits, consumption of alcohol, appetite, BMI, living alone, education, adulthood socioeconomic status and total fat consumption, the risk remained statistically significant (Table 8, Model 2). Among participants in the lowest tertile of cobalamin, pyridoxine and riboflavin intakes, the risk of being depressed was not different from that of the men in highest tertile.

Table 8. Odds Ratios (OR) of depression in middle aged men according to intakes of vitamins

Vitamin	Lowest tertile Model 1[1] [Model 2[2]]	Middle tertile Model 1[1] [Model 2[2]]	Highest tertile (ref.)	p value[3] Model 1 [Model 2]
	OR (95% CI)	OR (95%CI)	OR	
Folate	1.67 (1.19-2.35)	1.30 (0.91-1.86)	1.0	0.003
	[1.46 (1.01-2.12)]	[1.23 (0.85-1.79)]		[0.044]
Cobalamin	0.88 (0.63-1.23)	0.89 (0.64-1.24)	1.0	0.445
	[0.79 (0.56-1.13)]	[0.82 (0.58-1.16)]		[0.196]
Pyridoxine	0.88 (0.62-1.27)	1.07 (0.77-1.48)	1.0	0.446
	[0.79 (0.55-1.13)]	[1.04 (0.74-1.47)]		[0.189]
Riboflavin	1.06 (0.75-1.48)	1.14 (0.82-1.60)	1.0	0.757
	[1.24 (0.87-1.78)]	[1.23 (0.87-1.74)]		[0.237)]

[1] Logistic regression adjusted for age and examination years. [2] Logistic regression adjusted for age, years of study, smoking habits, consumption of alcohol, appetite, BMI, living alone, education, adulthood socioeconomic status and total fat consumption. [3] For difference between lowest and highest tertiles

5.2 Folate intake and the risk of depression (II)

The baseline characteristics of the study subjects with depression (n = 47) and the rest of the cohort are presented in Table 9. For the whole cohort, the mean intake of folate was 256 μg/day (SD: 76) and of cobalamin 9.54 μg/day (SD: 9.48). At baseline only 24.6% of the participants reached the Finnish recommended daily folate intake of 300 μg/day, while 98.7% of the participants reached the recommended daily cobalamin intake of 3 μg/day.

Table 9. Characteristics of the study population at baseline according to receiving a discharge diagnosis of depression during the follow-up (mean ± SD or %)

	Subjects hospitalised due due to depressior (n = 47)	Subjects not hospitalised due to depression (n = 2260)	p-value for difference
Energy-adjusted folate µg/d	233.4 (57.8)	256.0 (56.9)	0.007[1]
Total folate, µg/d	255.8 (84.5)	256.4 (75.7)	0.958[1]
Energy-adjusted cobalamin, µg/d	10.0 (15.9)	9.5 (9.1)	0.707[1]
Total cobalamin, µg/d	10.8 (16.0)	9.5 (9.3)	0.343[1]
Total energy, MJ/d	11.05 (2.94)	9.92 (2.59)	0.003[1]
Alcohol/week, g	73.0 (99.7)	72.7 (135.3)	0.761[2]
Age, years	52.2 (5.4)	53.0 (5.2)	0.190[1]
Marital status: living alone, %	12.5	12.5	0.995[3]
Education: graduated from high school, %	22.9	18.2	0.418[3]
Smoking, %	37.5	31.2	0.351[3]
HPL depression score	1.8 (1.5)	1.3 (1.3)	0.019[2]
Poor appetite, %	6.1	3.8	0.866[3]
Body mass index, kg/m^2	26.9 (3.3)	26.8 (3.5)	0.914[1]
Waist to hip ratio	0.93 (0.05)	0.95 (0.05)	0.100[1]

[1]Student's t –test, [2]Mann-Whitney U-test, [3]Chi-squared test. HPL = human population laboratory depression scale.

Participants were divided to two groups according their daily median intake of folate. Participants in the lower (≤ 246 µg/d) folate intake group had a three times higher risk of receiving a discharge diagnosis of depression than those in the higher folate group (Risk Ratio, RR : 3.04, 95%, Confidence interval, CI: 1.58- 5.86, $p < 0.001$; Table 10, Model 1). In multivariate analysis adjusted for adulthood socioeconomic status, baseline HPL depression score, the energy-adjusted daily intake of fibre and vitamin C, and total fat intake, the risk remained two-and-a-half-fold higher (RR: 2.53, 95% CI: 1.17- 5.48, $p = 0.019$; Table 10, Model 2). Further adjustment for

marital status, education, current smoking habits and weekly alcohol consumption did not alter this association (RR: 2.51, 95% CI: 1.16 - 5.45, p = 0.020).

Table 10. Relative risk of depression according to energy-adjusted mean intake of folate and cobalamin.

Vitamin	Cases in lower half[1]	Cases in upper half	Relative risk	95% CI	p value for difference
			Model 1[2] (Model 2[3])	Model 1 (Model 2)	Model 1 (Model 2)
	n	n			
Folate	35	12	3.04 (2.53)	1.58 – 5.86 (1.17 – 5.48)	<0.001 (0.019)
Cobalamin	22	25	0.85 (0.40)	0.48 – 1.51 (0.43 – 1.39)	0.590 (0.401)

[1]According to the median, [2]Adjusted for age and examination year, [3]Adjusted for age and examination year, current socioeconomic status, baseline HPL depression score, energy-adjusted daily intake of fibre and vitamin C, and total fat intake.

We also repeated our analysis excluding participants who had been discharged with a diagnosis of depressive disorder during the first two years of follow-up. This left 44 incident cases to be analysed. The RR of depression in the lower folate intake group remained almost the same (Model 1. RR: 3.13, 95% CI: 1.58- 6.19, p = 0.001; Model 2. RR: 2.57, 95% CI: 1.16- 5.70, p = 0.021). Furthermore, we repeated the analysis excluding also those participants who had been diagnosed as having a mental disorder prior to the baseline. This left 40 cases to be analysed. However, the results did not change significantly (Model 1. RR: 3.16, 95% CI: 1.54 –6.48, p = 0.002; Model 2. RR: 2.53, 95% CI:1.17-5.48, p = 0.019).

There was a significant difference in the total energy intake between the depressed and the other participants (Table 9). However, adjustment for the total energy intake

had little effect on the association between folate intake and the incidence of depression (Model 1) (RR: 3.01, 95% CI: 1.56- 5.82, p = 0.001). In order to eliminate the possible bias of other folate-related diseases causing depression, we repeated the analyses excluding those participants who had a history of cancer or cardiovascular disease (n = 846), which left 35 cases to be analysed, but it had little effect on the results (Model 1. RR: 2.82, 95% CI: 1.35 –5.89, p = 0.006; Model 2. RR: 2.51, 95% CI:1.06-5.95, p = 0.036).

Participants were also divided into two groups according the median daily intake of cobalamin. However, the intake of cobalamin and the incidence of depression were not associated (RR: 0.85, 95% CI: 0.48-1.51, p = 0.59; Table 10, Model 1). Furthermore, splitting the cohort into tertiles or quartiles still revealed no association between cobalamin intake and an increased risk of depression.

We also split the participants further into quartiles according to folate intake (Figure 3). The results were essentially similar to those after division according to the median intake of folate.

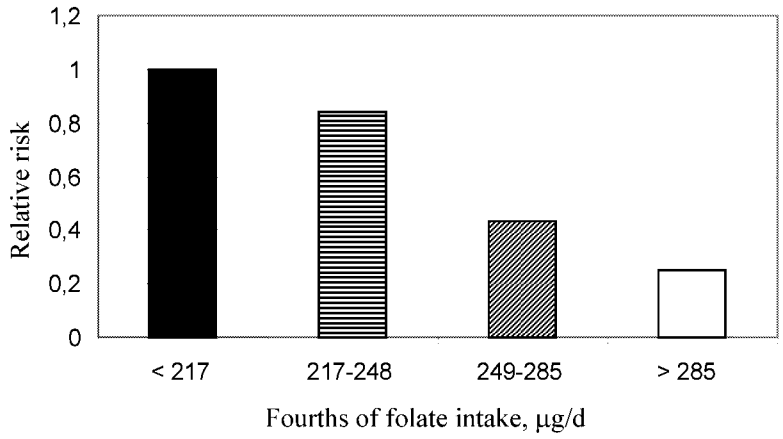

Figure 3. Relative risk of depression in participants divided into quartiles of folate intake. (With permission from Anu Ruusunen; Ruusunen 2004)

5.3 Association between serum homocysteine and depressive symptoms (III)

The characteristics of the study subjects with (n = 109) and without depression (n = 762) are presented in Table 11. Depressed participants had a history of IHD and poor appetite more often than the others, and they also had higher serum levels of tHcy.

Table 11. Characteristics of the study population of middle-aged men divided according to the presence (HPL ≥5) or absence (HPL< 5) of depressive symptoms.[1,2]

	Subjects with depressive symptoms	Subjects without depressive symptoms	p-value
N	109	762	
Age, y	57.0 ± 6.2	55.9 ± 6.8	0.128[3]
Alcohol intake, g/wk	91.7 ± 133.4	74.6 ± 123.5	0.351[4]
Body mass index, kg/m^2	27.5 ± 3.5	27.6 ± 3.7	0.703[3]
Education: graduated from high school, %	15.6	11.8	0.260[5]
Marital status: living alone, %	10.1	11.2	0.740[5]
Smoking, %	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]
Mean serum tHcy, µmol/l	11.9 ± 5.0	10.8 ± 3.1	0.003[3]

[1]The values are means ± SD or %.[2]HPL is Human Population Laboratory Depression Scale. [3]Student's t –test. [4]Mann-Whitney U-test. [5]Chi-squared test. IHD = ischaemic heart disease. tHcy = total homocysteine.

Participants were divided into tertiles according to their serum levels of tHcy. The characteristics of the study population according to the tertile of serum tHcy are summarized in Table 12. Higher levels of tHcy were associated with an older age, living alone, and a poor appetite.

The serum tHcy concentration was elevated (cut-off level >11.9 µmol/l) in 36.7% of the depressed participants and in 26.4% of the others (P = 0.024). The mean HPL depression scores were significantly higher among those with an elevated serum tHcy level than among other study subjects (mean HPL score 1.6, SD = 1.9 vs 1.3, SD = 1.7; P = 0.005).

Table 12. Characteristics of the study population in tertiles of serum tHcy.[1]

	Lower tertile	Middle tertile	Upper tertile	p-value
Mean serum tHcy, µmol/l	8.8 1.0	10.4 ± 0.5	14.0 ± 4.3	-
Range of serum tHcy, µmol/l	2.8 – 9.5	9.6 – 11.3	11.4 – 51.2	-
Age, y	54.9 ± 6.6	55.9 ± 6.8	57.6 6±.5	<0.001[2]
Alcohol intake, g/wk	80.2 ± 113.3	70.7 ± 101.9	78.9 ± 156.1	0.198[3]
Body mass index, kg/m^2	27.7 ± 3.6	27.5 ± 3.5	27.5 ± 3.5	0.584[2]
Education: graduated from high school, %	10.7	11.4	15.1	0.104[4]
Marital status: living alone, %	9.0	9.4	14.4	0.035[4]
Smoking, %	28.8	28.6	25.1	0.314[4]
Poor appetite, %	2.0	3.1	6.5	0.005[3]

[1]The values are means ± SD or %. [2]ANOVA, p-value is for the trend. [3]Kruskal-Wallis test. [4]Chi-squared test, p for the linear trend.

Depression was most common among participants in the upper tertile of serum tHcy. Furthermore, there was a linear trend in the proportions of depressed participants according to serum tHcy concentration (Table 13).

Table 13. Numbers and proportions of depressed participants and serum concentration ranges in the tertiles of serum total homocysteine (tHcy).

	Lowest tertile	Middle tertile	Upper tertile	p-value for linear trend[1]
Range of serum tHcy levels, µmol/l	2.8 – 9.6	9.6 – 11.4	11.4 – 51.2	
n (%) of depressed participants	23 (7.9)	37 (12.7)	49 (17.1)	< 0.001

[1]Chi-squared test

The risk of depression was over two-fold higher among men in the highest tertile of serum tHcy than in men in the lowest tertile (OR = 2.30, 95% CI: 1.35 - 3.90, p = 0.002, Table 14, Model 1). In multivariate analysis adjusted for IHD history, smoking habits, alcohol consumption, living alone, education and adulthood socioeconomic status the risk was at the same level (OR = 2.23, 95% CI: 1.30 - 3.83, p = 0.004; Table 14, Model 2). Adjusting Model 2 further for poor appetite slightly weakened the association (OR = 1.97, 95% CI: 1.14 - 3.41, p = 0.016).

Table 14. Odds Ratios for the risk of depression according to tertiles of serum total homocysteine (tHcy) levels.

Lowest tertile	Middle tertile	Highest tertile	p-value[3]
	Model 1[1]	Model 1[1]	Model 1
	[Model 2[2]]	[Model 2[2]]	[Model 2]
OR	OR (95% CI)	OR (95% CI)	
1.0 (ref.)	1.65 (0.95 - 2.86)	2.30 (1.35 - 3.90)	0.002
	[1.67 (0.96 - 2.91)]	[2.23 (1.30 - 3.83)]	[0.004]

[1]Logistic regression adjusted for age and the examination years. [2]Logistic regression adjusted for age, examination years, ischaemic heart disease history, smoking habits, alcohol consumption, marital status, education and adulthood socioeconomic status. [3]For difference between 1st and 3rd tertiles in logistic regression.

Adjustment of Model 2 for serum α-tocopherol, lycopene and total cholesterol did not significantly change the results of the analysis (OR = 2.21, 95% CI, 1.29 to 3.79, p = 0.004). We performed this adjustment to disclose the possibility of a commonly healthier diet and lifestyle among the non-depressed participants biasing the results, or of the other healthy characteristics of food explaining the association between depression and the tHcy.

We also repeated the analyses after the exclusion of all those participants who had a history of any cancer or any CVD except hypertension. We did not exclude those

participants who had hypertension because hypertension has not associated with tHcy in any previous studies. This left 658 participants to be analysed, of which 52 had a tendency towards depression. The results were essentially the same (Model 1: OR 2.20, 95% CI: 1.05 - 4.58, p = 0.037; Model 2: OR 2.24, 95% CI: 1.06 - 4.74, p = 0.035).

5.4 Serum cobalamin level and treatment outcome (IV)

At baseline, no patients had a low cobalamin level (<140 pmol/l), while 14 patients (12%) had a high cobalamin level (> 540 pmol/l). Twenty-one patients (18%) had a low erythrocyte folate level (< 315 nmol/l) and two (2%) had a high level (>850 nmol/l). On follow-up, two patients (2%) had a low and two (2%) a high cobalamin level. Baseline and 6-month cobalamin levels correlated significantly (r = 0.52, p < 0.001).

Haematological indices (Hb, MCV, RBC, Hcr) and the BMI did not correlate with cobalamin or folate levels. Neither were there significant differences in the levels of cobalamin or folate between patients with and without weight loss or gastrointestinal symptoms (data not shown). Furthermore, haematological indices did not associate with the treatment response (Table 15).

Table 15. Haematological indices, serum cobalamin and red-cell folate, and six-month treatment outcome in major depressive disorder (n = 115)

	Nonresponse (n = 40) Mean (SD)	Partial response (n = 34) Mean (SD)	Full response (n = 41) Mean (SD)
Haemoglobin (g/l)	141.7 (10.1)	141.1 (13.3)	139.1 (14.8)
Mean corpuscular volume (fl)	92.5 (6.0)	90.4 (5.8)	89.5 (6.6)
Red blood cell count (cell 10^{12}/l)	4.51 (0.46)	4.57 (0.39)	4.56 (0.42)
Hematocrit (%)	41.6 (3.1)	41.2 (3.7)	40.8 (4.0)
Cobalamin at baseline (pmol/l)[1]	347.2 (103.4)	396.0 (108.4)	439.1 (115.6)
Cobalamin on follow-up (pmol/l)[2]	280.4 (93.3)	316.2 (98.2)	338.8 (87.5)
Folate at baseline (nmol/l)	409.7 (126.0)	431.5 (114.8)	446.8 (244.8)
HDRS score at baseline[3]	16.8 (7.2)	22.3 (5.6)	17.8 (5.4)
HDRS score on follow-up[4]	17.6 (4.9)	13.8 (3.7)	4.6 (3.2)

ANOVA: [1]F = 7.17, p = 0.001. [2]F = 4.07, p = 0.02. [3]F = 8.10, p = 0.01. [4]F = 112.58, p < 0.001. HDRS = Hamilton Depression Rating Scale.

Significant differences were found in cobalamin levels between patients in full response, partial response and nonresponse groups (Table 15). The cobalamin level and the HDRS score did not correlate at baseline. Nevertheless, a positive correlation was found between both the cobalamin level at baseline ($r = 0.39$, $p < 0.001$) and on follow-up ($r = 0.26$, $p = 0.006$), and the decline in the HDRS score during six months of treatment.

There were no significant differences in the erythrocyte folate level at baseline between full response, partial response and nonresponse groups (Table 15). Nevertheless, the folate level at baseline correlated with both the baseline HDRS score ($r = 0.21$, $p = 0.021$) and the decline in the HDRS score during the follow-up ($r = 0.20$, $p = 0.037$).

The severity of depression (HDRS score) at baseline differed between full response, partial response and nonresponse groups. The highest score was in the partial

response group. Not surprisingly, a highly significant difference was also found between the groups in the follow-up HDRS score (Table 15).

Sixty-four patients (56%) had been on adequate antidepressive medication and 83 (72%) had had an adjunct therapeutic relationship. However, only 49 of them (43% of the total sample) had been met at least weekly during three months. Fifteen (13%) had been treated as inpatients during the follow-up period. These treatment variables did not associate with haematological indices, the erythrocyte folate level, the serum cobalamin level or the baseline HDRS score (data not shown). Those who were in nonresponse and partial response groups had been treated as inpatients more often than those in the full response group. No other associations were found between treatment variables and response groups (Table 16).

Table 16. Treatment variables and six-month outcome in major depressive disorder (n = 115)

	Nonresponse	Partial response	Full response
	(n = 40)	(n = 34)	(n = 41)
	n (%)	n (%)	N (%)
Adjunct therapeutic relationship	30 (73)	24 (71)	29 (73)
Weekly psychotherapy [1]	20 (49)	11 (32)	18 (45)
Adequate drug therapy [2]	26 (63)	20 (59)	18 (45)
Treated as an inpatient [3]	9 (22)	5 (15)	1 (3)

[1]Duration at least three months. [2]See definition in paragraph 4.2.2. [3]Chi-Squared 6.87, df 2, p = 0.032.

Haematological indices did not associate with the decline in the HDRS score according to linear regression analysis (data not shown).

The relationship between the baseline cobalamin level and the decline in the HDRS score was found to be positive and linear in univariate regression analysis ($\beta = 0.39$, $t = 4.50$, $p < 0.001$). This relationship remained independent and significant ($\beta = 0.28$,

t = 3.40, p = 0.001) even after adjustment for age (years), sex, duration of the illness (years), family history of depression (yes/no), patterns of alcohol use (at least once a week/other), smoking habits (daily/other), BMI, weight loss (less than 3 kg, 3-5 kg, 5-8 kg, over 8 kg), gastrointestinal symptoms (yes/no), the severity of depression at baseline (HDRS score), adequate drug treatment (yes/no), weekly psychotherapy (yes/no) and inpatient treatment (yes/no). For the follow-up cobalamin level the figures were also statistically significant (univariate $\beta = 0.26$, $t = 2.80$, $p = 0.006$ and multivariate $\beta = 0.24$, $t = 2.85$, $p = 0.006$, respectively).

The baseline folate level and decline in the HDRS score associated weakly in univariate linear regression analysis ($\beta = 0.20$, $t = 2.11$, $p = 0.037$), but the association was no more significant when adjusted for other variables included in the multivariate analyses ($\beta = 0.08$, $t = 0.87$, $p = 0.389$).

6. DISCUSSION

6.1 Dietary folate and depressive symptoms (I)

Participants with a dietary folate intake in the lowest tertile had a 67% higher risk of having elevated depressive symptoms than those in the highest tertile of folate intake. Multivariate analysis supported the existence of an independent relationship between dietary folate intake and current depression.

There was no relationship between cobalamin intake and depression, even though some earlier cross-sectional studies have reported low blood levels of cobalamin in depressed patients (Abou-Saleh and Coppen 1989). The diet in Finland is quite high in dairy products and meat, which are rich sources of cobalamin, but many middle-aged men in Finland eat relatively few green vegetables and other sources of folate (National Committee of Nutrition 1998). Accordingly, in our cohort only 24% of the participants reached the RDA for folate, but 99% reached the recommended intake of cobalamin. The Finnish RDA of 300 μg/d for folate is also lower than that, for example, in the US (400 μg/d). Even the mean intake in the highest tertile was low (317.7 μg/d) compared with those higher recommendations. All of the participants had an adequate intake of pyridoxine and riboflavin. There was a slight difference between depressed and other participants in the absolute intake of pyridoxine, but this association was not significant in the logistic regression model using the energy adjusted intake of pyridoxine.

Lee et al. (1998) suggested that Chinese people in Hong Kong obtain so much folate from their vegetable-rich diet that even in those who have the lowest blood levels of folate, depressive symptoms are not aggravated. The association between folate intake and depression may therefore be determined by culturally patterned eating habits.

The total energy intake of the depressed participants was significantly lower than that of the other participants. This may be because of poor appetite or neglect of well-being resulting from depression.

The relation between dietary folate and depression could be explained by other beneficial components of a folate rich diet. However, if this was true, there might also be an association between depression and the dietary intake of certain other B vitamins that are available in food also commonly considered to be healthy. No significant associations were found in this study sample between the energy-adjusted daily intake of cobalamin, pyridoxine or riboflavin, and depression. Poor eating habits and many other risk factors for depression could also cluster in the same people. However, adjustment for several possible risk factors associated with lifestyle did not affect the main results supporting the idea that the association between low folate intake and depression is real.

No structured interviews were used to identify psychiatric disorders according to specific diagnostic criteria, as some other investigators have done (Morris et al. 2003; Tiemeier et al. 2002). Thus, some of the study participants who were 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 this study (i.e., 9.2% at baseline and 7.3% on 4-year follow-up, if those with a previous psychiatric history were included) and Lindeman et al.'s (2000b) estimate of the prevalence of major depressive episode (7.2%) among Finnish men aged 15-75 years may suggest that subjects who scored $\geq$5 on the HPL scale had experienced a clinically significant depressive episode. Nevertheless, I do not claim that an HPL $\geq$ 5 is exactly the same as a clinical diagnosis of depression. All men who had a HPL score $\geq$ 5 either at baseline or on follow-up were included in this study, because there is some evidence that depressive symptoms are persistent

and relatively stable over time (Hagnell and Grasbeck 1990). Respectively, the sub-threshold depressive symptoms also seem to be stable (Merikangas et al. 2003).

An association between the intake of folate and depression has been suggested to be most clearly apparent in ageing populations (Reynolds 2002). In our sample at the study baseline the mean age was only 53 years, and therefore the association between the folate intake and depression might have been stronger if an older population had been selected. The sample also included only men, while previous studies have detected an association between folate levels and depression both in men and women (Coppen and Bailey 2000; Jacques et al. 2002; Morris et al. 2003). Depression in Finland, as in many other countries, is more common among women (Lindeman et al. 2000b), which makes the generalization of the findings to women difficult in a sample consisting only of men. However, the homogeneity of our sample improves the possibility of identifying associations between depression and background variables, because the ethiology of depression is considered to be multi-factorial.

A low dietary folate intake could be a consequence, rather than a cause, of depression. This possible bias is difficult to eliminate in a cross-sectional study. There might also exist a vicious cycle in which folate deficiency aggravates depression, a symptom of which is poor appetite, and this in turn further lowers the folate intake and furthermore aggravates folate deficiency. However, the main results remained significant after adjustment for poor appetite, even though poor appetite was significantly more common among the depressed participants.

I excluded participants who had a previous psychiatric history, which also reduced the possible bias of the residual symptoms of depression or other psychiatric disorders affecting eating habits. However, it is possible that depressed subjects do not complete their food records as carefully as the others, which could have affected my results.

6.2 Dietary folate and prospective risk of depression (II)

Participants with a low dietary folate intake had a three-fold higher risk of receiving a discharge diagnosis of depressive disorder during the follow-up period than those in the higher folate intake group. The relationship remained significant and independent even after adjustment for several confounding risk factors for depression and low vitamin intake.

Depressed participants had a higher total energy intake than the others. However, there was no difference in appetite, BMI or waist to hip ratio between the groups in my study. Morris et al. (2003) did not find appetite loss, weight loss or being underweight to be related to blood folate levels, but being overweight was more common among the depressed participants. This could imply that other factors in the diet are also associated with depression.

It was also interesting that in the cross-sectional study there was a decreased energy intake among the depressed participants, which was contradictory to the prospective study. As a poor appetite is a typical symptom of depression it is quite natural that current depression is associated with a lower intake of energy. The increased energy intake of those who later became depressed is a more complex phenomenon. There was no difference in alcohol consumption between the groups, so alcohol cannot be the reason for this difference. It could be somehow be connected to an increased appetite, weight gain and increased eating as atypical features of depression. This hypothesis has indirect support from the findings of Morris et al. (2003). Unfortunately, none of these possible underlying mechanisms could be investigated with this data.

Unlike the food frequency method, food records are based on the actual intake of foods. A major strength of the food record method is that it does not rely on memory.

In the KIHD study, food records were collected over four consecutive days. One of our qualified nutritionists provided the necessary instructions and checked the completed food records with the study subject. They used pictures of the portions of food, which is easier for the participants than measuring the number of grams of food that has been eaten. The weakness of the food record method is that the day-to-day intake of foods is highly variable for many individuals. Although undereating could occur during record-keeping days, it is believed that four consecutive days are enough to reliably detect differences in energy intake between depressed and non-depressed subjects. Inclusion of a large number of participants also decreases the risk of this possible bias.

Changes in eating habits during the follow-up period could also have biased the results. However, earlier studies on this data have shown eating habits to remain relatively stable. Follow-up data for 440 of these men revealed that the average daily folate intake had decreased by 7.1% (from 268 to 248 μg/day) during 11 years of follow-up (Voutilainen 2000). Because the study is a prospective population study it can be more easily generalized to the general population than case-control studies. Naturally, however, I cannot generalize my findings to men in all age groups and to women. Nevertheless, the intake of folate seems to change quite slowly in the Finnish population in relation to age. There was also strong variation in the folate intake of our population. This made the differences between groups clearer.

Hospital diagnoses were obtained from the National Hospital Discharge Register, which covers the treatment episodes in general, mental, military, prisoned and private hospitals and the inpatient wards of local health centers nation-wide. Data from this register have been used in epidemiological research and shown to be reliable (Keskimäki and Aro 1991). Unfortunately, Finland has no national register of outpatients with depression, and the total number of cases of depression could not therefore be included in the analysis. Furthermore, many depressed people do not

seek treatment for their mental problems. For this reason these analyses were limited to severe depression requiring hospitalisation. This restriction and the exclusion of participants with depressive symptoms at baseline also strengthens the probability that a low dietary folate intake is a cause rather than a consequence of depression. In other words, this also eliminates the bias of poor appetite as a symptom of depression affecting dietary habits. Furthermore, poor appetite was quite rare in both hospitalised and non-hospitalised subject groups (6.1% vs. 3.8%), and there was no significant difference between them. Repeating the analysis and excluding those who were hospitalised during the first two years of follow-up should also minimise bias due to depression causing poor dietary habits.

The present prospective study had the same limitations as the cross-sectional study concerning the age of the subjects, lack of women in the sample and possible confounding factors connected to a healthy life-style. These limitations were already discussed in the previous chapter.

6.3 Plasma homocysteine and depressive symptoms (III)

In this study, participants with the highest levels of serum tHcy had a more than two-fold greater risk of being depressed than those in the lowest tertile. The results of the present study support the hypothesis that folate-tHcy metabolism is associated with depression in middle-aged Eastern Finnish men. As stated earlier, it is not known whether these findings can be generalized to women or to men of different ages.

Morris et al. (2003) found that blood folate levels, but not plasma tHcy levels, were associated with depression in a general population sample. However, the mean age of the subjects in that study was 26.2 years, whereas in this study the mean age was 56.1 years. It has been suggested that the association between depression and high levels

of tHcy should be especially apparent in an aging population (Bell et al. 1992b). As an older population has more often hyperhomocysteinemic individuals due to age-related deterioration of kidney function, it may support the hypothesis that tHcy is directly detrimental and a cause of depression. Furthermore, Morris and co-workers (2003) found lower mean tHcy concentrations in both depressed and non-depressed subjects (8.6 vs. 8.3 μmol/l) than were recorded in this study (11.6 vs. 10.8 μmol/l). The connection between depression and increased plasma tHcy may be seen more clearly in populations with a commonly low folate and high tHcy status.

Over a third of the depressed participants in our sample had high levels of tHcy (cut-off > 11.9 μmol/l). Bottiglieri et al. (2000) recorded increased levels of plasma tHcy in 52% of depressed inpatients using the same cut-off ours, whereas Fava et al. (1997) observed increased levels of tHcy in 20% of depressed outpatients (cut-off > 13.1 μmol/l). A comparison between the findings of Bottiglieri and co-workers and those of Fava and co-workers might indicate that patients having more severe depression requiring hospitalization also have higher levels of tHcy than outpatients. However, a high level of tHcy was also a common finding in our general population sample, which may be connected with low intake of folate in this study sample (National Committee of Nutrition, 1998; Voutilainen et al. 2000).

The serum tHcy samples were kept in storage for approximately 7 years at –20 °C before analysis. It is known that tHcy is stable for at least for one year at that temperature (Ueland et al. 1993). Furthermore, the distribution of values from stored samples is similar to those from assays of freshly drawn blood. Afthan and co-workers (1994) tested the stability of serum tHcy samples that had been kept in storage for 7 years at –20 °C and thawed twice without significant deterioration. The mean (range) serum tHcy concentration was 9.1 (6.9 to 13.2) μmol/L at baseline and 9.3 (7.5 to 13.2) μmol/L seven years later. This suggests that the deterioration of tHcy

samples was unlikely in this study, which is also supported by some other studies (Moller and Rasmussen 1995; Refsum et al. 2004).

An elevated level of serum tHcy may also be an indicator of an unhealthy lifestyle, which in turn leads to an increased risk of depression, rather than an independent risk factor. However, in our sample many other well-known risk factors for depression did not associate with an increased level of serum tHcy.

Several serious, long-term diseases have also been linked to hyperhomocysteinemia. Having such a disease may be a cause of depression. Connections of this kind which are currently unknown may bias the findings. To avoid this as far as possible, the analysis was also performed with the exclusion of all those participants who had any history of cancer or chronic heart disease. This exclusion did not change the results, which supports the main findings of this study. Nevertheless, this kind of bias cannot be completely excluded.

6.4 Serum cobalamin level and treatment outcome (IV)

As far as I am aware, there have been no previous studies that have suggested a positive relationship between the cobalamin level and the treatment outcome in patients with major depressive disorder who generally have normal or high serum cobalamin levels. Previous research has focused on possible associations between a low cobalamin level and a poor treatment response (Wesson et al. 1994). A low cobalamin level was less common in this sample than has been previously reported in patients with major depression (Mischoulon et al. 2000). I found no correlation between the severity of depression and the level of cobalamin at baseline. Surprisingly, the folate level and severity of depression displayed a weak positive correlation. Nevertheless, it is probably a false positive finding due to the relatively

small study sample. Engström and Träksman-Bendz (1999) found no correlation between the levels of folate or cobalamin and the severity of depression, but an inverse relationship between the level of folate and severity of depression has been reported in some other studies (Carney et al. 1990; Wesson et al. 1994).

A low folate level was relatively common (18%) among our patients with major depressive disorder, which is in accordance with previous studies (Alpert and Fava 1997). Nevertheless, a low folate level was not detected in German or Chinese patients with major depression (Wolfersdorf and Konig 1995; Lee et al. 1998). I observed only weak indications that a low erythrocyte folate level might be associated with a poor treatment outcome, which is contradictory to some previous studies (Fava et al. 1997; Wesson et al. 1994). It could be that culturally defined dietary habits influence the relationship between the folate status and depression in different societies (Lee et al. 1998). Moreover, the present study included only young and middle-aged outpatients with moderate depression, which may have influenced the results. The association between folate and depression may be more prominent in elderly subjects, among whom folate deficiency has been relatively common in some studies (Quinn and Basu 1996). Finally, there were no follow-up data on folate levels, which is a limitation of this study.

Poor appetite and inappropriate food intake as symptoms of depression could result in low levels of cobalamin and especially low levels of folate. However, I found no connection between BMI, HDRS items relating to gastrointestinal symptoms and weight loss, and blood vitamin levels. Why some depressed people have lower levels of these vitamins could be a topic for further investigation. It might reflect a lower intake of vitamins from food or assimilation from the gastrointestinal tract, or a higher rate of metabolism of these vitamins. Depression may also affect the quality of food in the diet. Morris and co-workers (2003) found that the levels of blood folate

had decreased after an episode of depression. However, a loss of appetite, weight loss and being underweight were not related to folate levels.

No correlations were observed between cobalamin and folate levels and the MCV, RBC or HCR, which is in line with previous studies (Penninx et al. 2000; Mischoulon et al. 2000). Haematological indices did not predict the treatment outcome, either. This indicates that haematological indices have no value in assessing depressed patients, while the levels of cobalamin in serum and folate in erythrocytes may have.

Multivariate analyses adjusted for age, sex, the family history of depression, the duration of the illness, the severity of depression at baseline and treatment variables during the follow-up period supported the existence of an independent relationship between the cobalamin level and the decline in the HDRS score. This was, however, a naturalistic follow-up study, and sociodemographic and clinical and treatment variables were not controlled a priori. This is the main shortcoming of this study. Further studies are suggested with controlled illness and treatment variables to confirm or to refute the present findings.

Our sample included more women (61%) than men, only one subject was aged over 65 years and 87% had only been treated as outpatients. All these variables may influence the results. Men and women may have different dietary habits. A low cobalamin level and cobalamin deficiency have been found to be common among older women (Penninx et al. 2000), but elderly men may have even lower cobalamin levels (Lindeman et al. 2000a). Finally, patients with psychotic depression may have lower cobalamin levels than non-psychotic depressives (Bell et al. 1992). Psychotic depression is a common indication for inpatient treatment. For all these reasons one should be careful about generalizing these findings to all groups of depressive patients.

The mean level of cobalamin on follow-up was lower than at baseline, which may indicate some deterioration of the samples during freezing. Previously, Kirke et al. (1993) have reported that levels of folate may decline by approximately 20% during six years of freezing. The length of the storage had little effect on this deterioration. Another explanation is that depression may also affect the quality of food in the diet, which lowers levels of blood vitamins during an episode of depression (Morris et al. 2003). Nevertheless, baseline and follow-up levels of cobalamin in the present study correlated significantly and both also associated highly significantly with the decline in the HDRS level during the follow-up, which supports my findings.

7. CONCLUSIONS

7.1 Conclusions

Low dietary folate may be associated with an increased risk of depression, especially in populations with a generally low mean intake of folate and a large variance in it. Furthermore, folate-tHcy metabolism may play role with regard to depression. Augmentation of antidepressive treatment with B vitamins might be warranted, at least in the case of a poor treatment response.

7.2 Implications for prevention and clinical practice

The preventive and clinical implications of the results of studies on depression and B vitamins currently remain unclear. On the basis of this work and previously published studies one can suggest the following:

1. This work together with previous studies supports general recommendations that an adequate amount of fresh vegetables, fruits and full grain products have a beneficial impact on our health. They may also have positive effects on mental health. It could be useful to increase the use of these healthy food products as a part of hospital food and in institutional catering services.
2. Reasonable augmentation strategies for antidepressive treatments are still unclear, but vitamins, especially folate, and perhaps also cobalamin, as an augmentation of antidepressive medication, may be useful. They may be warranted at least in cases of refractory depression because they are safe.
3. Determining levels of erythrocyte folate, serum cobalamin, and possibly serum tHcy might have clinical value in treatment resistent and elderly patients with

depression. B vitamin augmentation could also be used in sub-threshold deficiency. Another possibility might be to carry out augmentation with all elderly depressed patients. This would probably be less expensive than the measurement of blood levels. In some studies such augmentation has also been useful for those who have adequate blood levels of vitamins. Augmentation is also safe.

7.3 Implications for further research

On the basis of this work the following further studies are suggested:

1. Associations between folate should also be studied in populations where an adequate intake of folate is common. These studies should additionally determine whether these associations are linked to age, sex or diet or some other underlying biological factors.

2. Prospective follow-up studies should also be conducted to evaluate the possibility that depression could be prevented by changing to a diet including more of B vitamins.

3. More randomized, placebo-controlled clinical studies should be conducted to determine whether folate and cobalamin are effective augmentation strategies in treating depression.

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APPENDIX 1. DIAGNOSTIC CRITERIA OF A MAJOR DEPRESSIVE EPISODE ACCORDING TO DSM-III-R (American Psychiatric Association 1987)

A. At least five of the following symptoms have been present during the same two-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood, or (2) loss of interest or pleasure. (Do not include symptoms that are clearly due to a physical condition, mood-incongruent delusions or hallucinations, incoherence, or marked loosening of associations.)
   (1) depressed mood (or can be irritable mood in children and adolescents) most of the day, nearly every day, as indicated either by subjective account or observation by others
   (2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated either by subjective account or observation by others of apathy most of the time)
   (3) significant weight loss or weight gain when not dieting (e.g., more than 5% of body weight in a month), or decrease or increase in appetite nearly every day (in children, consider failure to make expected weight gains)
   (4) insomnia or hypersomnia nearly every day
   (5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
   (6) fatigue or loss of energy nearly every day
   (7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
   (8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
   (9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. (1) It cannot be established that an organic factor initiated and maintained the disturbance

(2) The disturbance is not a normal reaction to the death of a loved one (Uncomplicated Bereavement)

Note: Morbid preoccupation with worthlessness, suicidal ideation, marked functional impairment or psychomotor retardation, or prolonged duration suggest bereavement complicated by Major Depression.

C. At no time during the disturbance have there been delusions or hallucinations for as long as two weeks in the absence of prominent mood symptoms (i.e., before the mood symptoms developed or after they have remitted).

D. Not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Pshychotic Disorder NOS.

APPENDIX 2. DIAGNOSTIC CRITERIA OF A MAJOR DEPRESSIVE EPISODE ACCORDING TO DSM-IV (American Psychiatric Association 1994)

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

Note: Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.

(1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful). **Note:** In children and adolescents, can be irritable mood.

(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)

(3) significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. **Note:** In children, consider failure to make expected weight gains.

(4) Insomnia or hypersomnia nearly every day

(5) Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)

(6) Fatigue or loss of energy nearly every day

(7) Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)

(8) Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by other)

(9) Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide ateempt or a specific plan for committing suicide.

B. The symptoms do not meet criteria for a Mixed Episode

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physciological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

E. The symptoms are not better accounted for by Bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

APPENDIX 3. HPL DEPRESSION SCALE (Kaplan et al. 1987)

Items of HPL – depression scale. Possible answers were "never", "sometimes" and "often". Answers "never" or "often" (whichever is appropriate) were considered to be indicative of a depressed response (Kaplan et al. 1987).

Felt depressed or very unhappy
Appetite poor
Trouble getting to sleep or staying asleep
Felt lonely or remote from other people
Felt happiness
Little enjoyment from leisure time
Less energy than other people
Felt pleased about accomplishing something
Felt bored
Felt so restless that could not sit still long
Felt exited or interested in something
Cannot relax easily
Felt vaguely uneasy without knowing why
Feeling too tired to do the things wishing to do
Social withdrawal even from the people who are close to
Difficulties while being with others
Never satisfied with things which one has done
Getting tired easily

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