ONE-CARBON METABOLISM IN ADULTS WITH MAJOR DEPRESSION

by

Susan Osher

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Nutritional Sciences
University of Toronto

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Master of Science, 1999
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ABSTRACT

Several lines of evidence support a disturbance in one-carbon metabolism (OCM) in depression. The current study evaluated principal components and cofactors in OCM, and polymorphism of methyltetrahydrofolate reductase (MTHFR) in unmedicated depressed subjects (n=26) as compared with healthy controls (n=26). Neither group took vitamin supplements. Results indicated that compared with control subjects, depressed subjects had significantly lower methionine levels (p=0.012), and significantly higher serum folate levels (p=0.005). Among depressed subjects there was a significant negative correlation between methionine (p=0.001), glycine (p=0.027) and Hamilton Rating for Depression (HAM-D), and a positive correlation between red cell folate (p=0.005), S-adenosylhomocysteine (p=0.006), vitamin B12 (p=0.032) and HAM-D. Depressed subjects were more likely to be heterozygous for the MTHFR C667T allele as compared with controls (53.8% vs 28.0%, p=0.019). The pattern of dysfunction in OCM in this study suggests that a substantial disturbance in both methionine synthetase and MTHFR may exist in major depression.
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<td>BMI</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<td>L-DOPA</td>
<td>L-3,4-dihydroxy-phenylalanine</td>
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<td>MAT</td>
<td>methionine adenosine transferase</td>
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<td>MS</td>
<td>methionine synthase</td>
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<td>MTHF</td>
<td>methyltetrahydrofolate</td>
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<td>MTHFR</td>
<td>methyltetrahydrofolate reductase</td>
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<td>NTD</td>
<td>neural tube defects</td>
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<td>OCM</td>
<td>one-carbon metabolism</td>
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CHAPTER 1: INTRODUCTION
Major depression is a recurrent, syndromal illness (Gold, P. W. et al., 1988) with dire psychological, social and economic consequences (Wells, K. B et al., 1989). It is a widespread phenomenon, affecting 17.1% of adults at least once in their lifetime (Blazer, D. G. et al., 1994). The one-year prevalence of major depression in Ontario is 4.1% (Offord, D. R. et al., 1996). In recent years, there has been interest in the relationship between food or nutrients, and mood. There is growing evidence that nutritional factors may play a role in the etiology, progression and possible treatment of depression (Young, S. N., 1993). Of all the micronutrients that have been examined, folic acid, or folate, has demonstrated the most consistent and convincing results (Alpert, J. E. et al., 1997; Arinami, T. et al., 1997).

The association between depression and low folate status has been studied in both folate deficient populations and in depressed patients. There is a high prevalence of depression in patients suffering from folate-deficient anaemia as well as other patient populations at high risk for folate deficiency, such as alcoholics (Abouh-Saleh, M. et al., 1982; Majumdar, S.K. et al., 1981) and epileptics on anticonvulsants (Froscher, W. et al., 1995; Kutt, H. et al., 1980). Folate deficiency and low folate status is more prevalent in depressed adults than in other adults (psychiatric and non-psychiatric populations alike) (Carney, M.W.P., et al., 1990; Young, S.N. et al.; Reynolds, E.H. et al.. 1984; Carney, M.W.P., 1967). Even when depressed adults have normal folate levels, the severity of depression is inversely related to the folate levels (Levitt, A.J. et al., 1994; Levitt, A.J. et al, 1989; Abou-Saleh, M. et al., 1989; Coppen, A. et al., 1982). Furthermore, when folic
acid supplementation was given as an adjunctive treatment for depression, psychiatric patients exhibited mood improvement, better social functioning and were discharged from hospital more quickly than patients who did not receive folate supplementation (Carney, M. W. P. et al., 1986). Folate status improves if depression is treated effectively, regardless of the class of antidepressant medication (Fava, M. et al., 1997; Alpert, M. et al., 1996). While numerous studies have demonstrated a relationship between folate and depression, the mechanism underlying this relationship remains unclear.

Theoretically, the disturbance of folate in depression could occur at one or many of the following levels: dietary intake, folate absorption, transportation, storage, utilization and/or excretion (Swain, R.A. and St.Clair, L., 1997). Some researchers have suggested that dietary intake, or folate malabsorption (Botez, M.I. et al, 1984, Botez, M.I., 1979a) may be the link between folate and depression. There have been no studies to date that have assessed the absorption of dietary folate in depressed adults. Dietary intake in depressed inpatient populations has been assessed by indirect measures such as asking relatives about the patient’s intake or assessing body weight change (Christenesen, L. et al., 1994, Levitt, A.J. et al., 1989, Thornton, W.E. et al., 1978). Of all the studies conducted with regard to folate intake in depression, there is no evidence thus far to suggest that low folate intake is related to low folate blood levels in depressed adults.

In contrast, there is evidence that alterations in the function of folate at the cellular level may explain the relationship between depression and folate levels. One-carbon
metabolism, for example, is known to impact on neurotransmission. Mood disorders are thought to be associated with dysfunctional neurotransmission, that is, a deficiency of biological signal transduction in the central nervous system (Podell, R. N., 1983). When a signal entering the brain or traveling from one brain cell to another arrives at a synapse, it causes a neurotransmitter (such as serotonin, norepinephrine and dopamine) to be released into the synaptic gap. The compounds complete the synaptic transmission of the signal by interacting with specific receptors on the postsynaptic terminal of the cell.

There exists a substantial body of evidence that suggests that depression is caused by a neurochemical dysregulation of such neurotransmitters as serotonin or norepinephrine (Groff et al, 1995). One carbon metabolism is crucial for the function of neurotransmitters through its role in methylation.

The compound of most interest with regards the relationship between neurotransmission and one carbon metabolism is S-adenosylmethionine (SAM). SAM is the main methyl donor in numerous essential transmethylation reactions in the brain, including the synthesis of neurotransmitters such as norepinephrine, serotonin and dopamine (Bottiglieri, T. et al., 1995). SAM also functions as a methyl donor for the methylation of membrane phospholipids. Methylation of membrane phospholipids influences both membrane fluidity and receptor sensitivity for the transduction of biological signals (Destein, M. L. et al., 1991). Several studies have explored SAM levels in depression. For example Bell et al (1994), in a longitudinal study of depressed adults found that SAM levels increased in those patients who recovered from depression. SAM has also been used in clinical practice as an effective antidepressant medication (Rosenbaum, J.F. et al.,
SAM, therefore, appears to be involved either in the regulation of mood or in recovery from depression. Since SAM itself is formed from folate, it is possible that the previous relationship between mood and folate may be mediated by SAM.

Folate crosses the blood-brain barrier in a monoglutamate form, 5-methyl tetrahydrofolate (5-MTHF). 5-MTHF combines with homocysteine, transferring a methyl group, to generate methionine. Methionine combines with ATP to form S-adenosylmethionine (SAM). Both MTHF (Reynolds, E. H. et al., 1984) and SAM (Fava, M. et al., 1997) are present in high concentrations in the CSF and synaptic regions, suggesting an important functional role of these substances in neurotransmission.

Several possible defects in one-carbon metabolism, therefore, may be involved in the pathophysiology of depressive illness. Such defects might include disturbed enzyme function, increased metabolic requirements that deplete the system of one carbon metabolites, decreased supply of substrates, or a combination of these mechanisms (Smythies, J. R. et al., 1997). Due to heritability of depression, it is plausible that genetic polymorphism of one of the regulating enzymes could contribute to the disturbance in one carbon metabolism that may be associated with depression. Also, depression is a state of hypermetabolism as seen by increased elevated levels of both cortisol (Gold, P. W. et al., 1984) and thyroxine (Joffe, R. T. et al., 1993). An increased metabolic requirement in depression may influence the functioning of the cycle. In order to assess the possibility of the site or cause of perturbation in one-carbon metabolism in depressed
adults as compared to controls, it is essential to examine each compound and the regulating enzymes. However, many of the key compounds and regulating enzymes may be influenced by a number of external factors, and these external factors need to be controlled for to clearly delineate the possible disturbances.

There are several potential confounding external factors of particular importance in the study of one carbon metabolism in depression. Most of the published studies in the area of depression have investigated medicated inpatient depressed patients. This population represents the most severely depressed subjects and disturbances in one carbon metabolism reported in these studies could be the result of confounding external factors, rather than from the depression itself. The current thesis describes a study that focuses on a sample of non-institutionalized, unmedicated, depressed otherwise healthy adults. Metabolites of one-carbon metabolism and markers of enzyme activity were studied simultaneously. Also, the possible impact of diet on one-carbon metabolism was investigated.

The aim of this study was to exclude confounding variables and measure the concentrations of principal metabolites, co-factors and related compounds of OCM as well as markers of enzyme activity in the plasma of unmedicated otherwise healthy depressed adults as compared with healthy controls. The relationship between the severity of depression and one-carbon metabolites and related factors, was also evaluated. As depression is a hereditary disease, the proportion of depressed subjects with genetic mutations of a key regulatory enzyme, MTHF reductase, was determined. In order to see
whether diet plays a role in the folate-depression link, the proportion of subjects with low, medium or high folate intake was evaluated in both the depressed subjects and healthy controls. The feasibility of measuring folate absorption was also investigated in this study.
CHAPTER 2: BACKGROUND/ LITERATURE REVIEW
2.1. Introduction

This review will describe the clinical aspects and social sequellae of the syndrome of major depressive disorder. Evidence that there is a relationship between folate and depression will then be discussed by reviewing the prevalence of depression in folate-deficient populations and of folate deficiency in depressed populations. Folate and the recovery from depression will be discussed: both from the perspective of the relationship between folate status and severity of depression, even when folate concentrations are within the normal range; and from the perspective of the role of supplementation of folate and its derivatives in treatment of depression. In order to understand the mechanisms underlying the folate-depression relationship, various theories linking the two will be discussed. These include theories based on alteration of folate intake, absorption and one-carbon metabolism. The various enzymes regulating the cycle will be discussed in detail.

2.2. Depression: a common illness

Mood disorders, which include depression and mania, constitute the most common psychiatric problem for which people seek help or suffer without seeking help. Major depression is a heritable, recurrent, syndromal illness with both psychological and biological components. The DSM IV describes a major depressive episode as a period of at least two weeks during which there is a prominent and relatively persistent dysphoric, sad, depressed mood or the loss of interest or pleasure in nearly all activities (American Psychiatric Association, 1994). This is associated at least four of the following
symptoms: changes in appetite or weight; sleep and psychomotor activity disturbances; decreased energy; difficulty concentrating, planning or making decisions; feelings of worthlessness, self-reproach, inappropriate or excessive guilt, and recurrent thoughts of death or suicide ideation.

There are many lines of evidence that confirm the heritability of depression. First-degree relatives of patients with severe depression are 1.5-3 times more likely to suffer from depression than are members of the general population (American Psychiatric Association, 1994). Studies of monozygotic twins show a 65% concordance for the illness, as compared with 14% of dizygotic twins (Gold, P. W. et al., 1988). In the past 20 years, genetic researchers have tried to identify genetic markers of the disease. Few studies have shown that polymorphisms on chromosome 11 (Egeland, J. A. et al., 1987), 18 and 21 (Berretini, W., 1995; Gold, P.W. et al, 1988) may participate in vulnerability to depression. However, these findings have not been replicated (Nemeroff; C.B., 1998; Gold, P.W. et al, 1988). The site of genetic perturbation remains to be determined.

Depression adversely affects all areas of a person’s functioning. Indeed, depressive symptoms, even in the absence of a depressive disorder, have more adverse effects on physical, social and role functioning than other chronic medical conditions such as diabetes, hypertension, back problems or arthritis (Wells, K. B et al., 1989). Personal suffering due to depression is evident in its high mortality rate. Patients diagnosed with Major Depression carry an estimated lifetime risk of suicide ranging from 15-30% (Goldblatt M.J. et al., 1991).
In addition to personal suffering, depression can have dire economic consequences if it causes work impairment. According to a recent study, every month, mood disorders accounts for 25 work loss days and an additional 109 ineffectual work days per 100 workers (Kessler, R. C. et al., 1997).

People suffering from depression usually experience symptomatic periods lasting from 7 to 14 months, 20 percent of who have symptomatic periods that last two years or more. Furthermore, as many as 30 percent of depressed adults describe depressive symptoms between episodes (Gold, P. W. et al., 1988). Depression is a widespread phenomenon, affecting 17.1% of adults at least once in their lifetime (Blazer, D. G. et al., 1994). The one-year prevalence of major depression in Ontario is 4.1% (Offord, D. R. et al., 1996). Furthermore, there is an 11.8% one-year prevalence of 'subsyndromal symptomatic depression' in the general population, which is defined as the presence of two or more depressive symptoms (Judd, L. L. et al., 1994). Women suffer at twice the rate or more from depression as compared with men (American Psychological Association, 1990). The prevalence of depression is expected to increase since people are being affected at an earlier age and the overall risk of suffering from a depressive disorder is increasing (Hirschfeld, R. M. A et al., 1997). Furthermore, the majority of people who suffer from depression do not get treatment. Based on the results of the Harvard Medical Practice Study, it was reported that one third of people suffering from a major depressive disorder seek treatment for it. Only 10% of those who seek help, receive adequate treatment (Brennan, T. A. et al., 1991).
Depression has been studied extensively and various theories of the pathophysiology of depression have evolved. There is growing evidence that nutritional factors may play a role in the etiology, progression and possible treatment of depression. Folate has yielded consistent results over the past three decades in Europe and North America with regard its link to neuropsychiatric disorders, and depression in particular (Bottiglieri, T., 1996).

This review will first explore the evidence that suggests that depressive symptoms arise in folate-deficient populations. Second, studies indicating that low folate levels are relatively more prevalent in depressed patients than in other psychiatric populations and that changes in folate status is associated with improvement of depression will be reviewed. Finally, theories linking folate and depression will be discussed and the uniqueness of the current study will be presented.

2.3. Prevalence of Depression in Folate Deficiency

In the last few decades, there has been greater interest in the role of folate in neuropsychiatric disorders. In 1962, Victor Herbert confirmed that there was an association between depression and folate deficiency by depleting himself of folate. After four months of consuming a folate-deficient diet, he developed insomnia, irritability, fatigue and forgetfulness. His symptoms abated after folate replacement (Herbert, V., 1961). In addition to Herbert’s evidence, neurological symptoms associated with folate deficiency were consistently seen in other folate deficient patient populations. Once assays became available to distinguish vitamin B12 deficient anemia from folate
deficient anemia, it became evident that the latter was associated with affective
disturbance (Shovron, S. D. et al., 1980). The same association was observed in chronic
alcoholics in whom generalized vitamin deficiency is common. Chronic alcoholics with
folate deficiency suffered far more depressive morbidity than those with adequate folate

Folate levels in serum and cerebrospinal fluid (CSF) were found to be lower in patients
taking antiepileptic medication, which are folate antagonists. Depression was reported to
occur in over half of epileptic patients on anticonvulsant therapy (Kutt, H. et al., 1980).
Folate supplementation in these patients alleviated depressive symptoms. When the
supplementation was discontinued, the improvement of well-being arrested (Froscher,
W. et al., 1995). These studies support the notion that depression arises in people whose
folate levels are compromised.

2.4. Presence of Folate Deficiency in Depression

Surveys of psychiatric inpatients showed that from 10-30% have low serum folate levels
(Reynolds, E. H. et al., 1984). This is most commonly seen in depressed patients. In the
late 1960s, a major study on the prevalence of folate deficiency in psychiatric patients
was carried out by Carney (1967). Serum folate levels were measured in 423 psychiatric
patients. In this study, it was noted that the prevalence of low folate levels (below 2 μ
g/ml) was particularly high in depressed patients (29-35%). A review article by Young
and Ghadirian (1989) cited no less than 22 predominantly European studies ranging from
1966 to 1986 on the prevalence of folate deficiency in psychiatric patients. The prevalence of folate deficiency in depressed patients varied from 2% to 80% (Table 2.1). The vast discrepancy in reported folate levels could be due to the different sampling (geriatric versus adult, geographical regions) or analytic methods (microbiologic versus radioimmunoassay) or diagnostic rigour used to define the illness or, most importantly, folate deficiency (range less than 2 to less than 6 μg/ml) between studies. The predominant measure of folate status used in these studies, especially the earlier ones, was a microbiologic assay of serum folate, which is an unreliable indicator of folate status (Grinblat, J. et al., 1968). It has been replaced by the more reliable radioimmunoassay. Furthermore, serum folate, the predominant indicator in these studies, only indicates folate status at the time the serum sample was drawn (Herbert, V., 1987b). Fluctuations in daily dietary folate affect this measure (Chanarin, I., 1979) and may obscure the true folate status of a patient. Red cell folate reflects folate status over the previous three months or longer (Chanarin, I., 1979).

Studies examining the prevalence of folate deficiency in psychiatric populations in North America do not corroborate results of European studies. A study of 60 psychiatric in-patients in Los Angeles reported only one depressed patient with a serum folate level below 2.5 μg/ml (Gray, G. E. et al., 1986). Levitt et al (1989) have found that the prevalence of low serum folate levels (<5nmol/l) was only 2% in unmedicated, depressed out-patients. The vast difference in reported folate status in depressed patients in Europe and North America could be due to higher folate intakes in America, and/or the use of an
updated method of folate assessment (radioimmunoassay versus microbiologic (Grinblat, J. et al., 1968), and/or the effects of other factors that could decrease folate levels (major medical illnesses, folate-antagonistic medication, pregnancy or birth in the previous three months, alcohol abuse, antidepressant medication or psychotropics. For example, a more recent European survey using the radioassay of red cell folate, a more reliable, stable measure of folate status (Carney, M. W. P. et al., 1990), assessed folate status of 243 psychiatric patients. The prevalence of low red cell folate (below 2 μg/ml) in depressed patients (54%) was significantly higher than in euthymic (17%), manic (22%) and schizophrenic (17%) populations. However, of all the confounding factors that can influence folate status, only alcohol abuse was defined as an exclusion criterion. Hence, while there are some studies reporting very high prevalence of folate deficiency, the lack of standardization of study design has led to large variations in results.

While many studies have focussed on folate deficiency, studies of folate within the normal range are of even more interest, as depression is a symptom of many nutrient deficient states. The nature of the association between folate levels within the normal range and depression will be discussed in the following section.

2.5. Changes in Folate Status with Recovery from Depression

In addition to the observation that depression and folate deficiency are associated, there is evidence to suggest that folate status changes with alteration in mood even when folate levels are within the normal range (Carney, M. W. P., 1967). For example, within the
normal range the level of folate is inversely proportional to the severity of depression (Reynolds, E. H. et al., 1970) (Table 2.2). Abou-Saleh and Coppen found a negative correlation between folate levels and depressive symptoms in 107 euthymic out-patients with affective disorder on prophylactic lithium treatment, both at the time of the folate assay and during the previous two years (Coppen, A. et al., 1982). A more recent study by the same group of researchers (1989) found that this correlation remained significant when patients with major depressive disorders on medication were excluded (n = 95) (Abou-Saleh, M. et al., 1989).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Fluid</th>
<th>Type of patient</th>
<th>Definition of Folate Deficiency (nmol/l)</th>
<th>Country</th>
<th>n</th>
<th>% with follicular deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Carney, M. W. P., 1967)</td>
<td>serum</td>
<td>depressed psychiatric admissions</td>
<td>&lt;2</td>
<td>U.K.</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>(Shulman, R., 1967)</td>
<td>serum</td>
<td>geriatric psychiatric admissions</td>
<td>&lt;6</td>
<td>Canada</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>(Callaghan, N. et al., 1969)</td>
<td>serum</td>
<td>psychiatric inpatients</td>
<td>&lt;2</td>
<td>Ireland</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>(Hallström, T., 1969)</td>
<td>serum</td>
<td>psychiatric inpatients</td>
<td>&lt;2.5</td>
<td>Sweden</td>
<td>84</td>
<td>51</td>
</tr>
<tr>
<td>(Kallström, B. et al., 1969)</td>
<td>serum</td>
<td>psychiatric inpatients</td>
<td>&lt;3</td>
<td></td>
<td>115</td>
<td>21</td>
</tr>
<tr>
<td>(Reynolds, E. H. et al., 1970)</td>
<td>serum</td>
<td>depressed inpatients</td>
<td>&lt;2.5</td>
<td>U.K.</td>
<td>91</td>
<td>24</td>
</tr>
<tr>
<td>(Reynolds, E. H. et al., 1971)</td>
<td>serum</td>
<td>psychiatric inpatients</td>
<td>&lt;2.5</td>
<td>U.K.</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>rbc*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>(Carney, M. W. P. et al., 1978)</td>
<td>serum</td>
<td>depressed psychiatric admissions</td>
<td>&lt;2</td>
<td>U.K.</td>
<td>110</td>
<td>28</td>
</tr>
<tr>
<td>(Thornton, W. E. et al., 1978)</td>
<td>serum</td>
<td>psychiatric admissions</td>
<td>&lt;5.9</td>
<td>USA</td>
<td>269</td>
<td>30</td>
</tr>
<tr>
<td>(Ghadirian, A. M. et al., 1980)</td>
<td>serum</td>
<td>depressed inpatients</td>
<td>&lt;2</td>
<td>Canada</td>
<td>16</td>
<td>50</td>
</tr>
<tr>
<td>(Gray, G. E. et al., 1986)</td>
<td>serum</td>
<td>psychiatric admissions</td>
<td>&lt;2.5</td>
<td>USA</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>(Levitt, A. J. et al., 1989)</td>
<td>serum</td>
<td>unmedicated depressed outpatients</td>
<td>(5.2nmol/L = pt)</td>
<td>Canada</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>(Abou-Saleh, M. et al., 1989)</td>
<td>rbc</td>
<td>unmedicated depressed in- &amp; outpatients</td>
<td>&lt;2.5</td>
<td>U.K.</td>
<td>95</td>
<td>16</td>
</tr>
</tbody>
</table>

* rbc – red blood cells
Levitt and co-workers studied a sample of 44 depressed patients whose folate levels fell generally within the normal range (Levitt, A. J. et al., 1989). Folate levels were negatively correlated with duration of a current episode of depression, and the severity of depression contributed significantly to the overall variance in folate levels. In a separate sample, the same group found a significant negative correlation between red cell folate and severity of depression among 99 depressed patients treated with desipramine, an antidepressant medication, for five weeks (Levitt, A. J. et al., 1994). Red blood cell folate was measured at baseline and at the end of five weeks of treatment. Not only was the severity negatively correlated with changes in folate status, but those who responded to the drug, showed mean increases in folate levels. Non-responders showed a mean decrease in red blood cell levels. There were significantly more responders (79%) than non-responders (21%) who had an increase in folate status. In another recent study on a group of 25 depressed subjects, the percent change in red cell folate across a four-week trial of antidepressant medication was significantly negatively correlated with percent change in severity of depression. (Levitt, A. J. et al., 1998). In all three studies by Levitt et al., most patients had normal folate levels with the rate of folate deficiency being less than ten percent. Similar observations have been reported for the association between S-adenosylmethionine and the severity of depression. A longitudinal study on levels of SAM in patients being treated for major depression found that as the severity of depression decreased, SAM levels increased (Bell, K. M. et al., 1994). The finding that folate and SAM levels increase in conjunction with recovery from depression becomes more compelling in view of the fact that folate and/or its derivatives may have antidepressant qualities.
2.6. **Impact of Supplementation with Folate and its Derivatives on Depression**

The association between low folate levels and depression has led to interest in the influence of folate supplementation on recovery from depression. Studies have demonstrated that if patients are folate-deficient at the start of antidepressant treatment, treatment efficacy is affected. Baseline folate deficiency rendered response to the selective serotonin reuptake inhibitor, fluoxetine (Fava, M. et al., 1997) and sertraline (Alpert, M. et al., 1996), less effective. In the Fava and colleagues' study (1997), subjects with folate levels below the normal range showed a risk of non-response to 8 weeks of antidepressant treatment 2.2 times that of subjects with normal folate levels. When folic acid supplementation was given as an adjunct treatment of depression, psychiatric patients exhibited mood improvement, better social functioning and were discharged from hospital more quickly than patients who did not receive folate supplementation (Carney, M. W. P. et al., 1986). Carney and Sheffield (1970), through retrospective chart review of 92 psychiatric patients, demonstrated that the 39 patients that were supplemented with folic acid had shortened hospital stay and better recovery.

The same authors conducted a placebo-controlled study of 24 depressed patients. Results demonstrated that folate supplemented patients recovered more quickly and showed significant improvement in memory and performance on psychometric tests as compared to patients who received no supplementation (Carney, M. W. P., 1979). While these studies indicate the benefit of
<table>
<thead>
<tr>
<th>Author and Date</th>
<th>No. of subjects</th>
<th>Effects of Low Folate on Depressive Illness</th>
<th>Description of Depressed Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Abou-Saleh, M. et al., 1982)</td>
<td>107</td>
<td>↑ severity</td>
<td>medicated euthymic</td>
</tr>
<tr>
<td>(Abou-Saleh, M. et al., 1989)</td>
<td>95</td>
<td>↑ severity</td>
<td>Unmedicated</td>
</tr>
<tr>
<td>(Levitt, A. J. et al., 1989)</td>
<td>44</td>
<td>↑ severity &amp; ↑ duration</td>
<td>medicated</td>
</tr>
<tr>
<td>(Levitt, A. J. et al., 1994)</td>
<td>99</td>
<td>↑ severity</td>
<td>&gt; 5 weeks medication</td>
</tr>
<tr>
<td>(Levitt, A. J. et al., 1998)</td>
<td>25</td>
<td>↑ severity</td>
<td>&gt; 4 weeks medication</td>
</tr>
</tbody>
</table>
oral supplementation of folate in the polyglutamate form in depressed adults with low or deficient folate status, it is not clear whether there are any benefits of supplementation in depressives with normal folate status.

Methyl tetrahydrofolate (MTHF) is the form of folate, which is actively transported across the blood-brain barrier (Spector, R. et al, 1975; Levitt, M. et al, 1971). When depressed patients with borderline or definite folate deficiency (red cell folate below 200 µg/l) were administered 15mg/day of MTHF for six months as an adjunct to their antidepressant medication, they exhibited better overall symptomatic and social recovery as compared to those given placebos. Also, the benefits of its administration increased with time, with the difference in recovery between groups becoming greater over the study period (Godfrey, P. S. A. et al., 1990). MTHF has been used as an antidepressant medication on its own. Geriatric depressed patients were given 50mg of MTHF per day for four weeks. 81% of those responded to treatment, showing a 50% or greater fall in HAM-D score, a measure of the severity of depression (Guaraldi, G.P. 1993).

The folate-dependent metabolite that has been most studied with regard to its antidepressant potential is S-adenosylmethionine (SAM). One-carbon metabolism will be discussed in detail in the next section. In brief, methionine, which is generated from the conversion of methyl tetrahydrofolate to tetrahydrofolate, combines with adenosine triphosphate to form SAM. SAM has a crucial role in mood regulation as it increases serotonin turnover, inhibits the reuptake of norepinephrine, and augments dopaminergic
activity (Rosenbaum, J. F. et al., 1990). Administration of SAM in patients with major depression has proved to be as effective an antidepressant, whether administered intravenously (Fava, M. et al, 1995; Janicak, P.G. et al, 1989) or orally (Rosenbaum, J. F. et al., 1990), as a number of standard treatments (particularly tricyclic antidepressants). The effectiveness of SAM as an antidepressant has been clearly supported by a meta-analysis by Bressa (1994). Furthermore, some depressed patients, resistant to other antidepressant medications, responded favorably to SAM administration (Rosenbaum, J. F. et al., 1990).

Folate and SAM are inextricably linked in this process as folate levels increase with SAM administration (Reynolds, E. H. et al., 1983). Also, just like folate, blood SAM levels increased significantly concomitantly with clinical improvement in response to antidepressant drug treatment (Bell, K. M. et al., 1994).

Folate and its derivatives seem to influence recovery from depression in two ways: firstly, folate supplementation in patients with low or deficient folate status corrects the deficiency and improves outcome. Secondly, folate is essential for one-carbon metabolism and administering folate may influence methylation. It is unclear whether folate, in its polyglutamate form, influences methylation as it cannot enter the brain without being converted to a monoglutamate, namely 5-MTHF. This could explain why both MTHF and SAM, both of which can cross the blood-brain barrier, are effective antidepressant agents, whereas dietary folate is not.
Folate status in depressed patients not only affects antidepressant outcome, but 5-MTHF and SAM have been shown to be efficacious in treating depression. These conclusions are congruous with the empirical observations of the emergence of depressive symptoms in folate-deficient populations and the relatively high prevalence of low folate levels in clinically depressed patients. Furthermore, evidence that folic acid has a vital role in mood regulation is supported by the finding that folate status affects mood, not only in patients with severe depression or with folate deficiency, but also in less severely depressed people and in those with normal folate levels.

2.7. Theories of the Relationship Between Low Folate and Depression

The relationship between folate and depression could occur at one or many of the following levels: decreased intake of dietary folate; decreased absorption; defective folate transportation; decreased folate storage capacity; increased utilization of folate or excessive excretion of folate (Swain, R. A. et al., 1997). In the literature related to folate, three of these factors have been proposed as the link between folate and depression: namely dietary folate intake, folate absorption and folate metabolism. It is unknown whether these factors are an integral part of the etiology of depression or whether depression somehow leads to alterations in folate intake, absorption or metabolism. Alternatively, it is possible that some other as yet undetermined factor is an essential link in the relationship between folate status and depression.
2.7.1 Folate Intake Theory

Changes in appetite and weight are characteristic features of depression (American Psychiatric Association, 1994). Therefore it seems reasonable that a disorder that potentially affects intake-related factors may cause generalized nutritional deficiency, including folic acid deficiency. Furthermore, depressed people often select carbohydrates in preference to other foods (Wurtman, J. J., 1993). Folate-rich foods such as dark, green leafy vegetables and liver, may be excluded from the depressive’s diet. Hence people with major depression have the potential to eat insufficient amounts of folate-rich foods. Decreased folate intake may play a role in the etiology or exacerbation of depression via decreasing the supply of substrate for one carbon metabolism. However, there is no study to date that has made a direct, comprehensive evaluation of folate intake in depressed adults.

The presence of weight loss or appetite change is an indirect measure of dietary intake. When these variables were measured with regard to folate in depression, no association was found between folate status and weight change or appetite (Levitt, A.J. et al., 1989; Abou-Saleh, M., 1989). However, very few studies have attempted to directly measure dietary folate adequacy in psychiatric patients. Most of these have used the patient’s overall diet adequacy on admission as the measure of folate intake (Thornton, W.E. et al., 1978; Reynolds, E.H. et al., 107; Shulman, R., 1967; Carney, M.W.P., 1967). The results found no association between dietary histories and folate status. Conclusions drawn from these studies should be viewed with caution as all these studies used the patient’s 24-hour dietary recall as the assessment tool, which is an unreliable method (Thompson, F. E. et
al., 1994) and will not necessarily reflect chronic intake. One of these studies assessed diet adequacy using a four-item rating scale including dietary recall from the patients' family members, which is also not optimal (Thornton, W. E. et al., 1978). A recent study used a 3-day diet record on 29 currently depressed adults (Christenesen, L. et al., 1994). There was considerable variation in the dietary intake of these subjects, indicating that diet records over a brief period are not a reliable method of studying food intake. Carney and co-workers (1967) reported that 23% of a psychiatric population with decreased folate levels were "malnourished", but dietary intake was not measured (Carney, M. W. P., 1967). Also, serum folate was used as the indicator of folate status, which has daily variation (Herbert, V., 1987b). In addition to these studies, there is indirect evidence that dietary factors are unlikely to be important in the genesis of depression. For example, the prevalence of folate deficiency, even in major depressive disorder, is very low in North America (Levitt, A.J., et al., 1989, Gray, G.E. et al., 1986; Ghadirian, A.M. et al., 1980) and China (Lee, S. et al; 1998) whereas the prevalence of major depression does not differ substantially. This suggests that there are geographical and cultural determinants that may be more influential on folate status than the presence or absence of depression.

Decreased dietary folate intake could lead to lowered folate concentrations and consequent depression. Alternatively, depression could lead to decreased dietary intake, including reduced folate intake, resulting in low folate levels. However, the balance of the, albeit limited, data available suggests it is unlikely that dietary folate intake is the crucial link between folate and depression. Prospective studies measuring folate intake
using a reliable assessment tool are, nonetheless, needed for expanding our understanding of dietary folate's relationship to depression.

2.7.2 Folate Malabsorption Theory

It has been hypothesized by Botez et al. that the low folate observed in depression is integrally related to malabsorption (Abouh-Saleh, M.T. et al, 1986; Botez, M.I. et al., 1984). Their theory evolved from the observation that intestinal malabsorption or past long-standing gastrointestinal disorders such as colitis, irritable colon and gastritis, existed in patients with depression. They suggested that an initial stress or a transient endogenous depressive episode could cause a gastrointestinal disorder, which compelled the individual to eat a low-fiber diet. This diet results in folate malabsorption due to morphological changes in the jejunal mucosa, which results in megaloblastic anemia. The consequent lassitude and depression perpetuate the vicious cycle of a diet low in dietary fiber and folate with consequent malabsorption. Hence, according to these authors, the long-standing association between folate levels and depression is due in part to a malabsorption syndrome. It has also been hypothesized that mental stress and neurohormonal changes, which occur in psychiatric disorders, may alter gastrointestinal function and decrease folate absorption (Botez, M. I. et al., 1979a). Indeed, subjective gastrointestinal complaints relating to gastric (9-18% prevalence) and intestinal (5-16%) disorders are not uncommon in depression (Hochstrasser, B. et al., 1996). However, there is no direct evidence to date, to prove the malabsorption hypothesis. Body weight change is an indirect indicator of malabsorption. Data from existing studies have not
found a significant association between weight change and folate status (Levitt, A. J. et al., 1989). Furthermore, although little is known concerning the stability of folate during its passage through the different microenvironments in the intestinal mucosa, the small intestine is very effective in absorbing folate in healthy humans (Herbert, V., 1987a). If depression is integrally related to folate malabsorption, according to the theory proposed, all depressed people would have a low intake of food and especially fibre, have a history of gastrointestinal complaints and suffer other folate deficiency symptoms. These characteristics are not homogeneous across depressed adults. No study to date has tested this hypothesis.

2.7.3 Folate Metabolism Theory

It is possible that depression is associated with neurochemical changes that affect folate metabolism, perhaps increasing the demand for this vitamin. Alternatively, primary alterations in folate metabolism could result in depression. Interest in the relationship between folate and depression has stemmed from the knowledge of the role of folate in methylation and the observation that the concentration of folate in cerebrospinal fluid in humans is three times greater than in serum (Reynolds, E. H. et al., 1972). In order to examine this relationship, the metabolism of folate and its possible influence on mood via methylation needs to be understood.

Folate naturally occurs in food in polyglutamate forms. Once ingested, folate is absorbed throughout the small intestine, but the most efficient absorption occurs in the jejunum. Before the polyglutamate forms can be absorbed, they must be hydrolyzed by conjugases.
to monoglutamate forms. The majority of the naturally occurring folates are converted to 5-methyl tetrahydrofolates (MTHF) and stored in the liver. The enzyme that converts 5,10-methylene tetrahydrofolate to 5-MTHF is methyl tetrahydrofolate reductase (MTHFR). MTHF is actively transported into the nervous system in the monoglutamate form, where it is present in cerebrospinal fluid and synaptic regions in high concentrations. In order for folate to become a functional enzyme, accepting and transferring one-carbon fragments from various degradative reactions in amino acid metabolism and transferring them to a variety of synthetic reactions, MTHF must be converted back to tetrahydrofolate (THF). Refer to Figure 2.1 for all major components of one-carbon metabolism. This conversion requires vitamin B12-dependent methionine synthetase (MS) which is rate-limiting and a key regulatory enzyme in this pathway. Through this reaction, the methyl group of methyl THF is transferred to homocysteine, generating methionine and THF. Once it is in the THF form, it can be conjugated to the polyglutamate form and retained within the cell or CSF. Methionine then combines with adenosine triphosphate to form S-adenosylmethionine (SAM). This reaction is catalyzed by methionine adenosine transferase (MAT). SAM has three main metabolic pathways (Carney, M.W.P., 1986):

1. About two thirds is metabolized by donating its methyl groups to over 35 transmethylation reactions involving amines, neurotransmitters, nucleoproteins, proteins, membrane phospholipids, and monoamines (Bottiglieri, T. et al., 1995).
Abbreviations:
- S-AM = S-adenosylmethionine
- S-AH = S-adenosylhomocysteine
- THF = tetrahydrofolate
- MTHF = methyl tetrahydrofolate
- B6 = co-factor vitamin B6

Four regulatory enzymes:
- MS = vitamin B12-dependent methionine synthase
- 5-MTHFR = 5-methyl tetrahydrofolate reductase
- MAT = methionine adenosyltransferase
- CBS = cystathionine β-synthase

*Figure 2.1: One-carbon metabolism*
2. SAM may be decarboxylated followed by aminopropylation, giving rise to polyamine synthesis.

3. SAM may undergo cleavage of the bond between the sulphur atom and carbon 4 of the amino acid chain, producing methyl adenosine and homocysteine thiolactone.

Once SAM has donated its methyl group, it becomes S-adenosylhomocysteine (SAH), the product of biological transmethylation. SAH is metabolized by the enzyme SAH hydrolase to yield adenosine and homocysteine. Homocysteine has two fates: it is either recycled to form SAM or it is degraded to cystathionine and then cysteine via cystathionine $\beta$-synthase (Fowler, B., 1997).

SAM is a regulator of the enzymes of one-carbon metabolism. SAM is a potent inhibitor of MTHFR when the cell is replete with SAM or when the SAM:SAH ratio is high, thereby decreasing the production of its precursor MTHF (Matthews, R.G., et al., 1998). SAM is also an essential regulator of MS. It donates its methyl group to vitamin B12 to reactivate it after every 100 – 2000 turnovers of MS (Goulding, C.W. et al., 1997). Furthermore, SAM has a negative feedback effect on MAT, the enzyme that catalyses the formation of SAM (LeGros, L.H. et al., 1997).

Since folate is necessary for the continued function of one-carbon metabolism, the methylation process may be the link between folate and the monoamines in affective
disorders. According to the monoamine hypothesis, depression may result from a
deficiency in transmission of serotonin and/or noradrenaline (Podell, R. N., 1983).

MTHF generates methionine for SAM production, which then donates its methyl group
for the synthesis of these neurotransmitters. Furthermore, SAM provides methyl groups
for the methylation of neuronal membrane phospholipids (Bottiglieri, T. and Hyland, K.,
1994), which may influence both membrane fluidity and receptor sensitivity for the
transduction of biological signals through membranes (Destein, M. L. et al., 1991).
Methylation of phospholipids can alter membrane-bound receptors, second-messenger
systems, and ion channels (Hartley, D.M. and Sondgrass, S.R., 1990). Subtle changes in
any of these components of cellular metabolism might lead to amplified or cumulative
effects in the CNS. Indeed, MTHF (Reynolds, E. H. et al., 1984) and SAM (Fava, M. et
al., 1997) are present in high concentrations in the CSF and synaptic regions, suggesting
an important functional role in neurotransmission. Studies have shown that folate and
SAM concentrations are correlated with CSF serotonin and 5-hydroxyindole acetic acid
(5HIAA) levels, both of which reflect serotonergic function which is believed to
influence mood (Alpert, J.E. et al., 1997; Botez, M.I. et al., 1979a).

Another proposed mechanism for the role of folate in regulating mood is that folate
increases tetrahydrobiopterin (BH4) concentration, which is required for the biosynthesis
of dopamine, norepinephrine, and serotonin. Folate may influence the rate of synthesis of
dopamine, norepinephrine, and serotonin by promoting the synthesis of
tetrahydrobiopterin (BH4), a cofactor in the hydroxylation of phenylalanine and
tryptophan. These two amino acids are converted to the monoamine neurotransmitters
such as dopamine, norepinephrine and serotonin, through rate-limiting hydroxylation steps (Coppen, A. et al., 1989) (Figure 2.2).

If insufficient amounts of MTHF are available to facilitate methylation, mood may be adversely affected and depression may result. This could be due to the direct role of MTHF and SAM in methylation and/or via BH4.
No studies to date have supported the theories of decreased folate intake or absorption being the crucial link between folate and depression. In contrast, the theory of altered metabolism of folate in depression seems plausible due to its crucial role in neurotransmission via one-carbon metabolism. There are at least to two possible ways in which one-carbon metabolism is disturbed in depression (Smythies, J. R. et al., 1997). Disturbance of one-carbon metabolism could be due to a defective enzyme(s) such as MS, MAT or MTHFR or due to a breakdown in the cycle due to excessive demands on the cycle, such as an increased need for methylation. One-carbon cycle disturbance in depression could be a combination of the two or due to a hormone or molecule particular to depression that may influence the cycle function. As depression is a heritable disorder (Gold, P.W., Goodwin, F.K. et al. 1988), it is possible that a defect in one of the key regulatory enzymes of one-carbon cycle is inherited. Alternatively, depression is a state of hypermetabolism (Gold, P.W., Chrousos, G.P. et al. 1984; Joffe, R.T. and Levitt, A.J. 1993) which may place excessive demands on the one-carbon cycle resulting in dysfunction. In the following sections the possibility of defective enzyme function due to genetic polymorphism, and hypermethylation will be discussed.

2.8. One-Carbon Metabolism Enzyme Polymorphism and Depression

There are many lines of evidence that confirm the heritability of major depressive illness (Gold, P. W. et al., 1988). While there is strong evidence that depression runs in families, the nature of the heritability has not yet been determined. It is possible that some defect
of a regulatory enzyme that is vital for mood regulation is inherited. A genetic mutation that reduces the function of any of the enzymes that are involved in one-carbon metabolism could predispose an individual to depression by decreasing the supply of methyl groups required for the optimal neurotransmission. While this hypothesis has not been extensively studied in depression, defective enzyme function or genetic polymorphism has been found in other heritable diseases, namely neural tube defects (NTDs) and cardiovascular disease (CVD). While these are two apparently unrelated hereditary diseases, NTDs and CVD, share a common characteristic moderately elevated homocysteine levels, a metabolite of one-carbon metabolism (Van der Put, N. M. J. et al., 1997). Enzymes and co-factors involved in homocysteine metabolism, that is one-carbon metabolism, are currently being investigated for their contribution to the disease. It is possible that they share a common inherited defect of a regulatory enzyme. Of particular interest is the finding of increased CVD in major depression. A recent meta analysis of 57 studies concluded that depression substantially increases the risk of cardiovascular disease (Wulsin, L.R. et al., 1999). Also, major depression occurs with higher than expected prevalence in patients with CVD (Barefoot, J. C. et al., 1996). If major depression and CVD are heritable, and CVD is significantly more common in major depression, it is possible that they share similar inherited enzymatic disturbances. This hypothesis has not yet been tested.

Although multiple gene patterns and numerous environmental factors will influence whether an individual will develop a complex disease, genetic mutations which decrease the activity of enzymes required for regulation of neurotransmission have been identified.
A discussion of recent advances in our understanding of genetic polymorphisms of the four major key-regulatory enzymes of one-carbon metabolism will follow. This is of particular interest to depression, as a disturbance in one-carbon metabolism due to a defective enzyme may result in neurotransmission and hence, depression.

There are four key regulatory enzymes in the one-carbon cycle (Figure 2.1):

1. 5,10-methylene tetrahydrofolate reductase (MTHFR) which converts 5,10-methylene-THF to 5-methyl THF

2. methionine synthase (MS), a vitamin B12 dependent enzyme, which converts 5-MTHF and homocysteine to THF and methionine

3. methionine adenosyl transferase (MAT) which converts methionine to S-adenosyltransferase

4. cystathionine β-synthase (CBS) which catalyses the condensation of homocysteine with serine

2.8.1 Methylenetetrahydrofolate Reductase

MTHFR synthesizes 5-MTHF, which provides the methyl group for methionine synthetase to methylate homocysteine to methionine (Miner, S. E. S. et al., 1997).

Methionine combines with ATP to form SAM, which is the main methyl donor required for neurotransmission. Complete MTHFR deficiency is rare and results in hyperhomocysteinaemia and premature cardiovascular disease (Verhoef, P. et al., 1998). A less severe genetic disorder results from MTHFR mutations. Fourteen rare mutations of the MTHFR gene have been cloned and sequenced. (Weisberg I. et al., 1998).
The most prevalent mutation of this gene is a C→T polymorphism at nucleotide position 677 which is characterized by reduced enzyme activity and thermolability (Langman, L. J. et al., 1998). Mild hyperhomocysteinaemia has been detected in 30% of homozygotes (Guglielmina, P. P., 1998). If however serum folate levels are ≤10ng/ml, all homozygotes have higher homocysteine concentrations (Brown, C. A. et al., 1998), suggesting that environmental factors, such as dietary folate intake, may influence the consequence of the mutation. Another mutation in the same gene, the 1298 (A→C) mutation has more recently been reported to result in decreased MTHFR activity, albeit to a lesser degree than the C677T mutation (Weisberg L. et al., 1998). A third silent genetic variant, T1317C, has been identified in black people and is virtually absent in other racial groups. T1317C is also associated with hyperhomocysteinaemia.

In a recent local study by Weisberg and colleagues (1998), the prevalence of homozygous mutant individuals of the C677T gene was reported to be approximately 10% in North America. The prevalence of homozygous mutant A1298C individuals in Canada was similar. Approximately 15% of Canadians were heterozygous for both C677T and A1298C – resulting in decreased enzyme activity. No cases of simultaneous homozygous C677T and A1298C were seen, perhaps due to the fatality of the co-existence of these two mutations. In the same study, the prevalence of T1317C was 39% in black individuals, while only 5% in the whole sample (Weisberg L. et al., 1998).
Hyperhomocysteinaemia is recognized as an independent risk factor for arteriosclerotic vascular diseases (Fletcher, O. et al., 1998) and is considered an increased risk for neural tube defects (Miner, S.E.S. et al., 1997; Whitehead, A.S. et al., 1995). In 1997, a study in Japanese depressed patients found that the odds ratio of having the C677T mutation was 2.8 (p<0.005) (Arinami, T. et al., 1997) as compared to controls, indicating increased risk in major depressive disorder.

Due to limitations of resources and availability of analysis techniques, we were not able to study all four regulatory enzymes. We chose to investigate the genetic polymorphism of MTHFR for the following reasons: first, folate enters the brain in the form of MTHF. MTHFR is required for the synthesis of this compound. Hence, a disturbance of MTHFR function would affect any subsequent one-carbon metabolic reactions. Second, MTHF is an effective antidepressant (Godfrey, P. S. A. et al., 1990), implicating that there may be a disturbance in MTHF production in depression. Third, the C677T mutation in the MTHFR gene has been recently identified in patients with major depression in Japan (Arinami, T. et al. 1997) and in two other diseases with altered one-carbon metabolism, namely NTD (Miner, S.E.S. et al. 1997; Whitehead, A.S., Weir, D.G. et al. 1995) and cardiovascular disease (Fletcher, O. and Kessling, A.M., 1998).

2.8.2 Methionine Synthase

Methionine synthase, which is probably present in all mammalian tissues, catalyses the transfer of a methyl group from MTHF together with homocysteine to produce
methionine. MS requires vitamin B12, or cobalamin, as a co-factor. In order for enzyme activity, cobalamin must be in its reduced form (Fowler, B., 1997). The enzyme, betaine-homocysteine methyltransferase, can also remethylate homocysteine to methionine, but this enzyme is present only in the liver. MS is essential for maintaining adequate intracellular methionine and tetrahydrofolate pools, as well as for ensuring that homocysteine concentration does not reach toxic levels. The gene for this enzyme has recently been cloned. MS consists of at least four different functional regions. The first region is the C-terminal fragment that binds SAM and is essential for reactivation of the inactive cob(II)alamin form of MS. The second region at the N-terminal 98kDa fragment binds the methylcobalamin cofactor. If this region were dysfunctional, the methyl group from 5-MTHF would not be transferred to form methionine, leading to an accumulation of serum folate, the major component of which is 5-MTHF (Lucock, M.D., 1999). The third region at amino acids 2-253 comprises a region responsible for binding and activation of homocysteine. A dysfunction of the third region may lead to accumulation of homocysteine. The fourth region, amino acids 345-649 are thought to bind and activate 5-MTHF (Goulding, C.W., Postigo, D. et al. 1997; Goulding, C.W. and Matthews, R.G., 1997). Genetic polymorphisms may affect any or all of the four regions and mutations, which result in dysfunction of human MS. For example, Goulding et al. (1997) have described mutations of Cys310Ala and Cys311Ala in the third region of MS which completely abolish methyl transfer from exogenous methylcob(III)alamin to homocysteine (Goulding, C.W., Postigo, D. et al. 1997) resulting in increased homocysteine levels.
Mutations resulting in severe deficiency of MS activity cause the inborn error of metabolism cblG characterized by megaloblastic anaemia, developmental delay, hyperhomocysteinaemia and hypomethioninaemia. The gene has been mapped to 1q43, near the telomere of chromosome 1 (Gulati, S. et al., 1996). An A→G substitution at bp 2756 has been associated with cblG patients (Van der Put, N. M. J. et al., 1997).

It is possible that other heritable diseases, such as NTDs and CVD, also have an inherited enzyme defect, albeit not as severe as cblG. Because elevated homocysteine levels characterize both NTD and cardiovascular disease, it has been postulated that defective MS (Leclerc, D.A. et al. 1998) could cause both diseases, at least in part. Even after exclusion of individuals homozygous for the C677T mutation in the MTHFR gene, significantly elevated homocysteine levels have been observed in NTD patients and their parents (Van der Put, N. M. J. et al., 1997) and in patients with cardiovascular disease (Kluijtmans, L. A. J. et al., 1996). While the possibility of defective MS activity in major depressive disorder has not been investigated to date, the prevalence of MS polymorphism in bipolar affective disorder has been studied (Li, T. et al., 1997). Li, et al. (1997) studied 93 Chinese subjects with bipolar affective disorder and 98 controls for the presence of an MS polymorphism, Val158Met allele, which is thought to express itself as low activity of MS. A significant positive correlation was found between this polymorphism of MS and bipolar depression (p = 0.004). Bipolar disorder is another heritable affective disorder and is related to unipolar depression. While there have been
no studies to date exploring MS polymorphism in depression, it is possible both affective disorders share a common dysfunction.

2.8.3 Methionine Adenosyl Transferase
Methionine adenosyl transferase (MAT) is a key enzyme in one-carbon metabolism as it catalyzes the formation of SAM from methionine and ATP. Perturbations in MAT activity is likely to affect the formation of SAM and hence the generation of neurotransmission. While there is not much known about MAT polymorphisms, MAT activity has been measured by assessing the enzyme's kinetics (Tolbert, L.C. et al., 1990; Alarcon, R.D. et al., 1985) and by assessing MAT subunit concentrations (Langkamp-Henken, B. et al., 1994; LeGros, L.H., Jr. et al., 1997), each of which have characteristic activity levels. Previous studies have examined MAT activity by kinetic methods, in depressed, manic and schizophrenic patients (Smythies, J. R. et al., 1986). Results revealed that depressed and schizophrenic patients showed significant lowering of Vmax (enzyme activity) and significantly higher values in mania (high Vmax). Furthermore, studies and case reports suggest that MAT activity increases with recovery from depression (Tolbert, L. C. et al., 1990). These studies suggest that the alterations in one-carbon metabolism enzymes vary with depressive states. Enzyme disturbances could be a consequence of a biochemical change associated with depression or a cause of depression. There is not enough data to make conclusive comments.

2.8.4 Cystathionine β-synthase
Cystathionine β-synthase (CBS) a pyridoxal 5'-phosphate (vitamin B6)-requiring enzyme, catalyses the condensation of homocysteine with serine. Forty point mutations
have been published. G919A alteration has been linked to homocystinuria in patients of Irish descent (Fowler, B., 1997).

CBS deficiency could be a cause of moderate hyperhomocysteinaemia in NTD, but evidence for defective CBS has not been found in mothers of children with NTD (Steegers-Theunissen, R. P. M. et al., 1994) or in patients with vascular disease (Fowler, B., 1997; Kluijtmans, L.A.J. et al., 1996). Therefore, mild hyperhomocysteinaemia seems not to be caused by heterozygosity for CBS. However, decreased CBS activity due to some other, possibly subtle, change affecting expression of the enzyme remains to be excluded (Fowler, B., 1997).

There have been several recent studies on the key regulatory enzymes of one-carbon metabolism. If defects of these enzymes from genetic polymorphism are identified, the cause and possible treatment of inherited diseases such as CVD, NTD and major depression may be discovered. Another possible reason for one-carbon metabolism disturbance is an increased demand on the cycle due to hypermetabolism associated with depression. In the following section, this theory will be elaborated.

2.9. Evidence for increased metabolic turnover in depression

Perturbations in one-carbon metabolism could be due to an overload of the cycle due to increased demand for methyl groups for neurotransmission. Depression is thought to be
due to neurotransmission deficiency (Podell, R.N., 1983). If the body attempts to compensate for this deficiency, SAM's methylation function may increase, as methylation is crucial for neurotransmission. Methylation is involved in neurotransmitter synthesis and providing methyl groups for the methylation of neuronal membrane phospholipids (Bottiglieri, T. et al. 1994), which may influence both membrane fluidity and receptor sensitivity for the transduction of biological signals through membranes (Destein, M. L. et al., 1991). There is evidence that depression is a state of hypermetabolism, which may result in hypermethylation, as most enzyme systems are accelerated in hypermetabolism. Two hormones that may impact on metabolism and are disturbed in depression are thyroid hormones and cortisol. Evidence for disturbance in these two key regulatory hormones will be discussed in the following paragraphs.

A consistent finding in biologic psychiatry is that patients with major depression (particularly melancholia) often have hypercortisolism. High cortisol concentrations in the depressed state, in the magnitude of levels of patients with Cushing’s disease (Gold, P. W. et al., 1984), is thought to result from hypersecretion of corticotropin-releasing hormone (Gold, P. W. et al., 1988).

Another hormone that is increased in depression is thyroid hormone. Changes in thyroid function have been shown to be associated with both depressive episodes and recovery from depression (Levitt, A. J. et al., 1993). In particular, many studies concur that there are relative increases in measures of thyroxine, T4, within the normal range, in people who are depressed (Joffe, R.T. and Levitt, A.J., 1993). This observation appears to hold
true whether depressed subjects are compared to controls (Joffe, R. T. et al., 1993) or whether longitudinal studies are done during depressed and euthymic periods (Joffe, R.T. and Levitt, A.J., 1993). It is uncertain whether these relative increases in T4 are of etiological importance or secondary to the state of depression.

While there have been no studies on cortisol and folate in depression, thyroid hormone was found to be related to folate in depression. Joffe et al. (1993) found a significant negative correlation between thyroid hormones and both B12 and red cell folate in depression, a correlation which persists following antidepressant treatment. The fact that there is a correlation between thyroid and folate levels may imply that folate (and perhaps one-carbon metabolism) may be influenced by the metabolic state. Hence, there is some evidence of increased cortisol and thyroxine levels in depression – both of which increase metabolism and may influence one-carbon metabolism. It is possible that these regulatory enzymes may be the link between depression and alterations in one-carbon metabolism.

In conclusion, there had been insufficient evaluation of folate status in depression to make definite conclusions about whether insufficient dietary folate intake, malabsorption or altered one-carbon metabolism is a cause or a result of depression. At this stage, we have ascertained that a relationship between folate and depression exists, but the precise nature of this association remains to be discovered.
2.10. Limitations of Previous Studies

The vast interest in the role of folate in depression has generated studies in both Europe and North America. While these studies have been very informative, most studies have limitations with regard to the theories outlined above. These limitations include the following:

1. Previous studies have focused primarily on inpatient clinical populations of depressed patients (Froscher, W. et al., 1995) (Young, S. N. et al., 1989; Carney, M. W. P., 1967). The findings of these studies cannot be generalized to other depressed people because clinical populations:

- reflect the most severe cases.
- reflect those who seek treatment.
- are frequently medicated with antidepressants and other medications which may affect the absorption, metabolism or excretion of vitamins involved in one-carbon metabolism (Cervantes, P. et al, 1999; Coppen, A. and Abouh-Saleh, M.T., 1982).
- are often treatment resistant.
- receive prescribed food and do not therefore reflect the intake of free-living depressed adults.

dietary assessment tools used were unreliable. No studies have measured dietary folate intake in depression prospectively, using acceptable dietary assessment tools.

3. Folate status has often been assessed according to serum folate levels. Serum folate only indicates folate status at the time the serum sample was drawn (Herbert, V., 1987b). Fluctuations in daily dietary folate affect this measure (Chanarin, I., 1979) and may obscure the true folate status of a subject.

4. No studies have measured one-carbon metabolites with regard to the function of folate in depression.

5. Definitions of low folate levels differ substantially (range less than 2-6 µg/ml) (Young, S. N. et al., 1989) making the interpretation and comparison of data infeasible.

6. Most studies have focused on folate-deficient depressed populations. Very few depressed patients are folate-deficient in North America (Gray, G. E. et al., 1986); (Levitt, A. J. et al., 1989). Relatively few studies have measured the association of folate function within the normal range and the presence and severity of depression range (Levitt, A.J. et al., 1998; Levitt, A.J. et al., 1994).
7. No study to date has measured dietary folate intake and compounds involved in folate metabolism concurrently.

Our study will measure all three postulations regarding reduced folate levels in depression i.e. decreased intake, decreased absorption and increased one-carbon metabolism. Furthermore, we will be studying a non-institutionalized, unmedicated sample of depressed adults. This group will be compared to non-psychiatrically ill, healthy controls.
CHAPTER 3: OBJECTIVES AND HYPOTHESES
3.1 Primary Objectives

(Hypermethylation in depression)

1. To measure the concentrations of principal metabolites, co-factors and related compounds of OCM as well as markers of enzyme activity in the plasma of unmedicated otherwise healthy depressed adults as compared with healthy controls.

2. To evaluate the relationship between the severity of depression and concentrations of principal metabolites, co-factors and related compounds of OCM as well as markers of enzyme activity, in depressed subjects.

3.2 Secondary Objectives

(Inadequate supply of substrates for optimal OCM function in depression)

3. (a) To evaluate the proportion of subjects with depression and healthy controls with low, moderate and high intakes of folate.

(b) To compare the intake of dietary compounds related to OCM in depressed adults and healthy controls.

(c) To measure folate absorption in depressed subjects and healthy controls.

4. To measure the proportion of depressed subjects and healthy controls with genetic polymorphism in a gene that controls the activity of a critical enzyme in one-carbon metabolism.
3.3 Primary Hypotheses

1. In a non-institutionalized sample, the concentrations of principal metabolites, co-factors, related compounds and markers of enzyme activity in one-carbon metabolism will be significantly different between depressed versus healthy controls. In particular, SAM will be significantly lower and SAH will be significantly higher in depressed subjects as compared to controls and SAM: SAH ratio will be significantly lower, reflecting increased methylation in depression. Also, red cell folate, the precursor required for the formation of SAM, will be significantly lower in the depressed group as compared with healthy controls.

2. In depressed subjects, the severity of depression will be significantly, negatively correlated with SAM and red cell folate levels and significantly, positively correlated with SAH levels, reflecting increasing methylation with increasing severity of depression.

3.4 Secondary Hypothesis

3. The supply of substrates required for one-carbon metabolism efficiency via dietary intake or absorption will not differ significantly between depressed subjects and healthy controls.

4. There will be significantly more MTHFR C677T homozygous and/or heterozygous individuals in the depressed sample as compared to healthy controls.
CHAPTER 4: METHODS
4.1 Recruitment

Two methods were used to recruit depressed and control subjects. The first method of recruitment was by random selection using telephone numbers. This method was designed to randomly select a group of depressed individuals living in the community. The second method involved a newspaper advertisement. The second method was employed only when an insufficient number of subjects were recruited using the first method.

4.1(a) Telephone Recruitment

A community sample was randomly selected from East York, a district of Toronto consisting of citizens with varying social and economic demographics. This region was chosen as the recruitment site as it is in close proximity to the investigation site. Also, the prevalence of depression has been studied (Katz, R. et al., 1995) and was found to be representative of the prevalence of depression in Ontario (Offord, D. R. et al., 1996). To ensure random selection of subjects, we used the following procedure. Random residential phone numbers with East York area codes were generated from the Ontario tele-direct directory. The numbers were called by a team of interviewers with market research expertise at Goldfarb Consultants. The person who answered the phone was probed for eligibility by a brief questionnaire. Households were eligible for inclusion in this study if (1) at least one member of the household was over 17 years old AND (2) at least one member of the household was able to speak English. Since females were more
likely to answer the telephone, a method, which ensured equal gender sampling, was used. Therefore, of those eligible household members, the person with the next birthday was asked to be interviewed. There were five possible outcomes of a telephone call:

1. The household was not residential or did not fulfil both the aforementioned eligibility criteria.

2. The household was eligible and the respondent agreed to be interviewed.

3. The household was eligible but the respondent refused to participate. In this case, a market researcher attempted the number at another time in order to complete the interview. Those who agreed were included and the previous refusal was recorded as 'refuser-conversions'.

4. If the household was eligible, but the respondent would not answer all the questions, the interview was still considered valid and the missing item/s have been acknowledged in the results.

5. If there was no answer, the household was called four further times at different times of the day. At least one attempt was made during a weekday day time; another during a weekday night and another attempt was made on the weekend. If there was no contact after all four attempts, an alternate, randomly selected number replaced the non-response number.

*Inclusion Criteria*

Once a suitable call recipient was contacted, he or she was told that we were doing a study on the effect of food on mood. The interviewee was considered a potential subject if he or she:
was between 18-65 years of age
was not suffering from bipolar depression or an eating disorder
was not on micronutrient supplementation
was not suffering from an active medical condition that may affect folate status such as liver disease, inflammatory bowel disease or diabetes mellitus
had not been treated with antidepressant medication in the last four weeks
was not on any medication that could affect folate status such as anticonvulsants, methotrexate, isoniazid, steroids, or a recent change in thyroid hormone therapy
was not pregnant or had given birth in the past three months

The call recipient was administered the screening and an abridged version of the depression section of the Structured Clinical Interview for DSM-IV Diagnoses (SCID). The interview took approximately three minutes for those subjects without depression and ten minutes for subjects diagnosed with current depression. Subjects were told that they were being assessed over the phone for eligibility to participate in the study. If they did not have depression, and were not selected for the control group, they were told they were not eligible. They were thanked for their help, but were given no clinical information by the lay interviewer. If they wished to discuss the interview with someone they were put in contact with one of the investigators. If they had depression, they were told that they are eligible to participate in the following stage of the study. There were three possible dispositions once the person was recognized as eligible; they refused to continue, they agreed to continue but did not show up, or they agreed and showed up.
(a) If a subject had depression, but elected not to proceed they were offered the opportunity to talk to one of the investigators for feedback. The clinician provided only a general indication of the possible diagnosis and a list of general treatment options. The clinician did not enter into a therapeutic relationship with the person, nor did he or she know the person's name. If there was any concern for the person's safety, the clinician took appropriate action. The investigators provided subjects with the names of crisis lines or the nearest hospital emergency room.

(b) If a subject agreed and did not show up, no further action was taken.

(c) When a subject did show up for the face-to-face interview, the person received the package of interviews and questionnaires. If the subject was positive for depression on either the phone or the face-to-face interview, he or she was seen by a study clinician, and was given general feedback and general treatment options.

Potential subjects were told that they would be compensated for their time. If the interviewee consented to participate in the study at the end of the phone call, his or her telephone number was given to the researcher. The researcher contacted the subject within two days to arrange the first face to face meeting.

4.2(b) Newspaper Advertisement

The second method of recruitment namely, newspaper advertisement, involved placing an advertisement in community newspapers and a daily newspaper. The advertisement asked readers between 18 and 65 years who were interested in the relationship between food and mood and were suffering for at least two weeks from a number of listed
depressive symptoms to call a researcher at the Sunnybrook Health Science Centre. An
appointment was made for those callers who qualified for the study. The same exclusion
criteria used for telephone recruitment were employed in the newspaper recruitment
method. All potential subjects were assured that they could withdraw from the study at
any stage. Clinicians were available for counselling throughout the study period in the
event that the study caused the subject distress. All individuals with depression were
seen by a clinician. Individuals who were at risk for suicide would be managed
appropriately, either with referral to their family doctor, to their own psychiatrist or to a
community psychiatrist. Alternatively, they were offered admission if the risk was severe
and imminent. Sunnybrook Health Science Centre Ethics Approval Board approved this
study.

Hospital Visit

During the first visit, the subject was given information about the study and was asked to
give informed consent. Each subject was advised that he or she could leave the protocol
at any stage. The subject was assured that the information gathered would be kept strictly
confidential.

In total, out of 8000 randomly generated telephone numbers, 826 completed the telephone
screening. From previous studies using this recruitment method, we expected to conduct
3000 interviews, i.e. 3.6 times more interviews than those completed. From these
interviews we anticipated that 3-4% would have current depression (approximately 100
subjects). Of these 100, about half would likely be excluded, and of the remaining 50, 30 would agree to be interviewed. In the survey, 62 interviewees were identified as having experienced a major depression in the past year, i.e. 7.5% one-year prevalence, somewhat higher than the rates previously reported for this population (Offord, D. R. et al., 1996). Of these 62, 12 were excluded due to current antidepressant treatment and 18 were excluded due to another major medical illness, recent birth, pregnancy or regular vitamin supplementation. Of the 32 eligible depressed subjects, only nine depressed subjects were recruited. Thirty control subjects were recruited via this method. Interviewees eligible for controls were invited to participate in the study until 30 controls had undergone the study protocol.

Due to the low number of depressed subjects recruited via this method, newspaper advertisement was adopted as an alternative recruitment method.

One hundred and twenty six people called in response to the newspaper advertisements (Figure 4.1). The questionnaire and eligibility criteria cited above were used to screen callers. Of the 126 callers, 22 were eligible for participation in the study. The others were ineligible for one or more of the following reasons:

- taking antidepressant medication
- suffering from other major medical illnesses
- not enough depressive symptoms to be depressed
- not currently depressed
- manic symptoms
- taking vitamin supplements
- eating disorders
- recent birth

Four eligible depressed subjects from the newspaper advertisements cancelled their visits without explanation. Subjects recruited from the two methods did not differ with respect to age, sex or BMI.

Depressed subjects (n = 26) and control subjects (n = 30) were age, sex and BMI matched. In the matching process, four control subjects were dropped. Amongst the depressed subjects, there was no difference in age or gender distribution according to method of recruitment.
Newspaper Recruitment (Depressed) – Telephone Recruitment (Depressed and Control)

8000 telephone numbers called

126 respondents to advertisements

826 interviews conducted

30 eligible controls recruited

62 depressed identified

20 eligible to participate*

32 eligible to participate*

16 depressed subjects recruited**

10 depressed subjects recruited**

* Met criteria for major depressive disorder
    Free of bipolar depression, other medical conditions, vitamin supplementation, alcohol abuse, recent birth or pregnancy

** Agreed to participate and arrived for scheduled appointment

Figure 4. Recruitment of Subjects
4.2 Assessment of Presence and Severity of Depression

The subjects were interviewed by a trained researcher to confirm that the diagnosis of depression or anxiety disorder was accurate and that the healthy controls were free of psychiatric morbidity. The SCID IV (First, M. B. et al., 1994) was used for this purpose. A depressed subject was included if he or she was diagnosed as currently suffering from major depression without psychiatric comorbidity. In order to establish the severity of depression, the HAM-D (Hamilton, M., 1960) was administered. In order to ensure homogeneity in the depressed group, a subject was considered ‘depressed’ if his or her HAM-D score was 14 or greater, indicating that the subject was suffering from at least mild to moderate depression.

4.3 Dietary Folate Intake Assessment

The food frequency questionnaire (FFQ) was administered to each subject. The questionnaire inquired about the average intake of 101 food items during the previous year. This questionnaire was based on the validated 1994 Ontario Health Survey Food Frequency Questionnaire (Bright-See, E. et al., 1994). A list of folate-rich foods was added to the original questionnaire (Cuskelly, G. J. et al., 1996). Foods that are not particularly high in folate but were found to be major food sources of folate in the US diet (there is no Canadian data available), such as orange juice, white breads, green salad and ready-to-eat breakfast cereals were included (Subar, A. F. et al., 1989). In addition to the
structured questions on food intake, subjects were asked to name specific brands of breakfast cereals. On completion, the researcher reviewed the FFQ with the subject to confirm the accuracy. The average folate intake was computed by multiplying the frequency of consumption of each food item by the nutrient content of the listed portion sizes (Bright-See, E., 1992). The questionnaires were analyzed using the CANDAT software (Version 3.0, 1995, Godin London Incorporated), which uses Canadian Nutrient File food compositions for nutritional analysis. This food analysis program was used for the Ministry of Health Survey (Bright-See, E., 1992). In order to improve the representativeness of longitudinal dietary intake over time, each subject was mailed a second FFQ one week after his or her visit and the average of both FFQs for the analysis of dietary intake. The subject was encouraged to complete the questionnaire and mail it back to the researchers as soon as possible.

4.4 Anthropometric Measures

Fasting body weight and height were measured after removal of heavy clothing and shoes.

4.5 Blood Sampling

Fasting blood samples were taken for the analysis of vitamin B12, red cell folate, serum folate, SAM, SAH, methionine, homocysteine, serine, glycine and serum MMA and polymorphism of the MTHFR gene.
4.5.1 Homocysteine and Methionine

Blood samples were collected in EDTA tubes. They were centrifuged at 3000 rpm for 5 min. The plasma was removed and frozen at -70°C until analysis. Samples were thawed for capillary gas chromatography and mass spectrometry analysis. 30 μL of 100g/L tris(2-carboxyethyl) phosphine (TCEP) (Pierce Chemical Co.) was added to 300 μL of plasma and gently mixed with a rotating stirrer at room temperature for 30 minutes. Then, 1170 μL of mobile phase (150 mmol/L NaClO₄, 100 mmol/L HCLO₄; and 50mL.L CH₃CN) were added and centrifuged at 10 000g for 5 min. The supernatant was passed through a C₁₈ solid-phase extraction cartridge (Evrovski, J. et al., 1995) and 50 μL of the filtrate was injected directly. Altering the valve-switch times to 1 min and 2 min generated a “heart-cut” of the eluting peaks, with homocysteine eluting at 7.9 min and methionine at 11.3 min (Cole, D. E. C. et al., 1998).

4.5.2 S-adenosylmethionine and S-adenosylhomocysteine

SAM and SAH samples were collected in heparinised test tubes. Immediately after collection, the blood was spun for 5 min at 3000 rpm. The serum was removed and a few of milliliters of saline was added to the test tubes and the blood and saline were gently rocked from side-to-side to mix. The sample was then centrifuged again at 3000 rpm for 5 min, after which the saline is removed. The washing with saline was repeated again.
The red blood cells (RBCs) were then frozen at -70°C. The SAM and SAH were measured by HPLC using the method of Wise and Fullerton (1995) (Wise, C. K. et al., 1995) and Wise et al. (1997) with slight modifications. 200 µL of 0.1 M sodium acetate, pH 6.0, and 60 µL of 40% trichloroacetic acid were pipetted into a 1.5 ml Eppendorf tube. The tube was kept on ice and 400 µL of previously frozen RBCs that had been thawed and vortexed were added. The mixture was again vortexed and then centrifuged in a refrigerated centrifuge at 4°C at 25 000g for 15min. The supernatant was transferred to a clean tube. The solution was extracted twice with a equal volume of diethyl ether and was filtered through a Ultrafree-MC filter (0.45µm; Millipore, Bedford, MA, USA).

4.5.3 Serine and Glycine

Serine and glycine samples were collected in heparinised test tubes. Immediately after collection, the blood was spun for 5min at 3000 rpm. The serum was removed and frozen at -70°C until analysis. The samples were analyzed by automated amino acid chromatography (Beckman 7300 Amino Acid Analyzer).

4.5.4 Serum MMA

Serum MMA samples were collected in heparinised test tubes. Immediately after collection, the blood was spun for 5min at 3000 rpm. The serum was removed and frozen at -70°C until analysis. Trio-2A mass spectrometry was used. Gas isotopically labeled MMA was used as an internal standard for quantitating levels of MMA in samples.
4.5.5 Vitamin B12, Serum Folate and Red Blood Cell Folate

Blood samples for the analysis of red cell folate, vitamin B12 and serum folate were collected in EDTA test tubes, refrigerated and analyzed within one week. Analysis of RCF and vitamin B12 was measured by radioimmunoassay (Quantaphase II; Bio-Rad, Richmond, CA). Radioimmunoassay is a more precise and sensitive technique than the microbiological assay (Grinblat, J. et al., 1968).

4.5.6 MTHFR Polymorphism

Blood was collected in EDTA test tubes. The sample was then frozen at -70°C until analysis. The DNA was isolated from the samples. C677T was genotyped by HinfI cleavage of a 198bp PCR product beginning at bp644 and ending in an intronic sequence. A1298C was genotyped by mutation selective PCR (MS0PCR) and the PCR products were digested with BbsI for detection of the T1317C polymorphism (Langman, L. J. et al., 1998).

4.6 Oral Folate Absorption Test

At a second visit four depressed and two control subjects were prepared for the Oral Folate Absorption Test (OFAT) as described by Steger and coworkers (Steger, G.G. et al., 1994). Each subject was given 1.5mg of folate intramuscularly in order to saturate body folate stores in preparation for the OFAT, which followed two days later.
Two days after the second meeting, the OFAT was carried out. Subjects came in fasting. Subjects were weighed again. Forty μg/kg body weight of folic acid was given with 10ml of tap water orally to the subjects in fasting state. Blood specimens were drawn at 0, 60, 120 minutes from a vein. The samples were then centrifuged and the serum was stored at -20°C until analysis. Serum folate levels were determined by radioimmunoassay.

Each subject received compensation for the time and effort that they devoted to the study. Subjects were given feedback on their interview, blood results and dietary assessment. The various treatment options for depressed subjects were presented by a psychiatrist.
CHAPTER 5: DATA ANALYSIS
5.1 Sample Size Calculation

A large effect size was anticipated based on previous studies on OCM in depression. In a study by Bell et al (1994) that evaluated SAM concentrations between depressed and non-depressed subjects, the effect size was 0.59. In a later study by Levitt et al. (1994) that evaluated folate status in depressed and non-depressed subjects, the effect size was 0.35. Using the principal hypothesis, 6 variables were entered into a sample size calculation ($\alpha = 0.05; \beta = 0.2$). Accordingly, 31 subjects were needed to find a significant difference between groups (Streiner, D.L., 1994).
5.2 Principal Analysis

The principal metabolites of OCM, that is SAM, SAH, methionine and homocysteine, were analyzed using MANOVA. Separate MANOVA tests were done on co-factors and related substances of one-carbon metabolism (vitamin B12, serine, glycine and MMA) and markers of enzyme activity; that is, ratios of red cell folate/ methionine (MS activity), serum folate/ methionine (MS), methionine/ SAM (MAT activity) and SAM/SAH (methylation). If MANOVA was significant (p<0.05), post-hoc analyses were undertaken. Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and all of the above variables in depressed subjects only. Variables whose bivariate analyses were significant were entered into a forward multiple regression analysis to examine the independent contribution of these variables to variance in the severity of depression.
5.3 Secondary Analysis:

Differences in dietary intake were analyzed defining low, moderate and high folate intake according to tertiles of the whole sample (n = 52). The proportion of subjects in each of the intake groups was compared across depressed and controls using non-parametric $\chi^2$ tests. MANOVA tests were used to compare mean dietary intake of macro- (fat, protein and carbohydrate) and micronutrients (methionine, vitamin B12, vitamin B6, and folate) related to OCM in depressed and control subjects. If MANOVA was significant ($p<0.05$), post-hoc analyses were undertaken. Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and components of dietary intake. Also, this test was used to assess the relationship between dietary intake of various nutrients related to OCM and the blood level of the metabolites. Variables whose bivariate analyses were significant were entered into a forward multiple regression analysis to examine the independent contribution of these variables to variance in the severity of depression. Statistical Package for the Social Sciences (SPSS) was used for the analyses.

Non-parametric $\chi^2$ tests were used to test for differences in presence of MTHFR C667T polymorphisms in depressed and control subjects.

The measurement of OFAT was designed to test the feasibility of doing this test in depressed subjects and the raw data was reviewed descriptively.
CHAPTER 6: RESULTS
6.1 Demographics of Depressed and Control Subjects

Using the MANOVA, no significant differences in age, weight, height, sex or body mass index (BMI) were found (See Table 6.1) between depressed and controls.

Table 6.1: Demographics of depressed and control subjects

<table>
<thead>
<tr>
<th></th>
<th>DEPRESSED</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>SEX (male/ female)</td>
<td>9/17</td>
<td>9/17</td>
</tr>
<tr>
<td>AGE (years)</td>
<td>40.08 ± 10.06</td>
<td>41.04 ± 9.99</td>
</tr>
<tr>
<td>WEIGHT (kg)</td>
<td>73.39 ± 15.42</td>
<td>74.24 ± 15.07</td>
</tr>
<tr>
<td>HEIGHT (m)</td>
<td>1.64 ± 0.08</td>
<td>1.66 ± 0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.33 ± 5.13</td>
<td>26.83 ± 4.64</td>
</tr>
</tbody>
</table>

mean ± standard error of the mean
6.2 The Difference between Depressed and Control Subjects in Principal Metabolites of One-Carbon Metabolism

The difference in mean levels of principal metabolites of one-carbon metabolism between depressed and control subjects was analyzed using MANOVA. The MANOVA demonstrated a trend towards significance (λ = 0.780, d.f. = 6; 43, p = 0.083). It is possible that λ was not significant because there was one subject with missing serum folate and one other subject with missing red cell folate values, thus reducing the number of subjects that could be included and hence the power of the MANOVA. Because λ showed a trend towards significance, individual compounds were compared between groups using independent student t-tests. Table 6.2 reports the means of each one-carbon metabolite in the two groups. Independent student t-tests revealed that depressed subjects had significantly lower methionine levels (p = 0.012) and significantly higher serum folate levels (p = 0.005) than control subjects. Graphical representations of the mean difference between groups and the standard error of the mean in methionine and serum folate can be seen in Figures 6.2a and 6.2b.
Table 6.2: The difference between depressed and control subjects in principal metabolites of one-carbon metabolism

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>DEPRESSED</th>
<th>CONTROL</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM (nmol/ml)</td>
<td>2.29 ± 0.14</td>
<td>2.37 ± 0.21</td>
<td>0.384</td>
<td>0.768</td>
</tr>
<tr>
<td>SAH (nmol/ml)</td>
<td>0.55 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>0.624</td>
<td>0.417</td>
</tr>
<tr>
<td>METHIONINE (µmol/L)</td>
<td>13.8 ± 1.8*</td>
<td>20.1 ± 1.6</td>
<td>5.974</td>
<td>0.012</td>
</tr>
<tr>
<td>HOMOCYSTEINE (µmol/L)</td>
<td>10.9 ± 0.8</td>
<td>10.9 ± 0.9</td>
<td>0.205</td>
<td>0.967</td>
</tr>
<tr>
<td>RBC FOLATE (nmol/L)</td>
<td>727 ± 46*</td>
<td>703 ± 36</td>
<td>0.295</td>
<td>0.674</td>
</tr>
<tr>
<td>SERUM FOLATE (nmol/L)</td>
<td>31.3 ± 1.8*</td>
<td>23.9 ± 1.8</td>
<td>9.458</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean

* n = 25

MANOVA, λ = 0.780, d.f. = 6; 43, p = 0.083 (trend)

* t = -2.596, F = 0.644, by student t-test

b t = 2.940, F = 0.249, by student t-test
Figures 6.1 and 6.2: Difference between mean methionine concentrations and mean
6.3 The Difference in Methionine and Serum Folate Concentrations between Control, Past Depressed and Current Depressed Subjects

Within the group of depressed subjects ($n = 26$), there were 4 subjects who had experienced a major depressive disorder within the 12 months preceding the study, but were not currently depressed. In order to see whether there was a difference between controls ($n = 26$), past depressed ($n = 4$) and currently depressed ($n = 21$) subjects in mean levels of compounds found to be significantly different in the primary analysis of principal one-carbon metabolites, ANOVA was undertaken. Currently depressed subjects had significantly lower methionine levels than controls ($p < 0.05$) as seen in Table 6.3 and Figure 6.3a. Also, currently depressed subjects had significantly higher serum folate levels than control subjects ($p < 0.01$) as seen in Table 6.3 and Figure 6.3b.
Table 6.3: The difference in methionine and serum folate concentrations between control, past depressed and current depressed subjects

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n = 26)</th>
<th>PAST DEPRESSED (n = 4)</th>
<th>CURRENT DEPRESSED (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHIONINE (µmol/L)</td>
<td>20.1 ± 1.6\textsuperscript{a}</td>
<td>25.2 ± 4.3\textsuperscript{a}</td>
<td>12.0 ± 1.8\textsuperscript{b}</td>
</tr>
<tr>
<td>SERUM FOLATE (nmol/L)</td>
<td>23.9 ± 1.8\textsuperscript{c}</td>
<td>24.0 ± 3.2 \textsuperscript{cd}</td>
<td>32.2 ± 1.9\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Means ± standard error of the mean

* n= 20

by post-hoc test, values with different letters are significantly different (a vs b, p < 0.05, c vs d, p < 0.01)
ANOVA, $F = 6.120$, d.f. = 48, $p = 0.004$
Bars with different letters are significantly different at a level of $p < 0.05$

*Figure 6.3a:* Difference between mean methionine concentrations ± 2 SEM in control, past depressed and current depressed subjects

ANOVA, $F = 5.341$, d.f. = 48, $p = 0.008$
Bars with different letters are significantly different at a level of $p < 0.01$

*Figure 6.3b:* Difference between mean serum folate concentrations ± 2 SEM in control, past depressed and current depressed subjects
6.4 Correlation between Severity of Depression (HAM-D) and the Principal Metabolites of One-Carbon Metabolism in Depressed Subjects

In order to assess the association between the severity of depression and principal metabolites of one-carbon metabolism, Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and the metabolites (Table 6.4). Variables whose bivariate analyses were significant, namely S-AH (p = 0.006), methionine (p = 0.007) and red blood cell folate (p = 0.005), were entered into a forward multiple regression analysis to examine the contribution of these variables to variance in the severity of depression (Figures 6.4a-c). The overall $R^2$ of the model was 0.61. ANOVA for the model was significant $F = 10.7$, d.f. = 3; 21, p < 0.001. Of the individual variables entered SAH ($\beta = 0.36$, $t = 2.1$, p = 0.04) and red cell folate ($\beta = 0.45$, $t = 3.3$, p = 0.004) were significant. Methionine was not significant ($\beta = -0.27$, $t = -1.6$, p = 0.13).
Table 6.4: Correlations between severity of depression (HAM-D) and the principal metabolites of one-carbon metabolism in depression subjects

<table>
<thead>
<tr>
<th>OCM METABOLITE</th>
<th>PEARSON CORRELATION</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFFICIENTS BETWEEN HAM-D SCORE AND METABOLITE (r)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAM</td>
<td>-0.004*</td>
<td>26</td>
<td>0.985</td>
</tr>
<tr>
<td>SAH</td>
<td>0.528</td>
<td>26</td>
<td>0.006</td>
</tr>
<tr>
<td>METHIONINE</td>
<td>-0.517</td>
<td>26</td>
<td>0.007</td>
</tr>
<tr>
<td>HOMOCYSTEINE</td>
<td>0.103</td>
<td>26</td>
<td>0.617</td>
</tr>
<tr>
<td>RBC FOLATE</td>
<td>0.548</td>
<td>25</td>
<td>0.005</td>
</tr>
<tr>
<td>SERUM FOLATE</td>
<td>0.294</td>
<td>25</td>
<td>0.153</td>
</tr>
</tbody>
</table>
Figure 6.4a: Scatterplot relationship between S-AH and severity of depression with linear trend line

Figure 6.4b: Scatterplot relationship between methionine and severity of depression with linear trend line

Figure 6.4c: Scatterplot relationship between red cell folate and severity of depression with linear trend line
6.5 The Difference between Depressed and Control Subjects in Co-factors and Related Compounds of One-Carbon Metabolism

Differences in mean levels of co-factors and related compounds of one-carbon metabolism between depressed and controls subjects were analyzed using MANOVA. As seen in Table 6.5, there were no significant differences between the groups.
Table 6.5: The difference between depressed and control subjects in co-factors and related compounds of one-carbon metabolism

<table>
<thead>
<tr>
<th></th>
<th>DEPRESSED</th>
<th>CONTROL</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITAMIN B12 (pmol/L)</td>
<td>290 ± 29</td>
<td>290 ± 30</td>
<td>0.044</td>
<td>0.835</td>
</tr>
<tr>
<td></td>
<td>n = 23</td>
<td>n = 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLYCINE (µmol/L)</td>
<td>213 ± 13</td>
<td>210 ± 11</td>
<td>0.006</td>
<td>0.940</td>
</tr>
<tr>
<td></td>
<td>n = 23</td>
<td>n = 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERINE (µmol/L)</td>
<td>92 ± 4</td>
<td>94 ± 4</td>
<td>0.474</td>
<td>0.495</td>
</tr>
<tr>
<td></td>
<td>n = 23</td>
<td>n = 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMA (nmol/L)</td>
<td>297.84 ± 42.46</td>
<td>372.54 ± 65.23</td>
<td>0.562</td>
<td>0.458</td>
</tr>
<tr>
<td></td>
<td>n = 23</td>
<td>n = 21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: not all data was available for all subjects
MANOVA; $\lambda = 0.963$, d.f. = 4; 34, p = 0.859
6.6: Correlations between Severity of Depression (HAM-D) and Co-factors and Related Factors of One-Carbon Metabolism in Depressed Subjects

In order to assess the association between the severity of depression and co-factors and related compounds of one-carbon metabolism, Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and the co-factors and related compounds (Table 6.6). Variables whose bivariate analyses were significant, namely vitamin B12 (p = 0.032) and glycine (p = 0.027), were entered into a forward multiple regression analysis to examine the contribution of these variables to variance in the severity of depression (Figures 6.6a-b). The overall R² of the model was 0.37.

ANOVA for the model was significant F = 5.2, d.f. = 2;18, p < 0.02. Of the individual variables entered glycine was significant (β = -0.42, t = -2.2, p = 0.04). Vitamin B12 trended towards significance (β = 0.35, t = 1.8, p = 0.08).
Table 6.6: Correlations between severity of depression (HAM-D) and co-factors and related factors of one-carbon metabolism in depressed subjects

<table>
<thead>
<tr>
<th>OCM CO-FACTOR OR RELATED FACTOR</th>
<th>PEARSON CORRELATION COEFFICIENT BETWEEN HAM-D SCORE AND CO-FACTORS OF OCM (r)</th>
<th>n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITAMIN B12</td>
<td>0.448*</td>
<td>23</td>
<td>0.032</td>
</tr>
<tr>
<td>SERINE</td>
<td>-0.398</td>
<td>23</td>
<td>0.060</td>
</tr>
<tr>
<td>GLYCINE</td>
<td>-0.480</td>
<td>23</td>
<td>0.027</td>
</tr>
<tr>
<td>MMA</td>
<td>-0.247</td>
<td>23</td>
<td>0.257</td>
</tr>
</tbody>
</table>

* Values represent Pearson correlation coefficients
**Figure 6.6a:** Scatterplot relationship between vitamin B12 and severity of depression with linear trend line

Rsq = 0.2004
Rsq (excluding outlier) = 0.2782

**Figure 6.6b:** Scatterplot relationship between glycine and severity of depression with linear trend line

Rsq = 0.2129
6.7 The Difference between Depressed and Control Subjects in Markers of Enzyme Activity in One-Carbon Metabolism

MANOVA was used to test for the difference in the markers of enzyme activity (using ratios over substrates) in depressed subjects and controls. Methionine: red cell folate was a measure of MS activity, SAM: methionine was a measure of MAT activity and SAH: SAM was a measure of methylation (Table 6.7). Because λ for the MANOVA was significant (λ = 0.836, d.f. = 3; 47, p = 0.036), individual compounds were compared between groups using independent student t-tests. Independent student t-tests revealed that depressed subjects had significantly lower MS activity (p = 0.028) and significantly higher MAT activity (p = 0.003) than control subjects.
Table 6.7: The difference between depressed and control subjects in markers of enzyme activity in one-carbon metabolism

<table>
<thead>
<tr>
<th></th>
<th>DEPRESSED (x 10⁻²)</th>
<th>CONTROL (x10⁻²)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 26</td>
<td>n = 26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS activity[^a]</td>
<td>2.05 ± 3.18*</td>
<td>2.97 ± 2.53</td>
<td>5.144</td>
<td>0.028</td>
</tr>
<tr>
<td>MAT activity[^b]</td>
<td>23.54 ± 2.73</td>
<td>13.98 ± 1.56</td>
<td>9.470</td>
<td>0.003</td>
</tr>
<tr>
<td>Methylation[^c]</td>
<td>26.76 ± 2.19</td>
<td>27.51 ± 2.81</td>
<td>0.012</td>
<td>0.914</td>
</tr>
</tbody>
</table>

[^a] METHIONINE / RED CELL FOLATE
[^b] SAM / METHIONINE
[^c] SAH / SAM

mean ± standard error of the mean

MANOVA, λ = 0.836, d.f. = 3; 47, p = 0.036
6.8 Correlations between Severity of Depression (HAM-D) and Markers of Enzyme Activity in One-Carbon Metabolism in Depressed Subjects

Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and enzyme activity (Table 6.8). One variable demonstrated a significant coefficient, namely MS activity (p = 0.001). When MS activity was entered into a forward multiple regression analysis to examine the independent contribution of this variable to variance in the severity of depression (Figure 6.8), the overall $R^2$ of the model was 0.443. ANOVA for the model was significant ($F = 18.3$ d.f. = 1;24, p < 0.001). MS activity had a significant coefficient ($\beta = -0.665, t = -4.3, p = 0.001$).

Red cell folate, methionine and MS activity all contributed to the variance in severity of depression. The most appropriate tests to determine the independent contribution of these related variables to severity of depression would have been a regression analysis. However, as MS activity was a constructed variable from the two other variables (red cell folate and methionine), collinearity diagnostics were performed. Results revealed unacceptable multicollinearity. Therefore, the magnitude of the Pearson correlation coefficient of each variable was compared. MS activity had the greatest correlation coefficient ($r = 0.66$) as compared to its two component parts, that is red cell folate ($r = 0.54$) and methionine ($r = -0.52$).
Table 6.8: Correlations between severity of depression (HAM-D) and markers of enzyme activity in one-carbon metabolism in depressed subjects

<table>
<thead>
<tr>
<th>MARKERS OF ENZYME ACTIVITY</th>
<th>PEARSON CORRELATION COEFFICIENT BETWEEN HAM-D SCORE AND MARKERS OF ENZYME ACTIVITY (r)</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS activity(^a)</td>
<td>-0.665*</td>
<td>25</td>
<td>0.001</td>
</tr>
<tr>
<td>MAT activity(^b)</td>
<td>0.307</td>
<td>26</td>
<td>0.127</td>
</tr>
<tr>
<td>Methylation(^c)</td>
<td>0.278</td>
<td>26</td>
<td>0.170</td>
</tr>
</tbody>
</table>

RCF = red blood cell folate
SFOL = serum folate
* Values represent Pearson correlation coefficients
\(^a\) METHIONINE / RCF
\(^b\) SAM / METHIONINE
\(^c\) SAH / SAM

Figure 6.8: Scatterplot relationship between methionine synthetase and severity of depression with linear trend line
6.9 Proportion of Homozygous and Heterozygous MTHFR C667T Subjects in Depressed and Control Groups

Non-parametric $\chi^2$ tests were used to analyze the difference in presence of MTHFR C667T polymorphisms in depressed and control subjects. The percentage of MTHFR C677T heterozygous depressed subjects (versus no polymorphism or homozygosity) was significantly higher than control subjects (Figure 6.9).

* $\chi^2 = 5.538$, d.f. = 1, $p = 0.019$
6.10 Proportion of Depressed and Control Subjects with Low, Moderate and High Folate Intakes (per BMI)

Differences in dietary intake were analyzed defining low (dietary folate intake/ BMI < 9.60), moderate (dietary folate intake/ BMI 9.61 – 14.60) and high (dietary folate intake/ BMI > 14.61) folate intake according to tertiles of the whole sample (n = 52). Dietary folate intake per BMI as BMI is a ratio of weight and height and is a rough estimate of blood volume. A more accurate estimation of dietary folate per blood volume would have been attained by measuring body fat and hence, fat-free mass. As body fat analysis was not undertaken in this study, dietary intake could not be related to fat-free mass.

The proportion of subjects in each of the intake groups was compared across depressed and controls subjects using non-parametric $\chi^2$ tests. No differences were found between groups (Figure 6.10).
Figure 6.10: Comparison of dietary folate intake between depressed and control subjects

\[ \chi^2 = 0.038, \text{ d.f.} = 2, p = 0.981 \]

**Low Intake:**
- DIETARY FOLATE INTAKE/ BMI < 9.60

**Moderate Intake:**
- DIETARY FOLATE INTAKE/ BMI 9.61 – 14.60

**High Intake:**
- DIETARY FOLATE INTAKE/ BMI > 14.61
6.11 Difference between Macronutrient Intake (Total, per Kilocalories, per Body Mass Index and as a Percentage of Total Caloric Intake) in Depressed and Control Subjects

MANOVA was used to compare mean dietary intake of macronutrients related to OCM in depressed and control subjects. Each macronutrient was analyzed as (1) absolute dietary intake, (2) per unit BMI and (3) as a percentage of total energy intake (Table 6.11). No significant differences were found between groups using any of these calculations.
Table 6.11: Difference between macronutrient intake (total, per kilocalories, per body mass index and as a percentage of total calories) in depressed and control subjects

<table>
<thead>
<tr>
<th></th>
<th>DEPRESSED</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 26)</td>
<td>(N = 26)</td>
</tr>
<tr>
<td><strong>CALORIES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>µg</td>
<td>µg</td>
</tr>
<tr>
<td></td>
<td>2368.3 ± 195.2</td>
<td>2123.4 ± 149.2</td>
</tr>
<tr>
<td>per BMI</td>
<td>µg/kg/m²</td>
<td>µg/kg/m²</td>
</tr>
<tr>
<td></td>
<td>88.1 ± 7.1</td>
<td>81.4 ± 6.45</td>
</tr>
<tr>
<td><strong>CARBOHYDRATE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>µg</td>
<td>µg</td>
</tr>
<tr>
<td></td>
<td>303.4 ± 26.3</td>
<td>257.2 ± 19.0</td>
</tr>
<tr>
<td>per kcal</td>
<td>µg/kcal</td>
<td>µg/kcal</td>
</tr>
<tr>
<td></td>
<td>129.4 ± 4.0</td>
<td>121.6 ± 4.5</td>
</tr>
<tr>
<td>per BMI</td>
<td>µg/kg/m²</td>
<td>µg/kg/m²</td>
</tr>
<tr>
<td></td>
<td>11.3 ± 0.9</td>
<td>9.9 ± 0.9</td>
</tr>
<tr>
<td>% of energy intake</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>51.7 ± 1.6</td>
<td>48.6 ± 1.8</td>
</tr>
<tr>
<td><strong>FAT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>mg</td>
<td>mg</td>
</tr>
<tr>
<td></td>
<td>85.0 ± 6.2</td>
<td>82.5 ± 5.9</td>
</tr>
<tr>
<td>per kcal</td>
<td>mg/kcal</td>
<td>mg/kcal</td>
</tr>
<tr>
<td></td>
<td>37.3 ± 1.7</td>
<td>39.3 ± 1.6</td>
</tr>
<tr>
<td>per BMI</td>
<td>µg/kg/m²</td>
<td>µg/kg/m²</td>
</tr>
<tr>
<td></td>
<td>3.2 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>% of energy intake</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>14.9 ± 0.7</td>
<td>15.7 ± 0.7</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean, BMI = body mass index

Macronutrient (total intake per subject): MANOVA, \( \lambda = 0.948 \), d.f. = 4:47, \( p = 0.631 \)

Macronutrient (total intake per kcal): MANOVA, \( \lambda = 0.965 \), d.f. = 3:48, \( p = 0.635 \)

Macronutrient (total intake per BMI): MANOVA, \( \lambda = 0.954 \), d.f. = 4:47, \( p = 0.570 \)

Macronutrient (% of total calories): MANOVA, \( \lambda = 0.965 \), d.f. = 3:48, \( p = 0.636 \)
6.12 Difference between Micronutrient Intake (Total, per Kilocalories, per Body Mass Index and as a Percentage of Total Calories) in Depressed and Control Subjects

MANOVA was used to compare mean dietary intake of micronutrients related to OCM in depressed and control subjects. Each micronutrient was analyzed as (1) absolute dietary intake, (2) per unit BMI and (3) per 1000 calories (Table 6.12). No significant differences were found between groups.
Table 6.12: Difference between micronutrient intake (total, per kilocalories, per body mass index and as a percentage of total calories) in depressed and control subjects

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>DEPRESSED (N = 26)</th>
<th>CONTROLS (N = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FOLATE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>337.78 ± 27.79</td>
<td>322.42 ± 125.61</td>
</tr>
<tr>
<td>per Kcal</td>
<td>148.42 ± 7.94</td>
<td>158.22 ± 8.13</td>
</tr>
<tr>
<td>per BMI</td>
<td>12.70 ± 1.06</td>
<td>12.69 ± 1.12</td>
</tr>
<tr>
<td><strong>VITAMIN B12</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>5.26 ± 1.25</td>
<td>4.54 ± 3.21</td>
</tr>
<tr>
<td>per Kcal</td>
<td>2.35 ± 0.64</td>
<td>2.17 ± 0.25</td>
</tr>
<tr>
<td>per BMI</td>
<td>0.21 ± 0.05</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td><strong>VITAMIN B6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>1.99 ± 0.14</td>
<td>1.73 ± 0.72</td>
</tr>
<tr>
<td>per Kcal</td>
<td>0.89 ± 0.06</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td>per BMI</td>
<td>75.1 ± 6.11</td>
<td>67.9 ± 6.20</td>
</tr>
<tr>
<td><strong>METHIONINE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>1.60 ± 0.11</td>
<td>1.59 ± 0.66</td>
</tr>
<tr>
<td>per Kcal</td>
<td>0.71 ± 0.04</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td>per BMI</td>
<td>59.9 ± 4.19</td>
<td>62.6 ± 5.52</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean, BMI = body mass index
Micronutrient (total intake per subject): MANOVA, $\lambda = 0.944$, d.f. = 4;47, $p = 0.595$
Micronutrient (total intake per Kcal): MANOVA, $\lambda = 0.857$, d.f. = 4;47, $p = 0.115$
Micronutrient (total intake per BMI): MANOVA, $\lambda = 0.935$, d.f. = 4;47, $p = 0.517$
6.13: Correlations between Severity of Depression (HAM-D) and One-Carbon Metabolism-Related Micronutrient Intake per BMI in Depressed Subjects

Pearson correlation coefficients were used to test for significant bivariate relationships between HAM-D scores and components of dietary intake/ BMI (Table 6.13). There was no significant relationship between OCM-related micronutrient intake and HAM-D scores.
Table 6.13: Correlations between severity of depression (HAM-D) and one-carbon metabolism-related micronutrient intake per BMI in depressed subjects

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Correlation Coefficient</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOLATE /BMI</td>
<td>-0.127*</td>
<td>26</td>
<td>0.536</td>
</tr>
<tr>
<td>VITAMIN B12 /BMI</td>
<td>-0.095</td>
<td>26</td>
<td>0.647</td>
</tr>
<tr>
<td>VITAMIN B6 /BMI</td>
<td>0.166</td>
<td>26</td>
<td>0.417</td>
</tr>
<tr>
<td>METHIONINE /BMI</td>
<td>-0.065</td>
<td>26</td>
<td>0.751</td>
</tr>
</tbody>
</table>

* Values represent Pearson correlation coefficients
6.14 Correlations between Blood Concentrations and Dietary Intake of One-Carbon Metabolism-Related Micronutrients per BMI in Depressed and Control Subjects

Pearson correlation coefficients were used to assess the relationship between dietary intake of an OCM-related nutrient and the blood level of that metabolite (Table 6.14). Significant bivariate relationships were found between plasma methionine levels and (1) dietary folate intake per BMI (p < 0.01), (2) dietary methionine intake per BMI (p < 0.05) and (3) dietary vitamin B12 intake per BMI (p < 0.05). These variables were entered into a forward multiple regression analysis to examine the independent contribution of these variables to variance in plasma methionine level (Figures 6.14a-c). The overall R² of the model was 1.7. ANOVA for the model was significant F = 3.3, d.f. = 3.48, p < 0.02. However, none of the individual β values for the individual variables were significant.
Table 6.14: Correlation coefficients of blood concentrations and dietary intake of one-carbon metabolism-related micronutrients per BMI in depressed and control subjects

<table>
<thead>
<tr>
<th></th>
<th>DIET FOL / BMI</th>
<th>DIET METH / BMI</th>
<th>DIET VIT B12 /BMI</th>
<th>DIET FOL / KCAL</th>
<th>DIET METH / KCAL</th>
<th>DIET VIT B12 / KCAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC FOLATE</td>
<td>0.221</td>
<td>0.002</td>
<td>-0.031</td>
<td>-0.045</td>
<td>-0.272</td>
<td>-0.059</td>
</tr>
<tr>
<td>SERUM FOLATE</td>
<td>-0.011</td>
<td>-0.117</td>
<td>0.021</td>
<td>-0.116</td>
<td>-0.208</td>
<td>0.013</td>
</tr>
<tr>
<td>PLASMA METH</td>
<td>0.360**</td>
<td>0.291*</td>
<td>0.345*</td>
<td>0.283*</td>
<td>0.083</td>
<td>0.286*</td>
</tr>
<tr>
<td>BLOOD VIT B12</td>
<td>-0.188</td>
<td>0.003</td>
<td>-0.082</td>
<td>-0.327*</td>
<td>-0.053</td>
<td>-0.107</td>
</tr>
</tbody>
</table>

RBC = red blood cell  
METH = methionine  
VIT B12 = vitamin B12  
FOL = folate  
* Correlation is significant at the 0.05 level (2-tailed)  
** Correlation is significant at the 0.01 level (2-tailed)  
Values represent Pearson correlation coefficients
Plasma Methionine Concentration

Figure 6.14a: Scatterplot relationship between dietary intake of vitamin B12 and plasma methionine concentration with linear trend line.

Figure 6.14b: Scatterplot relationship between dietary intake of methionine and plasma methionine concentration with linear trend line.

Figure 6.14c: Scatterplot relationship between dietary intake of folate and plasma methionine concentration with linear trend line.

R^2 = 0.1081
R^2 (excluding outlier) = 0.2343
6.15 Oral Folate Absorption Test

Figure 6.15 shows the serum folate concentration of 4 depressed subjects and 2 control subjects that underwent a pilot Oral Folate Absorption Test. As seen in this graph, all starting serum folate levels (after saturation with intramuscular folate injection 2 days prior to test) and peak serum folate levels were higher than normal levels as defined by Steger and colleagues (Steger, G.G., et al., 1994). This data was collected to determine the feasibility and technical requirement of doing the OFAT in depressed subjects, and so no formal analyses were conducted.
Figure 6.15: Serum folate levels after ingestion of oral folate supplement in depressed and control subjects.
CHAPTER SEVEN: DISCUSSION
The following discussion will focus on first one-carbon metabolites, second, genetic polymorphism, third, the impact of dietary factors and finally, on absorption.

7.1 One-Carbon Metabolites

7.1.1 Difference between depressed and controls
The most consequential finding of this study with regard to the difference between depressed and control subjects, is a significantly lower plasma methionine level in depressed adults. Also, serum folate was significantly higher in the depressed group. Folate circulating in the blood is found mostly in the monoglutamate form of 5-MTHF (serum folate). Although almost two-thirds of the folate in the blood is bound to protein, up to one-third is free monoglutamate folate. 5-MTHF is transported into tissue cells (such as red cells) and is converted into a polyglutamate form to become a functional coenzyme, accepting and transferring one-carbon fragments. Therefore, serum folate and red cell folate both reflect 5-MTHF concentrations, but in different compartments. Both these findings (low methionine and high serum folate in depressed versus control) point to a decreased production of methionine and an accumulation of its substrate, 5-MTHF in depression, which could be explained by a disturbance in methionine synthase (MS). MS is a rate-limiting enzyme in one-carbon metabolism that catalyzes the formation of methionine. Further evidence for dysfunctional MS in depression may be implied from this finding that MS activity as calculated by the ratio of methionine: serum cell folate (product: substrate) was significantly lower (p = 0.007) in depressed subjects versus controls. If the ratio is entered into a MANOVA along with serum folate and methionine, the ratio is still significant and in fact has the largest F score (MS = 9.3, MTHF = 8.5,
methionine = 7.3). These findings point to a possible defect in a key regulatory enzyme of one-carbon metabolism in depression.

Although methionine levels were lower in depressed subjects, there were no significant differences in depressed versus controls in SAM or SAH levels as originally hypothesized. Mean SAM levels were 3% lower in depressed subjects as compared to controls, but this difference was not significant. It is possible that this study did not have the power to demonstrate significant differences between groups. It may be possible that methylation was not significantly altered in depression. Alternatively, there may have been an increase in methylation, but SAM levels may have been maintained by increasing the conversion of methionine to SAM. There may be indirect evidence to support this hypothesis: calculated MAT activity was significantly higher in depressed subjects, suggesting an increased conversion of methionine to SAM. Furthermore, the calculated methylation activity (that is SAH: SAM ratio) was 7.2% higher in depressed subjects, although this difference failed to reach significance. Taken together, these findings might suggest that depression is associated with depleted methionine levels as a result of impaired MS activity and that depression is associated with an increased requirement for methionine to be converted to SAM to support a modest increase in methylation. To confirm this explanation future studies would have to include a functional measure of methylation such as examining methylated neurotransmitters. Also, the inclusion of a measure of SAM decarboxylation would be helpful in determining alternative fates of SAM in depression. The extent of our data does not allow comment to be made on the fate of SAM.
7.1.2. **Effect of severity of depression on one-carbon metabolites**

Within the depressed group of subjects, the principal findings were that with increasing severity, 5-MTHF (as measured by red cell folate), SAH and B12 levels increase and methionine and glycine decrease significantly. In addition, there was a trend for serine to decrease with increasing severity. These findings suggest that with increasing severity of depression, there is increasing disruption in MS activity and increasing methylation. With regard to change in MS activity with severity, these data demonstrate that there is an accumulation of the substrate of MS (that is 5-MTHF), a decline in its product (that is methionine) and an accumulation of the “unused” co-factor of MS (that is vitamin B12). In addition, calculated MS activity (red cell folate: methionine) was significantly, negatively correlated with severity of depression. MS is more powerful in predicting variance in HAM-D scores than either red cell folate or methionine. Therefore, not only is MS activity disturbed in depressed versus control subjects, the MS activity decreases with increasing severity of depression.

With regard change in methylation with severity, there is an accumulation of the methylated by-product (that is SAH) and a decline in the amino acid co-factor (that is glycine). SAM levels do not decline as originally hypothesized. There are two possible explanations for SAM levels remaining constant in the face of increasing methylation. First, SAM levels could be maintained by increasing the conversion from the methionine pool. Second, other fates of SAM, such as decarboxylation, could be diminished in depression in order to preserve SAM levels for methylation. These possibilities have been
discussed previously (see Section 7.1.2.).

7.1.3 Comparison of current versus past depression: are changes in one-carbon metabolites a “state-dependent” phenomenon?

It is unconfirmed as to whether the metabolic perturbations observed during an episode of depression are characteristic of the state of depression or are a longitudinal characteristic of depressed individuals, that is a trait. The current study provided some evidence that the metabolic disturbances observed in depression may be a “state” phenomenon. In the current study, a sub-group of four past depressed (currently euthymic) subjects within the depressed subjects had methionine levels that were no different from control subjects. However, methionine levels in the euthymic group were significantly higher than in the currently depressed subjects. The numbers were too few to make any generalizations. However, it is possible that the illness of depression is associated with a deficit in MS that is only manifest with worsening depression.

Furthermore, the finding that one-carbon metabolites fluctuate according to the severity of the illness provides further support for the state-dependency of these changes. The findings of the current study provide no evidence as to whether the disturbances in one-carbon metabolism result in depressive symptoms or whether one-carbon metabolism disturbances are a consequence of biochemical changes in depression. To test this properly a longitudinal study within and across groups should be conducted in the future.

In summary, these findings point to a disturbance in MS activity in depressed subjects compared to controls and a worsening impairment with increasing severity. In addition,
there is suggestive evidence that with increasing severity of depression there is increasing methylation. The following paragraphs will present supportive direct and indirect evidence from existing literature for the findings of the current study and will also describe how these findings differ from those of previous research.

7.1.4 Possible reasons for MS dysfunction in depression

The defect in the methionine synthase function described above could be due to either an inhibition or deactivation of MS by some factor that is abnormally high or low in depression or due to an inherited disorder of MS enzyme function. To evaluate the plausibility of these explanations, the structure and function of the enzyme itself needs to be understood. MS is a complex enzyme with numerous sites requiring binding with co-factors and activation. MS transfers a methyl group from methylcobalamin to homocysteine, to yield methionine and cob(I)alamin (Figure 7.1). The cob(I)alamin is remethylated by 5-MTHF to produce THF. Every 100-2000 turnovers, the cob(I)alamin species become oxidised to the inactive cob(II)alamin form (deactivation). In order to reactivate the co-factor, a methyl group and electron are needed. Reduced flavodoxin and a methyl group from SAM supply an electron to regenerate methyl-cobalamin. In the following section I will discuss the possible mechanisms for inhibition/deactivation.
Homocysteine  Methionine

Primary Turnover

CH$_3$

Co$^{III}$

N + 2e$^-$

SAH

SAM

Co$^{II}$

N + 2e$^-$

Deactivation/Reactivation

THF

5- MTHF

$e^-$

$2e^-$

Figure 7.1: Schematic summary of the reactions carried out by methionine synthetase

(Goulding, C.W. et al, 1997)
7.1.4.1 Inhibition or Deactivation of MS

One possible mechanism for inhibition involves the deactivation/reactivation of cob(I)alamin. The demand for SAM may increase in depression as SAM is required for methylation of, for example, neurotransmitters. There is some suggestive evidence of increased methylation in the current study. If methylation is increased, SAM may preferentially be used for this function, and not for other functions, or SAM may be used for the methylation of neurotransmitters and not for the methylation of other methyl-acceptors. For example, Hilton-Manz has shown that in the mouse, when there is increased demand for methyl groups (resulting from treatment with the methyl acceptor L-dopa) SAM decarboxylase tends to "switch off" (Hilton-Manz, J., 1994). It is also possible to speculate that with increased methylation of neurotransmitters, SAM may cease to donate a methyl group to deactivated cob(II)alamin for the reactivation of MS. If this occurred then MS activity would decline as methylation increased, and this would result in a reduction in methionine levels and an accumulation of B12.

Another possible reason for dysfunctional MS, is inadequate vitamin B12 - the co-factor that is vital for the working of MS. Results of the current study indicate that vitamin B12 levels were normal in the depressed group and actually increased with increasing severity of depression (p = 0.032). However, the measurement used, serum vitamin B12, does not distinguish between the cob(I-III)alamin. Hence, it is possible that the form of vitamin B12, which accumulated with increased severity of depression, was the inactivated cob(II)alamin form perhaps due to decreased methyl donation by SAM. Alternatively, it is possible that the MS enzyme is defective in its binding site for
B12 and so vitamin B12 is not being utilized by MS.

It is also possible that hormonal regulators of MS may be disturbed in depression. This may mean that there is an increase in inhibitory factors or a decrease in stimulatory factors. A model for the inhibition of MS in certain pathological conditions exists (Nicolaou, A. et al., 1997). It is believed, for example, that inflammation or sepsis may produce an increase in nitric oxide in the liver and that this results in inhibition of MS. The inhibition of MS and subsequent reduction in methylation may eventually lead to cellular death. There is no evidence that nitric oxide is increased in depression, but there remains the possibility that some inhibitory or stimulatory substance may be involved in the MS dysfunction observed in the current study. For example, thyroid hormone (Joffe, R. T. et al., 1993) and cortisol (Gold, P. W. et al., 1984) levels increase in depression and both these hormones may have a direct influence on various enzyme functions, including, possibly, MS. However, both hormones have stimulatory effects on metabolism, and the increased levels in depression would therefore result in an increase in enzyme activity. Hence, it is unlikely that either thyroid hormone or cortisol is involved in the inhibition of an enzyme such as MS in depression. This study did not measure cortisol or thyroid hormone nor have previous studies explored the role of these hormones in the regulation of MS in depression. No conclusion can be drawn regarding stimulatory or inhibitory influences on MS from the current data, but close scrutiny of potential factors is a reasonable focus for future studies.
7.1.4.2 Genetic Mutations of MS

The DNA structure of MS has been well-described (see Section 1.7). A dysfunction of any of the four distinct functional regions of MS could result in one-carbon metabolism disturbance. The fourth region of MS, amino acids 345-649 are thought to bind and activate 5-MTHF (Goulding, C.W., et al., 1997; Goulding, C.W. and Matthews, R.G., 1997). Dysfunction of the fourth region of MS would lead to an accumulation of serum folate and decreased production of methionine, similar to the findings of the current study. Alternatively, dysfunction of the first region, the C-terminal fragment that binds SAM, and is essential for reactivation of the inactive cob(II)alamin form of MS, may result in increasing levels of the inactive form of vitamin B12. If the increasing levels of vitamin B12 with increasing severity of depression are a reflection of the inactivated form cob(II)alamin, it is possible that this first region may not function optimally when there is an increased demand for methionine. Genetic polymorphisms may affect any or all of the four regions and mutations, which result in dysfunction of human MS. For example, Goulding et al. (1997) have described mutations of Cys310Ala and Cys311Ala in the third region of MS which completely abolish methyl transfer from exogenous methylcob(III)alamin to homocysteine (Goulding, C.W. et al., 1997) resulting in increased homocysteine levels. According to the site of the polymorphism, a specific pattern of one-carbon metabolism perturbation would be present.

As major depressive disorder is a hereditary disease (Gold, P.W. et al. 1988), it is plausible that a genetic polymorphism of MS resulting in dysfunction could be more
prevalent amongst the depressed group. While the prevalence of MS polymorphism in major depression has not been studied, polymorphism of the third region of MS, which is responsible for the binding and activation of homocysteine, has been found in a related disorder, bipolar affective disorder. Li et al. (1997) studied 93 Chinese subjects with bipolar affective disorder and 98 controls for the presence of an MS polymorphism, namely Val158Met allele on the third region, which is thought to express itself as low activity of MS (Li, T. et al., 1997). The frequency of the Met158 allele was significantly higher among subjects with bipolar depression as compared to controls (31% vs. 18%, p = 0.004). Furthermore, the frequency of the homozygous Met158 allele was also higher in bipolar subjects as compared with controls (9% vs. 3%, p = 0.01). This study suggests that adults with affective disorders may have a genetic defect in MS. Li’s study did not measure any blood levels such as homocysteine, methionine or folate, which would demonstrate how the polymorphism Val158Met is reflected in blood levels. Given the site of the polymorphism, one would expect elevated homocysteine levels in this group. This study raises the possibility that there is genetic polymorphism of MS in affective disorder. We did not examine MS DNA, nor has any other group examined all four regions. Furthermore, no study of MS polymorphism has included carefully diagnosed subjects with unipolar depression. Nonetheless, Li’s study provides some direct evidence for a genetic disturbance of a key regulatory enzyme of one-carbon metabolism in mood disordered patients.

Other indirect evidence for MS genetic polymorphisms manifesting in disease will be discussed in Section 7.2. The following section focuses on the second possible
disturbance in one-carbon metabolism in depression, that is methylation.

7.1.5 Evidence of increased utilization of SAM in depression

We hypothesized that the results of the current study would show that SAM levels and the SAM: SAH ratio were lower in depressed subjects as compared with controls. The data did not support this hypothesis. Several factors may influence SAM levels and these may have obscured the difference between depressed and controls. Specifically, age and gender have a powerful influence on SAM levels (Wise, C.K. et al. 1997). Indeed, in the current study SAM levels of males were significantly higher than those of females in this study ($t = 5.7, p < 0.001$) and there was a significant correlation between SAM levels and age ($r = 0.33, p = 0.016$). However, using MANCOVA to compare mean SAM levels in depressed versus controls with age and sex as co-variates, there was no change in the results. Similarly, using partial correlation for the relationship between SAM levels and HAM-D score (controlling for sex and age) there was no difference in the results. Hence, age and sex did not impact on findings regarding SAM levels and depression in this study.

Although SAM and SAH levels were not significantly different between the groups, they were in the anticipated direction of disturbance. Our inability to find difference may be the result of inadequate statistical power. However, the correlation between the HAM-D score and both SAH and glycine are supportive of increased methylation in depression. These findings suggest that there may be hypermethylation but only subtle depletion of SAM in the current depressed subjects. These data therefore provides preliminary
support for the hypothesis that depression is associated with hypermethylation.

In summary, results of the current study have provided evidence for more than one site of disturbance in one-carbon metabolism in depression, namely dysfunctional MS and possibly increased methylation. These are novel findings that have not previously been reported and in fact, some of these findings are at odds with previous research. The following section discusses where this study differs from earlier studies and possible reasons for these differences.

7.1.6 Inconsistencies between results of current and previous studies

This is the first study to propose a disturbance in MS in major depression. No previous study has examined all components of one-carbon metabolism simultaneously (see Table 7.1). The simultaneous measurement of the metabolites of the one-carbon cycle provides the possibility of identifying a pattern of functioning. In this fashion, levels of metabolites can be compared relative to their precursors, products and co-factors. The finding of low methionine levels in depressed subjects in the current study cannot be compared to previous studies as methionine levels have previously never been measured in adults suffering from major depressive disorder. Nor has there been a study comparing SAM levels in depressed versus healthy controls. Serum folate and red cell folate are the only one-carbon metabolites, to the best of our knowledge, that have been measured previously in depressed adults versus controls (Table 7.1). In many respects, our findings
are in stark contrast to most previous studies with respect to folate levels. Possible reasons for the difference between results of the current study and previous studies are outlined below.

7.1.6.1. Elevated folate levels

In the current study, folate status tended to be higher in depressed adults as compared with healthy controls. First, serum folate was significantly higher (31.3 ± 1.8 vs. 23.9 ± 1.8, p = 0.005) and second, red cell folate tended to be higher in the depressed group (727 ± 46 vs. 703 ± 36), but this difference was not significant. Serum folate and red cell folate concentrations have been measured in depressed patients for several decades. The results of the current study contradict most previous studies (Table 7.1). Ten out of thirteen previous studies have concluded that adults with depressive disorder have either high levels of folate deficiency, low folate levels as compared with controls, or that depressed patients with low folate status have longer duration of a depressive episode or are less likely to respond to treatment. Two studies found that folate status was normal in depressed adults. Only one study has found higher folate levels among depressed adults: a recent study by Lee et al. (1998) assessed folate status in 117 newly admitted inpatients with major depressive disorder in Hong Kong (Lee, S., Wing, Y.K., et al. 1998). In Lee and colleagues' study (1998), patients who had physical diseases, thalassaemia trait, were pregnant, alcoholic, on vitamin supplements, antibiotics or known folic acid antagonists were excluded. As compared to 72 healthy controls, patients had significantly higher red cell folate (801.8 ± 284.6 nmol/l vs. 699.5 ± 248.7 nmol/l). Few previous studies of folate levels in depressed subjects have been as fastidious with regards exclusion criteria. Table
7.1 summarizes the exclusion of the previous studies. It is clear that few studies have identified the important and specific factors that may impact on folate levels. The current study has attempted to be even more careful in its exclusion than the Lee and colleagues’ study. In the current study, non-institutionalized depressed adults were studied whereas in most previous studies, subjects were being treated either in an inpatient or outpatient setting. Also, the majority of previous studies included depressed subjects without controlling for factors that decrease folate status. In the current study, strict exclusion criteria ensured that no subjects had another major medical illness, were taking folate-antagonistic medication, were pregnant or had given birth in the previous three months, abused alcohol, or had been on antidepressant medication or psychotropics for a minimum of three months. Evidence for how these factors could affect folate will be discussed below. In general, however, it appears that the studies with few or no exclusion criteria, tended to find the highest prevalence of folate deficiency and low folate levels in depressed subjects.
Table 7.1: Comparison of screening methods, comparison groups, measurements of one-carbon metabolism in studies investigating folate in depression

<table>
<thead>
<tr>
<th>Study</th>
<th>Controls</th>
<th>Screening</th>
<th>Measurements</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carney, M.W.P., 1967</td>
<td>Healthy</td>
<td>-</td>
<td>SF, B12</td>
<td>↓ serum and red cell folate vs. controls</td>
</tr>
<tr>
<td>Callaghan, N. et al., 1969</td>
<td>Healthy</td>
<td>Psychotic epileptics &amp; non-psychotic</td>
<td>SF, B12</td>
<td>Serum folate normal in depressed subjects</td>
</tr>
<tr>
<td>Kallström, B. et al., 1969</td>
<td>N/A</td>
<td>-</td>
<td>SF, B12</td>
<td>2/3 had megaloblastic anaemia; 21% folate deficiency</td>
</tr>
<tr>
<td>Reynolds, E.H. et al., 1970</td>
<td>N/A</td>
<td>M</td>
<td>SF, RCF, B12</td>
<td>24% folate deficient; low serum folate levels associated with more severe cases</td>
</tr>
<tr>
<td>Carney, M.W.P. et al., 1978</td>
<td>N/A</td>
<td>P, AA</td>
<td>SF, RCF, B12</td>
<td>28% serum folate deficiency; all patients most severe cases</td>
</tr>
<tr>
<td>Thornton, W. E. et al., 1978</td>
<td>Healthy</td>
<td>AD, L, M</td>
<td>SF, B12</td>
<td>Serum folate of all psychiatric patients lower than controls</td>
</tr>
<tr>
<td>Ghadirian, A.M. et al., 1980</td>
<td>Other, medical patients</td>
<td>AD, L, I, M, P</td>
<td>SF</td>
<td>Subjects assessed one week after hospitalization. ↑ serum folate deficiency – low folate status associated with more severe depression</td>
</tr>
<tr>
<td>Gray, G.E. et al., 1986</td>
<td>Psychiatric patients</td>
<td>-</td>
<td>SF</td>
<td>No significant difference in folate status</td>
</tr>
<tr>
<td>Levitt, A.J. et al., 1989</td>
<td>N/A</td>
<td>AD, L, I, M, AA</td>
<td>SF, B12</td>
<td>Folate inversely correlated with duration of episode; no correlation with severity</td>
</tr>
<tr>
<td>Abou-Saleh, M. et al., 1989</td>
<td>Healthy, detoxified alcoholics, lithium treated</td>
<td>AD, L, M, AA</td>
<td>SF, RCF</td>
<td>↓ serum and red cell folate vs. controls; low folate status associated with more severe depression</td>
</tr>
<tr>
<td>Carney, M.W.P. et al., 1990</td>
<td>Euthymic, manic, schizophrenic, alcoholic</td>
<td>AA</td>
<td>RCF, B12</td>
<td>↓ red cell folate vs. controls</td>
</tr>
<tr>
<td>Levitt, Wesson, et al. 1994</td>
<td>N/A</td>
<td>AD, L, AA, I, M</td>
<td>SF, RCF, B12</td>
<td>1.4% folate-deficient; RCF not correlated with HAM-D; non-respondents to treatment had lower RCF</td>
</tr>
<tr>
<td>Bell, Potkin S.G., et al. 1994</td>
<td>N/A</td>
<td>I, AA</td>
<td>SAM</td>
<td>SAM levels significantly positively correlated with clinical improvement</td>
</tr>
<tr>
<td>Lee, Wing, et al. 1998</td>
<td>Healthy</td>
<td>P, AA, I, M, V</td>
<td>SF, RCF</td>
<td>↑ red cell folate vs. control folate levels not correlated with HAM-D</td>
</tr>
<tr>
<td>Current study, 1999</td>
<td>Healthy</td>
<td>I, V, AD, AA, L, M, P, RB</td>
<td>SF, RCF, B12, MET, HCY, SAM, SAH, SER, GLY</td>
<td>↑ serum folate and methionine vs. control; red cell folate, SAH, B12, MAT activity positively correlated with HAM-D; methionine, glycine, MS activity negatively correlated with HAM-D</td>
</tr>
</tbody>
</table>

Legend:
- I = major medical illness
- V = vitamin supplements (for >3 months)
- AD = antidepressants (for >3 months)
- AA = alcohol abuse
- L = psychotropics (e.g. lithium; for >3 months)
- M = medication affecting folate status
- P = pregnancy
- RB = recent birth
- RCF = red cell folate
- SF = serum folate
- B12 = vitamin B12
- MET = methionine
- HCY = homocysteine
- SAM = S-adenosylmethionine
- SAH = S-adenosylhomocysteine
- SER = serine
- GLY = glycine

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Inpatient status

The current study population was a group of non-institutionalized depressed adults. Most other studies were inpatients or new admissions. Inpatient status may affect folate status. Unlike non-institutionalized, depressed adults, inpatients represent more severe cases of major depressive disorder. From one perspective, inpatient status may increase folate levels as compared to free-living depressed adults as nutritionally adequate meals are prepared and served to inpatients, whereas free-living depressed adults may not have the appetite, motivation, energy or economic means to prepare nutritious meals. Also, inpatients may receive dietetic counselling as part of treatment. However, hospital food is often unfamiliar and unpalatable and may not be eaten by a depressed patient with a decreased appetite. Also, many studies measured folate status in new admissions. Depressive symptoms such as social withdrawal, loss of appetite, fatigue and decreased interest could reduce the purchase as well as preparation of and intake of folate-rich foods in severely depressed adults, especially in the period immediately preceding hospitalization. Furthermore, some studies reporting low folate status in depressed inpatients clumped depressed patients together with inpatients with other psychiatric disorders (Carney, M.W.P. and Sheffield, B.P., 1978; Carney, M.W.P., 1967). There is evidence that other psychiatric illnesses are associated with low folate status (Young, S.N. and Ghadirian, 1989; Hallström, T., 1969). Therefore, the study of depressed inpatients may be complicated by factors intrinsic to inpatient status, rather than depression itself.
7.1.6.1.2 **Major medical illnesses and folate-antagonist medications**

Few studies controlled for other major medical illnesses or known folate-antagonist medications. This study excluded potential subjects with major medical illnesses such as inflammatory bowel diseases (IBD) and diabetes mellitus. Folate deficiency is prevalent amongst patients with **inflammatory bowel disease** (IBD) (Cattaneo, M., et al. 1998; Cravo, M.L. et al. 1998). Approximately 15-25% of patients with Crohn’s have impaired folate absorption leading to a high prevalence of folate deficiency in this patient population (Cattaneo, M. et al. 1998; Steger, G.G. et al. 1994). Furthermore, hyperhomocysteinaemia has been reported in adults with IBD, indicating that one-carbon metabolism is affected. Cattaneo et al. (1998) found that IBD patients had significantly higher homocysteine levels than controls (12.2 ± 7.7 vs. 10.5 ± 4.6 μmol/L, p = 0.045).

There are no studies that have examined the prevalence of inflammatory bowel disease in depression, but there is extensive literature that demonstrates the depression is very common in IBD (Addolorato, G. et al. 1997; Porcelli, P. et al. 1996; Walker, E.A. et al. 1996). Another disease that may affect folate status is **diabetes mellitus** (DM). While the absorption of folate does not seem to be affected by DM (Botez, M.I. and Bachevalier, J., 1981), a drug commonly used for glucose control in NIDDM, metformin, reduces serum vitamin B12 and folate levels and increases homocysteine levels (Carlsen, S.M. et al., 1997; Aarsand, A.K. and Carlsen, S.M., 1998). In addition to the effects of metformin on one-carbon metabolites, DM itself may be associated with altered folate levels. Reddi and colleagues (1993) studied the distribution of water-soluble vitamins in tissues of
diabetic rats. The study found that diabetes significantly lowered folate in kidney, heart, brain and muscle. Although there have been no studies to date specifically evaluating the prevalence of diabetes among subjects with depression, depression is very common in diabetes (Garduno-Espinosa, J. et al. 1998; Peyrot, M. and Rubin, R.R., 1997). The prevalence of depressive symptoms amongst adults with diabetes has been reported as high as 41.3% (95% CI: 37.4-45.2%). For comparison the prevalence in the general population 10-20% (Peyrot, M. and Rubin, R.R., 1997). The number of diabetic complications increases the likelihood of depressive symptoms (Peyrot, M. and Rubin, R.R., 1997). It is therefore possible that if previous studies failed to carefully exclude subjects with medical illnesses such as diabetes or IBD, the study may have included a larger number of these subjects in the depressed group, and the inclusion of these subjects may have lowered the mean folate levels for the group.

Folate status is also affected by antifolate medication taken for the treatment of epilepsy. Folate levels in adults taking anti-convulsants are frequently lower than normal (Botez, M.I. and Young, S.N., 1991; Kutt, H. and Solomon, G.E., 1980; Froscher, W. et al., 1995) as antiepileptics interfere with folate metabolism. Also, many chemotherapeutic agents used for the treatment of cancer are antifolate drugs and act by mimicking natural folates to inhibit critical cellular biosynthetic pathways (Moscow, J.A., 1998). The use of steroids and thyroid medications (Levitt, A.J. and Joffe, R.T., 1993; Levitt, A.J. et al. 1998) also interfere with folate metabolism. Potential subjects with IBD, DM, cancer or taking anti-convulsants, steroids or varying doses of thyroid hormone were excluded from the current study. While a few studies have excluded subjects on some of the above-
mentioned medication, no study has included such an exhaustive list of diseases and medication that can interfere with folate metabolism.

7.1.6.1.3 Pregnancy or recent birth

Few studies reported exclusion of women who were pregnant or had recently given birth. Pregnant women are prone to becoming folate deficient because there is a significant increase in folate requirement during pregnancy and folate intakes of pregnant women are often insufficient (Pietrzik, K.F. and Thorand, B., 1997). Pregnancy-induced sub-normal folate levels may take a number of months postpartum to normalize. Furthermore, new mothers have a higher prevalence of depressive symptoms with 10-28% of women reporting postpartum depression after childbirth (Berggren-Clive, K., 1998; Bergant, A.M. et al. 1999). It is therefore also possible that previous studies included a disproportionate number of post-partum females in the depressed as compared with the control group. All pregnant women and women who had given birth in the past three months were excluded from the current study.

7.1.6.1.4 Alcohol abuse

Substance abuse was not controlled in many of the previous studies. Alcohol abuse is known to affect folate status due to the displacement of energy sources by alcohol (Hillers, V.N. and Massey, L.K., 1985) and due to the toxic effects of alcohol. In a study of 33 chronic alcoholics with no clinical or laboratory evidence of liver disease, 60.6% of subjects had low red cell folate values, in spite of adequate dietary folate intake (Gloria, L. et al. 1997). Animal studies suggest diverse effects of ethanol on intestinal absorption
(Fernandez-Borrachero, O. et al. 1996), hepatic metabolism and urinary excretion of folate (Collins, T.D. et al., 1992). In a study focused on the effect of ethanol on one-carbon metabolism, serum homocysteine, red blood cell folate, vitamin B12 and vitamin B6 concentrations of 32 chronic alcoholics were compared to 31 healthy controls (Cravo, M. et al. 1996). In chronic alcoholics, serum B6 and red blood cell folate concentration were significantly lower and homocysteine concentrations were twice as high as compared to control subjects. These results suggest that by interfering with folate or vitamin B6 metabolism, chronic alcoholic intake may impair the disposal of homocysteine through one-carbon metabolism. While the relationship between alcohol abuse and depression is a complex one, the comorbidity of these disorders has been well-described (Sexton, H. et al. 1999; Petty, F., 1992; Merikangas, K.R. and Gelernter, C.S., 1990). It is unclear whether it is more common for depression or alcohol dependence to be the primary disorder (Gorwood, P., 1999). In depressed adults, alcohol is sometimes used as self-medication for the treatment of depression (Abraham, H.D. and Fava, M., 1999). Also, alcoholism is often associated with negative affect (Johnson, P.B. and Gurin, G., 1994). It is thought that alcoholism is frequently undiagnosed amongst psychiatric patients (Woodward, B. et al., 1991). Thus, not controlling for alcohol abuse in previous studies may have led to the inclusion of more alcohol abusers in the depressed groups. This may also have contributed to an increased chance of finding low folate in the depressed group.

7.1.6.1.5 Vitamin supplementation

Few studies have controlled for vitamin supplementation. Carney (1967) excluded
inpatients that were prescribed vitamins. Gharidian et al. (1980) excluded any respondents who had taken multivitamins, folate, or B-vitamins in the previous three months (Ghadirian, A.M. et al. 1980). Lee et al. (1998) excluded those taking vitamin supplements at the time of admission. Subjects taking multivitamins, B-vitamins or folate supplements in the past three months were excluded from the present study. While there is no evidence regarding the metabolic effects of vitamin supplementation in depression, oral ingestion of vitamins may have an unpredictable effect on components of OCM, and the failure to exclude people taking supplements makes meaningful comparison among studies more difficult.

7.1.6.1.6 Antidepressant medication and psychotropics

Finally, very few studies have excluded depressed subjects taking antidepressants or psychotropics such as lithium. While little is known on the effect of antidepressant medication on the absorption, excretion and metabolism of folate, studies have shown that folate levels increase with successful antidepressant therapy (Coppen, A. and Abouh-Saleh, M.T., 1982; Levitt, A.J. et al. 1994; Levitt, A.J. and Joffe, R.T, 1989) and decline with unsuccessful treatment (Levitt, A.J. et al. 1994). There is some evidence that lithium treatment reduces folate levels in depressed patients. Coppen and colleagues (1982) studied 81 unipolar depressed patients attending a lithium clinic and found that patients on long-term lithium treatment had a significantly lower folate status as compared to healthy controls (Coppen, A. and Abouh-Saleh, M.T. 1982). Another recent study showed that serum lithium concentrations were significantly negatively correlated with red blood cell levels in a group of depressed patients on lithium treatment (r =-0.314,
p = 0.032) (Cervantes, P. et al. 1999). It seems reasonable to assume that many depressed subjects who participated in earlier studies, were being treated, or had recently been treated, for depression (especially inpatient populations). As many previous studies did not control for antidepressant and psychotropic (e.g. lithium) usage, folate levels may have been confounded and perhaps lowered by antidepressant treatments.

With the exception of Lee’s study, previous studies have found that depressed subjects have lower or similar folate levels to controls. It is possible that the discrepancy in results between previous studies and the current study is due to the lack of control of several crucial factors that influence folate status, generally lowering folate levels. These factors include inpatient versus outpatient status, major medical illnesses and folate-antagonist medication, pregnancy or recent birth, alcohol abuse, vitamin supplementation and antidepressant medication and psychotropics such as lithium.

7.1.6.2. Homocysteine levels

In the current study, there was no significant difference in homocysteine levels between depressed subjects and controls. If there is a possibility of MS dysfunction in major depression, it is reasonable to expect homocysteine levels may be elevated. Furthermore, other compounds required for methionine synthesis, namely folate and vitamin B12, were elevated in the current study. The fact that homocysteine levels are not elevated in the face of reduced MS activity is likely not explained by a compensatory underproduction of homocysteine. In fact, the accumulation of its precursor, SAH, suggests that there would
more likely be an accumulation or increased production of homocysteine. SAH levels are significantly, positively correlated with HAM-D scores ($p = 0.006$) which might lead to the expectation that homocysteine levels might also rise with increasing severity of depression. The distinction between SAH and homocysteine, however, is that SAH can only be hydroxylated to homocysteine, whereas homocysteine has several fates. In order to understand the finding of normal homocysteine levels in the presence of increased SAH, the metabolism of homocysteine must be understood.

Homocysteine is a metabolite at a critical branch point in one-carbon metabolism. Hyperhomocysteinaemia can result from defects in either MTHFR (insufficient 5-MTHF is produced) (Kluijtmans, L.A.J. et al. 1996; Verhoef, P. et al. 1998), MS (homocysteine can not be converted to methionine) (Fowler, B., 1997; Gulati, S. et al. 1996) or cystathionine β-synthase (homocysteine is not degraded) (Steegers-Theunissen, R.P.M. et al. 1994). However, in conditions associated with defects in MS or MTHFR, homocysteine may be shunted away from the one-carbon metabolism cycle via increased activity of cystathionine β-synthase (CBS). For example, it has been found that if MTHFR is inhibited, excess homocysteine is degraded by conversion to cystathionine (Fowler, B., 1997). Cystathionine is then converted to α-ketobutyrate, ammonia and cysteine (Matthews, R.G. et al., 1998), hence degrading homocysteine. It is possible that, in the current study, homocysteine was shunted to cystathionine, resulting in normal homocysteine levels. There is no direct evidence from this study, that homocysteine shunting did occur, as the by-products of cystathionine β-synthase action were not
Normal homocysteine levels in depression stand in contrast to the elevated homocysteine levels seen in other disease states associated with MS disturbance, such as neural tube defects (NTDs) and cardiovascular disease. (Van der Put, N. M. J. et al., 1997; Fowler, B., 1997; Mills, J. L. et al., 1995). The difference between the normal homocysteine levels in major depression and the elevated levels in NTDs and cardiovascular disease may possibly be explained by difference in functioning of the enzyme responsible for the degradation of homocysteine, that is, an inability of the one-carbon cycle to get rid of excess homocysteine via CBS. While we have indirect evidence that the production of homocysteine may have increased (SAH levels correlated with severity of depression), no conclusions about the degradation of homocysteine can be made as neither CBS function nor its by-products were measured in the current study.

7.1.6.3.  Elevated MAT activity

In the current study, calculated MAT activity was significantly higher in depressed subjects as compared to controls. The increased MAT activity in the depressed subjects in the current study may indicate an accelerated production of SAM, supporting our hypothesis of increased methylation in depression in spite of normal SAM levels. The finding of increased MAT activity in depression in the current study, however, contradicts previous studies on MAT activity in depression.
Alarcon and colleagues (1985) examined MAT activity by kinetic methods in depressed, manic and schizophrenic patients (Alarcon, R.D. et al., 1985). Results revealed that depressed patients showed significant lowering of Vmax (enzyme activity) as compared to healthy controls. The same group of investigators (Tolbert, L.C. et al. 1990) reported another case of decreased MAT activity during a depressive episode of a woman with bipolar affective disorder. MAT activity increased to normal levels upon recovery and was elevated in a manic phase. While both of Alarcon and colleagues’ studies indicate decreased MAT activity, it is possible that MAT was decreased due to treatment, rather than depression. The subjects in Alarcon’s group were being treated with antidepressant medication or electroconvulsive therapy, both of which could interfere with enzyme activity. It is difficult to compare MAT activity from previous studies and the calculated MAT activity from the current study, since the current sample had more exclusion criteria for factors that could confound enzyme activity, and MAT activity was not measured directly in the current study.

In summary, the results of the current study suggest a possible disturbance of MS activity as indicated by significantly higher methionine levels and significantly lower folate levels and calculated MS activity in depressed subjects as compared with controls. Furthermore, one-carbon metabolism disturbance may worsen with increasing severity as indicated by the significant, inverse correlation of calculated MS activity, methionine and glycine with HAM-D scores and the significant, positive correlation of SAH, 5-MTHF and vitamin B12 with HAM-D scores. While some of the results of the current study
contradict the findings of previous studies, this discrepancy could be explained in part by our strict exclusion of factors that may influence folate status.

7.2 MTHFR polymorphism

While MS polymorphism was not measured in the current study, another enzyme which is essential for the functioning of the one-carbon cycle, MTHFR, was assessed. MTHFR catalyzes the formation of the enzyme that converts 5,10 methylene tetrahydrofolate to 5-MTHF. In the current study, there were significantly more MTHFR C677T heterozygous depressed subjects than control subjects (53.8% vs. 28.0%, \( p = 0.019 \)), indicating a higher prevalence of genetic mutation amongst depressed adults. The high rate of heterozygosity of MTHFR in depressed subjects may explain some of the changes in methionine and folate reported above.

Results of a recent Japanese study suggests that MTHFR might be affected in depression. In 1997, a study of Japanese depressed patients found that the odds ratio of having the C677T mutation was 2.8 as compared with healthy controls (\( p<0.005 \)) (Arinami, T. et al., 1997), indicating increased prevalence of MTHFR mutations in major depressive disorder. In the current sample, the odds ratio for the presence of heterozygosity (compared to no polymorphism and homozygosity) in subjects with major depression was 3.2 as compared to controls. The odds ratio of being heterozygous for MTHFR polymorphism (compared to controls only) in depressed subjects was 2.6, which is similar to Arinami et al.'s study. These two studies both support an increased risk of
genetic polymorphism of the C677T mutation in major depression.

Of note, when the subjects are divided into those with the polymorphism (hetero or homozygous) and those without, different patterns of correlations between one-carbon metabolites and severity of depression emerge. In those without polymorphism, there are few differences to the correlations described in the whole group: that is glycine, methionine and MS activity are negatively correlated and SAH is positively correlated with severity. However, there is no significant correlation between red cell folate or vitamin B12 and severity in this group. In contrast, in the polymorphic group, the only significant findings were that red cell folate and vitamin B12 are significantly correlated with severity. This suggests that a defect in MTHFR leads to accumulation in MTHF and vitamin B12 with increasing severity, and that subjects without the defect display more evidence of hypermethylation and disturbed MS activity. These conclusions should be considered preliminary since the number of subjects was small. However, it is possible that while the whole group of depressed subjects appear to have a disturbance in MS, in reality, there may be two distinct groups: one with a disturbance of MTHFR and another group with hypermethylation. In the group of depressed subjects with a disturbance of MTHFR, increasing severity of depression may lead to increasing amounts of MTHF. In the group of depressed subjects with hypermethylation, increasing severity of depression may lead to depletion of methionine and accumulation of SAH. More extensive study is required to test this preliminary hypothesis. Of note, one homozygous subject with depression had baseline red cell folate levels substantially higher than normal levels. Furthermore, the subject reported that dietary folate supplementation led to nausea and
vomiting. When this outlier’s blood values were excluded from the analyses, results did not change.

It should be noted that the difference between the findings of the current study and previous work could be a result of an unusual group of subjects with a high proportion of polymorphism of MTHFR. As there are no other studies to date that have measured both one-carbon metabolites and MTHFR polymorphism in depression, this hypothesis cannot be confirmed.

The effects of the enzyme polymorphism/s may not be evident at all times. However, at times of stress on the one-carbon cycle such as increased requirement for production of metabolites due to a heightened need for neurotransmitters for mood regulation, the disturbed function of the enzyme may be a “weak link” resulting in insufficient neurotransmitter synthesis and function and hence in a major depressive episode. Therefore, it may be possible to have increased genetic polymorphism in depressed individuals (that is, a trait-phenomenon), but for these individuals to show a pattern of one-carbon metabolism disturbance only in the state of depression. This hypothesis has never been tested. A longitudinal study measuring one-carbon metabolites (including various forms of folate) during depressive and euthymic periods in a large sample of adults with major depression would explore this proposal.

The results of the current study suggest a disturbance in enzyme function in one-carbon metabolism. However, the results of the current study make it impossible to distinguish...
between MTHFR and MS as the cause of one-carbon metabolism disturbance. In the current study MS was assessed using a calculated ratio of product: substrate. MTHFR was assessed by measuring genetic polymorphism, but product: substrate ratios could not be calculated. In order to comprehend the limitations with regard to interpretation of our results it is necessary to understand the formation of different forms of folate.

5-MTHF enters the red blood cell and is converted via MS to THF. Thereafter five to seven glutamate residues are added to the monoglutamate form. The polyglutamate form is thus trapped within the cell (Groff, G.L. et al., 1995). The polyglutamate form of folate is the preferred substrate of both MTHFR and MS (Lucock, M.D., 1999). If there is a disturbance of MTHFR, the polyglutamate forms of folate, 5,10 methylene THF and THF would accumulate. The accumulation of the MTHFR substrates may drive the cycle in the opposite direction, causing a decrease in methionine production and an accumulation of 5 MTHF. In the current study, red cell folate (polyglutamate form of folate) was significantly, positively correlated with HAM-D scores in depressed subjects and serum folate (monoglutamate form) was significantly higher in the depressed group. If MS was dysfunctional (as suggested above), 5-MTHF would accumulate and methionine concentrations would be lower than in subjects with normal MS function; that is, the levels would be virtually indistinguishable from those that result from MTHFR dysfunction. Hence, it is possible that either enzyme, MS or MTHFR, is disturbed in depression. The primary difference in the two scenarios is that THF would be higher in MTHFR disturbance due to a backlog, whereas its levels of THF would be lower in MS deficiency due to a lack of production. Unfortunately we did not measure THF levels in
the current study. It is therefore not possible to make any definite conclusions regarding the root of one-carbon metabolism disturbance in depression.

7.3 Diet

The current study was the first to attempt to undertake a thorough assessment of dietary intake in depressed adults. The intake of total energy, macronutrients (carbohydrate, fat, protein) and one-carbon related vitamins and amino acids (folate, vitamin B12, vitamin B6, methionine) of this group of non-institutionalized depressed adults did not differ from healthy controls. The percentage of subjects with low, moderate and high dietary folate intake (per unit BMI) did not differ significantly between depressed subjects and controls. The only previous study that at least attempts to evaluate in a limited fashion folate intake examined newly admitted Chinese depressed patients (n = 117). The investigators, in a somewhat gross measure, assessed folate intake compared to controls (n = 72) by asking patients whether they had intake of green vegetables for 5 days or more in a week (Lee, S. et al. 1998). They found that significantly fewer patients (n = 66.92%) than control subjects (n = 80.68%) had high intake of green vegetables. However, in the depressed group there was no significant difference in serum or red cell folate levels between high folate consumers (greens > 5 days per week) and low folate consumers (greens < 5 days per week). Hence, even though Lee found a higher dietary intake of folate-rich foods in control subjects, folate intake did not influence blood folate levels.

The calorie and macronutrient intake (total, per kcal, per BMI or as a percentage of total
energy intake) did not differ significantly between groups in the current study. Analysis of the intake of micronutrients that could influence one-carbon metabolism, namely folate, vitamin B12, vitamin B6, methionine (total, per unit BMI or per kcal), did not reveal significant differences between groups. There were no significant correlations between the dietary intake any of the micronutrients analyzed and severity of depression.

In order to assess whether dietary intake of compounds related to one-carbon metabolites has any influence on one-carbon metabolites, the correlation between blood levels and dietary intake of these compounds were calculated. A correlation matrix was calculated using Pearson correlation coefficients and was used to assess the relationship between dietary intake and blood values of compounds involved in one-carbon metabolism (methionine, homocysteine, red cell folate, serum folate, vitamin B12, serine, glycine, SAM and SAH). Using all 52 subjects (both depressed and control groups) a significant, positive correlation was found between plasma methionine levels and dietary intake of methionine, folate and vitamin B12. The correlations did not all remain significant once the groups were split into depressed and controls. In the control group, folate (per unit BMI and per kcal) was significantly, positively correlated with plasma methionine levels (p = 0.021, p = 0.022 respectively). Thus, the higher the dietary intake of folate in control subjects, the higher the level of methionine, a product of folate metabolism. In contrast, the main dietary determinant of plasma methionine levels in depressed subjects was vitamin B12, the coenzyme required for the functioning of MS. In depressed subjects, dietary intake of vitamin B12 (per unit BMI and per kcal) was significantly, positively correlated with plasma methionine levels (p = 0.012, p = 0.050 respectively).
This finding suggests that in depressed subjects, whose MS may not function optimally, sufficient dietary vitamin B12, the co-enzyme of MS, is a vital nutrient required for the production of methionine. In contrast, in control subjects, in whom MS is probably functioning adequately, the dietary intake of folate, the precursor of methionine, is the main determinant of methionine levels. Hence, although all subjects had adequate intake of all one-carbon metabolites, there were differential effects of dietary components on the plasma levels of methionine in the depressed as compared with control subjects.

While this is the first study that made a serious attempt to capture the dietary intake of a sample of depressed adults, the sample size of this study was not large enough to make generalizations to the entire depressed population. Furthermore, the FFQ has inherent limitations such as presenting respondents with a limited set of food and beverage items (although the FFQ used in this study did attempt to include an exhaustive list of folate-rich foods). While the correlations between dietary intake of one-carbon metabolites and blood concentrations of those metabolites were not large, the use of this kind of model may be helpful in selecting nutrients that might impact on one-carbon metabolism in different populations.

7.4 Folate absorption

The OFAT is a potentially valuable tool in evaluating absorption of folate. This preliminary investigation employed the OFAT in 4 depressed subjects and 2 controls. After the intramuscular injection of folate, all subjects had normal serum folate levels.
The mean peak serum value of all subjects was more than 8 times normal peak values as described by Steger and colleagues (Steger, G.G. et al., 1994). There was no consistent pattern in the serum folate curves (Figure 6.15) following consumption of folate per unit BMI in depressed or control subjects. Most serum folate levels had not peaked within the 2-hour post-ingestion period. While it has been hypothesized that folate levels in depression may be linked to malabsorption (Abouh-Saleh, M. T. et al., 1986; Botez, M. I. et al., 1984), it has never been measured. This preliminary study provided no indication of malabsorption of folate intake. Dietary intake of nutrients related to one-carbon metabolites were similar between depressed and control subjects. There were some significant correlations between blood levels and dietary intake of compounds related to one-carbon metabolism (see Dietary Intake, Section 7.3). Based on the preliminary oral folate absorption test results in the current study, it is unlikely that the correlations between blood levels and dietary intake are related to malabsorption in depressed subjects. However, the sample used to test folate absorption was very small, and in fact, the purpose of including this part of the study was to test the feasibility of the OFAT in depressed subjects. In the future, it may be useful to decrease the dose of intramuscular and oral folate given as well as extend the time after ingestion of the oral folate dose in a larger sample of subjects to more accurately assess oral folate absorption.
CHAPTER 8: CONCLUSIONS
8.1. Disturbance of one-carbon metabolism is evident in depressed subjects as compared to controls. Depressed adults may suffer from MS deficiency or dysfunction as evidenced by decreased methionine levels and increased folate levels. Also, MS activity as calculated by methionine: folate ratio was significantly lower in depressed subjects as compared to controls.

8.2. SAM levels and SAM: SAH ratio did not differ between groups. There was, however, indirect evidence of possible increased utilization of SAM in the depressed group, that is, HAM-D scores were significantly, positively correlated with SAH levels and significantly, negatively correlated with glycine levels.

8.3. There was significantly more heterozygosity of MTHFR C677T polymorphisms in the depressed group as compared to controls. This indicates that dysfunctional MTHFR may lead to some or all of the disturbances seen in one-carbon metabolism in depression.

8.4. Dietary intake did not differ between groups. However, the major predictor for plasma methionine levels in the control group was folate. In the depressed group, the major predictor of levels of plasma methionine was vitamin B12. The specific relationships between dietary intake of nutrients and plasma levels differ across subject populations.
8.5. The OFAT may be a reasonable tool to measure folate absorption in depression, but modifications to the current technique are required.
CHAPTER 9: LIMITATIONS AND FUTURE DIRECTIONS
9.1. Peripheral measures of biochemical compounds, including folate (Reynolds, E. H. et al., 1972), are correlated with CSF measurements. When analyzing the impact of certain compound concentrations on neurotransmission, it would be ideal to measure CSF levels. However, while taking blood is a simple procedure, taking CSF is more painful for the subject and more costly. Therefore blood samples were used in this study. Blood levels are nonetheless correlated with CSF levels. Future studies might include CSF levels to determine the central relationship between one carbon metabolism and mood.

9.2. We hoped to sample our subjects using random selection. As there were insufficient depressed respondents, advertisement was necessary. Future studies might increase the sampling pool or include more financial incentive for respondents in order to increase random recruitment.

9.3. Our sample size was not large enough to provide adequate power for some analysis of dietary intake and enzyme polymorphism. Nonetheless, the current study did yield interesting results for further investigation of these factors.

9.4. Due to financial limitations and the availability of analysis techniques, we were unable to analyse the various forms of folate. Also, only MTHFR
polymorphisms, rather than all the enzyme polymorphisms, were analysed. We were unable to measure enzyme function directly. The results of the current study suggest that future investigations need specifically to focus on direct measurement of enzyme function and activity.

9.5. This study was a cross-sectional study comparing depressed and control subjects. Longitudinal studies measuring one-carbon metabolites during depression and remission in the same individual would demonstrate any changes in metabolites in the state of depression.

9.6. The MANOVA included variables that are intercorrelated. However, the results from our tests for violation indicated that there was no important violations.

9.7. Very strict inclusion and exclusion criteria were used to select the subjects. The results of our study may, therefore, not be generalizable to the rest of the population. However, the selection was essential to ensure that changes in one carbon metabolism observed were specifically due to depression and not external factors. The mechanisms for changes in one carbon metabolism may be generalizable, although the presence of confounding factors needs to be taken into account. The importance of the inclusion and exclusion criteria is that they will allow for more accurate comparisons across study populations.
CHAPTER 10: REFERENCES


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