I, Charles D Duda, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
Dietary and Biochemical Markers of Folate in the Consideration of Depression

Student's name: Charles D Duda

This work and its defense approved by:

Committee chair: Graciela Falciglia, PhD

Committee member: Seung-Yeon Lee, PhD

2601
Dietary and Biochemical Markers of Folate in the
Consideration of Depression

A Thesis submitted to the
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by

Charles David Duda

Bachelor of Arts, John Carroll University, 2004

Committee Chair: Graciela Falciglia, PhD

Committee Member: Seung-Yeon Lee, PhD
Abstract

Depression is a leading cause of disability worldwide. Of the nutrients considered to have a role in the development and treatment of depression, folate is one of the most widely investigated. Twenty eight studies (eight case-control, 14 cross sectional, and six cohort studies) involving human subjects examining the link between folate and depression were reviewed. The body of data illustrates that low folate level, whether found through blood chemistry values or dietary analysis is associated with depression. The majority of the evidence comes from case-control and cross sectional studies. These studies have been very useful in establishing a relationship between folate and depression, it is now necessary to move beyond that point. Future research should be focused on the root cause of folate deficiency, once this is established it is necessary to examine possible treatment methodologies. Prospective studies and randomized-controlled trials examining therapeutic benefit of folate will be necessary to confirm or disprove a causal relationship between folate and depression.
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Depression is a large cause of disability and affects approximately 98.7 million people worldwide (World Health Organization, 2008). The illness is the leading worldwide cause of years of life lost due to disability and is one of the three leading causes of burden of disease (World Health Organization, 2008, 2009). Fifteen percent of populations from high income countries and 11 percent of populations from low and middle income countries are likely to get depression in their lifetime with 5.5 percent having had depression in the past year (Bromet et al., 2011). In the United States 12 month and lifetime prevalence rates of depression are approximately 12 percent for men and 24 percent for women (Kessler et al., 1994).

**Definition of Depression**

The Diagnostic and Statistical Manual of Mental Disorders (First, 2000) defines a major depressive episode as lasting at least two weeks during which there is depressed mood or the loss of interest or pleasure in nearly all activities. In children and adolescents the mood may be irritable rather than sad. The individual must also experience four or more additional symptoms including changes in appetite or weight, sleep, and psychomotor activity; decreased energy; feelings of worthlessness or guilt; difficulty thinking, concentrating, or making decisions; or recurrent thoughts of death or suicidal ideation, plans, or attempts. The symptoms must either be newly present or must have worsened compared with the person's pre-episode status. Symptoms must persist for the majority of the day, nearly every day, for at least 2 consecutive weeks. There must also be clinically significant distress or impairment in social, occupational, or other important areas of functioning with the episode. Some individuals experiencing milder episodes
may appear to be functionally normally but require markedly increased effort to do so (First, 2000).

**Treatment of Depression**

Current treatment regimes for depression include pharmacological and psychotherapeutic approaches which have been fairly effective (Imel, Malterer, McKay, & Wampold, 2008). Since the introduction of anti-depressive medication in the 1950s and subsequent development of novel pharmacologic therapies, there has been little change in the efficacy of medications used to treat depression (Murlow, & Williams 2000, Seitz, Gill, & Conn, 2010). Methods to increase efficacy in the treatment of depression and to build a mechanistic understanding of the disease have been explored for many years, with nutritional investigations being a focus of a large body of the research. Of the nutrients considered to have a role in the development and treatment of depression, folate is one of the most widely investigated. Low folate status has been linked to depression, malnutrition, physical illness (Carney & Sheffield, 1978). Folate deficiency is found in about one-third of depressed individuals, and there is some questions as to if folate values considered in the normal range are effective for the purpose of methyl donation and neurotransmitter synthesis, processes which are disrupted in depression (Miller 2008). Deficiency of folate can lead to serious health complications such as megaloblastic anemia, leukopenia, and thrombocytopenia. Folate is also important in the nervous system at all ages, but especially in the elderly where low folate level contributes to accelerated detrimental processes of the aging brain, increasing the risk of Alzheimer’s disease and vascular dementia (Reynolds, 2002).
**Dietary Allowance and Assessment of Folate**

Folate is a water soluble vitamin occurring naturally in foods such as green leafy vegetables, yeast, organ meats such as liver, and egg yolks. Folate occurs naturally in these foods, whereas folic acid is considered a synthetic form of the vitamin and is found in fortified foods such as breads, pasta, rice, and other cereals. The recommended dietary allowance of folate is 400 micrograms a day from all sources in the diet including folate found naturally in food, food fortified with folate, and folate from supplementation; the upper limit of folate intake is 1000 micrograms a day (Institute of Medicine, 1998). There are two main methods used to assess folate in the human body: folate level in the plasma by a folic acid test and red blood cell folate level. Folic acid level determines the circulating folic acid in the blood at any given time. This measurement is an indicator of circulating folic acid levels only, having no association with the body’s reserve of folate and can be confounded by a meal high in folate. The normal range of circulating folic acid varies slightly depending on the source and lab performing the analysis, but is generally thought to be between 3-13 nanograms per milliliter (ng/mL). Red blood cell folate level is an assessment of the overall folate store in the body. The normal values vary from lab to lab but are thought to be between 140-628 ng/mL.

**Metabolism of Folate**

Folic acid is not biologically active, requiring conversion to tetrahydrofolate by dihydrofolate reductase, a process which may be slow in humans, making the effects of high intake of folic acid limited (Bailey, & Ayling, 2009). Folic acid describes a group of compounds containing a pteridine nucleus, a pteroyl portion, with one or more glutamic acids attached to the pteroyl portion; polyglutamate molecules are the major type found in foods (Chanarin 1979; Stipanuk, 1979; Bailey, & Ayling, 2009).
Polyglutamate molecules must be converted to monoglutamates in the intestinal mucosa by a glutamyl transferase enzyme in order for absorption to occur. Monoglutamates are then transported by the bloodstream in the form of methylenetetrahydrofolate and are actively transported across the blood brain barrier, which makes folic acid levels in cerebrospinal fluid higher than in the serum (Spector, & Lorenzo, 1975; Stipanuk 2006).

When folate or folic acid intake is diminished, serum levels fall in a matter of days. Depletion of body stores takes a significantly longer amount of time, requiring one to six months to develop depending on the nutritional status and rate of utilization of the patient. There are many factors and conditions aside from dietary deficiency which can lead to folate deficient states including medications such as anticonvulsants, antibiotics, oral contraceptives, and antifolate cancer chemotherapeutics, malabsorption syndromes, chronic diseases such as rheumatoid arthritis, folate metabolism enzyme deficiencies, pregnancy, and alcoholism (Abou-Saleh & Coppen, 1986, Young & Ghadirian, 1989).

After absorption the tetrahydrofolate molecule is transferred to folic acid co-factors which can donate or accept one carbon methyl groups in order to catalyze enzymatic reactions. One of the most important enzymatic reactions occurs in DNA biosynthesis. During DNA synthesis methylenetetrahydrofolate donates a methyl group to uracil, converting it to thymine, which is used for DNA synthesis and repair; when folate levels are low, it increases the possibility of uracil being misincorporated into the DNA which can lead to DNA strand breaks, chromosome damage and cancer (Duthie, Narayanan, Brand, Pirie, & Grant, 2002).
Folic acid plays a major role in two main central nervous system pathways that pertain to depression. In one pathway folic acid is thought to affect the creation of neurotransmitters by promoting the synthesis of tetrahydrobiopterin (BH4). BH4 is a cofactor of tyrosine hydroxylase, which is the enzymatic rate limiting step in the biosynthesis of the catecholamine neurotransmitters dopamine, noradrenaline, and serotonin, which are targets of current pharmacotherapy for depression (Nagatsu, Levitt, & Udenfriend, 1964, Nagatsu, & Ichinose, 1999). BH4 may also have a role in regulation of the presynaptic release of neurotransmitters from nerve terminals, a mechanism which is thought to be altered in individuals with depression (Anderson, & Abou-Saleh, 1995). BH4 is formed by compounds called neopterins and broken down into compounds called biopterins, both of which are excreted in the urine. Measure of these compounds from urine has allowed researchers to assess changes in BH4 metabolism to identify reduced availability of BH4, which has been linked to depression (Coppen et al. 1989, Tiemeier et al. 2002, Tiemeier et al. 2006).
MTHF: Methyltetrahydrofolate, THF: Tetrahydrofolate

Figure 1. Biosynthetic pathway promoting creation of neurotransmitters through tetrahydrobiopterin.

The other mechanism involving folic acid thought to have an effect on depression affects methylation reactions in the central nervous system involving monoamine neurotransmitters, membrane phospholipids, and proteins. Folic acid is converted to 5-methyltetrahydrofolate, which then combines with homocysteine through a vitamin B12 dependent reaction to produce methionine. Methionine and adenosine triphosphate (ATP) are combined in a reaction catalyzed by methionine adenosine transferase to form a molecule of S-adenosyl methione (SAM). SAM is the only available molecule acting as a methyl donor in the central nervous system. Methylation reactions in the central nervous system form many products, including neurotransmitters thought to play a large role in the pathogenesis of depression. During
methylation reactions SAM is converted to S-adenosyl homocysteine (SAH) which is metabolized into homocysteine. The reaction is the only source of homocysteine in the human body. Folic acid and vitamin B12 allow the remethylation of homocysteine to methionine and subsequent creation of SAM for additional methylation reactions.


Figure 2. Biosynthetic pathway promoting methylation reactions involving folic acid.

If folic acid or vitamin B12 levels are deficient homocysteine levels can rise and metabolize to SAH which is a competitive inhibitor of methylation reactions required for monoamine neurotransmitter synthesis (Anderson, & Abou-Saleh, 1995). Bottiglieri has found that red cell folic acid, cerebrospinal fluid folic acid, and cerebrospinal fluid SAM were reduced in patients with depression and high homocysteine levels (1996). Recent studies have also linked folic acid
to homocysteine and depressive disorders (Tiemeier et al., 2002; Bjelland et al., 2003; Sachdev et al., 2005; Dimopoulos et al., 2007; Kim et al., 2008; Ng, Feng, Niti, Kua, & Yap, 2009; Nanri et al., 2010). Tests allowing the assessment of total plasma homocysteine have been developed which has allowed the identification of a biological subgroup of depression with functional folate deficiency. This subgroup has multiple factors contributing to depression including folate deficiency, impaired methylation, and impaired monoamine neurotransmitter metabolism can be diagnosed with routine blood counts of total homocysteine, greatly reducing the clinical quandary of which patients would benefit from vitamin replacement therapy (Bottiglieri et al., 2000). Overall, there is variation in the research linking the strength and degree of association between folate, homocysteine, and depression. While the mechanisms clearly show connections, it is not clear whether homocysteine acts as a cause of depressive symptoms or is a marker of folate deficiency (Bottiglieri, 2005).

Recent investigation has lead to the discovery of some genetic factors linking folate and depression. The MTHFR C677T mutation is the most common genetic mutation affecting the folate metabolic pathway resulting in differences in homocysteine and folate levels and may lead to increased susceptibility to depression (Lewis et. al., 2006). Single nucleotide polymorphisms (SNPs) have also been identified, the folate hydrolase SNP FOLH1 rs61886492 C>T being very recently found to be associated with increased depressive symptoms in those carrying the polymorphism (Ye et al., 2011). Genetic factors linking metabolic pathways and folate to depression will likely continue to be an area of interest in research as early results have showed some strong associations. While much research has gone into establishing metabolic pathways linking folic acid to depression, it is likely a combination of factors such as decreased appetite or
improper diet, decreased absorption, and increased utilization of folate stores that lead to
detrimental effects on the central nervous system (Abou-Saleh & Coppen, 1985).

There is accumulating evidence linking low folate status to depression. While there have been
some reviews and analyses examining portions of the evidence linking folate and depression, the
dissimilarities between studies make comparing data difficult, resulting in only those studies
with similar structure and method to be examined side by side. It is necessary to examine the
evidence as a whole so that some consensus can be made in order to identify gaps in current
research and direct future study. The purpose of this investigation is to examine current evidence
in order to illustrate the strengths and limitations of the available research in order to better
define the direction and need for future study.

Methods

Several major medical and scientific databases were searched to identify relevant studies
concerning folate and depression. Medline, Academic Search Complete, and Science Direct
databases were searched from 1965 to April 2012. Major search terms used were “folate and
depression” as well as “folic acid and depression.” Reference lists of retrieved studies were also
examined to find additional relevant studies. All cross sectional, case-control, and prospective
and retrospective cohort studies concerning the relationship between folate and depression were
sought. To be included, studies must have drawn a conclusion between folate status and
depression.

Search yielded 28 studies involving human subjects with examination of the link between folate
and depression being a main aim of the investigations. Of the available literature there were 8
case-control, 14 cross sectional, and 6 prospective or retrospective cohort studies.
Results

The 14 cross sectional studies were published between the years of 2000 and 2012 with the number of subjects ranging from 247 to 9670. Of the cross sectional studies there were two main methodologies used to assess folate status; either direct measure of folate through blood chemistry, or estimation of folate status through dietary measures. Table 2.1 summarizes the studies using blood chemistry as a marker of folate status.

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<td>Penninx et al. 2000</td>
<td>To examine vitamin B12 and folate deficiency and depression in physically disabled older women</td>
<td>Seven hundred disabled, nondemented women aged 65 years and over from the Women’s Health and Aging Study.</td>
<td>No association found between depression and folate deficiency.</td>
<td>Results only apply to disabled women. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels. Data on the duration of vitamin deficiency, the duration of depressive symptoms, associated metabolic disorders, and genetic predisposition to vitamin deficiencies was not available.</td>
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<td>Bjelland et al. 2003</td>
<td>To investigate folate, Vitamin B12, homocysteine, and the MTHFR 677C→T</td>
<td>Five thousand nine hundred and forty eight individuals 46 to 49 and 70 to 74 years old</td>
<td>High homocysteine levels and the MTHFR 677C→T Polymorphism were significantly related</td>
<td>Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status</td>
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<td>Ramos, Allen, Haan, Green, &amp; Miller, 2004</td>
<td>One thousand four hundred sixty three men and women of Latino descent over the age of 60 years from the Sacramento Area Latino Study on Aging (SALSA) cohort.</td>
<td>Prevalence of folate deficiency in the study population was under one percent. There was no association between depressive symptoms and folate levels in men. Low folate status was associated with depressive symptoms in women in the lowest folate tertile having an odds ratio of 2.04 (95% CI: 1.38, 3.02).</td>
<td>Study limited to elderly Latinos. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels. Plasma was frozen before analysis which can lower folate levels. Ethnic dietary factors were not examined to assess inclusion of folate fortified foods.</td>
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<td>Sachdev et al. 2005</td>
<td>Four hundred twelve men and women between the ages of 60 and 64 years from a large Australian community sample.</td>
<td>Low folic acid and high homocysteine were predictors of depressive symptoms in community-dwelling middle-aged individuals. This suggests a possible relationship between the mechanism of folic acid and homocysteine, which could overlap each other.</td>
<td>Narrow age range of study population. Exclusion of those who were already found to be depressed or have vitamin deficiency. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels. Plasma</td>
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<td><strong>Ng et al. 2009</strong></td>
<td>To examine Folate, Vitamin B12, Homocysteine, and Depressive Symptoms in a Population Sample of Older Chinese Adults</td>
<td>Six hundred sixty nine Community-living noninstitutionalized Chinese adults aged 55 and older.</td>
<td>Low serum folate and folate deficiency and were associated with higher risk of depressive symptoms in an elderly Chinese population. A significant linear trend was found suggesting the risk of depression increases with decreasing folate levels along a continuum. No significant relationships were found between depressive symptoms and vitamin B12 and homocysteine levels.</td>
<td>Blood samples were assessed for folate, vitamin B12, and homocysteine in a random one in three subsample. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels. Plasma was frozen before analysis which can lower folate levels.</td>
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<td><strong>Beydoun et al. 2010A</strong></td>
<td>To investigate the sex-specific role plasma folate has in mediating the association of dietary quality with depressive symptoms</td>
<td>Representative sample of 1681 African Americans and Whites 30 to 64 years of age living in Baltimore, Maryland from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study.</td>
<td>An inverse association was found between plasma folate and depressive symptoms in women. Women in the upper two tertiles of plasma folate were associated with and approximately 40 percent reduced odds of high number of depressive symptoms. The study illustrates that depressive symptoms in women.</td>
<td>Some selection bias as about one half the original HANDLS sample was included in the study. Data on supplemental intakes of folate was not included in the total intake of folate. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels.</td>
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<td>Beydoun, Schroff, Beydoun, &amp; Zonderman, 2010</td>
<td>To assess Serum Folate, Vitamin B-12, and Homocysteine association with depressive symptoms among US adults.</td>
<td>Two thousand five hundred twenty four adults aged 20 to 85 years from 2005 to 2006 NHANES data.</td>
<td>After adjusting for sociodemographic, life-style, and dietary factors, elevated depressive symptoms were inversely associated with folate only in women. When examining folate status in women, elevated depressive symptoms in the upper tertile folate status was approximately one third of those in the lowest tertile folate status.</td>
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<td>Nanri et al. 2010</td>
<td>To examine the correlation between serum folate and homocysteine and depressive symptoms among Japanese men and women.</td>
<td>Five hundred thirty male and female Japanese municipal employees aged 21 to 67 years who participated in a health survey at the time of a periodic checkup.</td>
<td>Higher serum folate was associated with a decreased prevalence of depressive symptoms in men. Folate was not associated with depressive symptoms in women. Overall, the study suggests that low serum folate could be related to increased prevalence of depressive symptoms in men. Study limited to municipal employees in Japan, may not be representative of the entire population. Plasma was frozen before analysis which can lower folate levels. Serum folate level was used to assess folate status which is not a reliable indicator of long term folate status as red cell folate levels.</td>
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Table 2.2 summarizes the studies using dietary measures as a marker of folate status.

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<tr>
<td>Tolmunen et al. 2003</td>
<td>To examine associations between dietary folate and depressive symptoms in middle age Finnish men.</td>
<td>Two thousand four hundred forty three men aged between 42 and 60 years from eastern Finland</td>
<td>Study participants with dietary folate intake in the lowest tertile had a 67% increase in risk of having elevated depressive symptoms than participants with folate consumption in the highest tertile. The results remained significant after adjustment for sociodemographic factors and fat consumption.</td>
<td>Study limited only to middle aged men. Study sample may have included some cases of minor depressive disorders and dysthymia as well as major depressive disorders.</td>
</tr>
<tr>
<td>Murakami et al. 2008</td>
<td>To examine Dietary intake of folate, other B vitamins, and omega three polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults.</td>
<td>Five hundred seventeen Japanese men and women aged 21 to 67 years</td>
<td>Folate intake in men related to a statistically significant inverse linear association to depressive symptoms. There were no statistically significant findings in the female group. Overall, higher dietary intake of folate was associated with a lower prevalence of</td>
<td>Study subjects were workers in two municipal offices, not a random selection from the population. Although study was adjusted for confounders, there still could be some residual confounding as occupational and leisure-time physical activities were only roughly assessed.</td>
</tr>
<tr>
<td>Payne et al. 2009</td>
<td>To examine the link between natural food folate and late life depression.</td>
<td>Two hundred forty seven participants over the age of 60 clinically diagnosed with depression compared to others in the same age category without psychiatric illness.</td>
<td>Naturally occurring food folate was inversely associated with depression after controlling for age, sex, race, education, and total energy. Other folate variables including total dietary folate (natural folate and folic acid from foods and supplements) were not significant for depression. The naturally occurring form of folate could be protective for depression and brain health.</td>
<td>Small sample size. Results pertain only to elderly individuals with depression receiving psychiatric treatment. Individuals may not have been depressed during the entire year duration of the study. Intake of folate from fortified food difficult to assess based on nature of food frequency questionnaire.</td>
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<tr>
<td>Sanchez et al. 2009</td>
<td>To investigate the association between folate, vitamin B6 and vitamin B12 intake and depression</td>
<td>Nine thousand six hundred seventy Spanish male and female college graduates from the Seguimiento University of Navarra (SUN) cohort study</td>
<td>Depression was associated with low folate intake among currently smoking men and men with low anxiety levels. Men with a high folate intake showed lower prevalence of depression.</td>
<td>Study limited to college educated individuals, not representative of entire population. Individuals with a diagnosis of depression prior to start of study were excluded from the study population.</td>
</tr>
<tr>
<td>Skarupski et al. 2010</td>
<td>To examine the association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time.</td>
<td>Adults over the age of 65 years from the Chicago Health and Aging project, an ongoing, population-based, biracial</td>
<td>No association was shown between depressive symptoms and food intake of folate before or after adjustment for smoking, alcohol use, widowhood,</td>
<td>Study limited to older adults. Study controlled for use of anti depressant medications, but not other medications that could affect depression.</td>
</tr>
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Cross sectional studies mainly focused on levels of folate either estimated from dietary evaluation or through blood chemistry to show a link between decreased folate and depression. Gender differences were illustrated in several studies where there was significant evidence illustrating the link between folate and depression gender and not the other. Three of the fourteen studies showed a link between low folate status and depression in men but not women (Murakami et al., 2008; Sanchez et al., 2009; Nanri et al., 2010). One study included only male subjects (Tolmunen 2003). Four of the 14 cross sectional studies illustrated significant findings in women but not men (Bjelland et. al., 2003; Ramos et al., 2004; Beydoun et al., 2010A;
Two studies showed no significant findings linking folate status to depression (Pennix et al., 2000; Skarupski et al., 2010). Four studies found a link between folate and depression in both men and women (Sachdev et al., 2005; Ng et al., 2009; Payne et al., 2009; Seppala et al., 2012). Meta-analysis of the studies before 2007 showed a significant relationship between folate status and depression (Gilbody, Lightfoot, & Sheldon, 2007).

The eight case-control studies were published between the years of 1990 and 2007. Table 2.1 summarizes the case control studies where the case was considered a depressed individual and the control a non-depressed individual.

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<tr>
<td>Carney et al. 1990</td>
<td>To examine red cell folate concentrations in psychiatric patients.</td>
<td>Two hundred forty three successively admitted in-patients to a psychiatric unit and 42 Euthymic outpatient controls. All patients from London, England.</td>
<td>Mean red cell folate in depressed patients was significantly lower than in controls. Fifty four percent of depressed patients had red cell folate levels under 200 ng/ml. Depressed patients with folate deficiency found to have more severe depression than those with folate in the normal range.</td>
<td>Study not controlled for confounders. Selection of subjects not random.</td>
</tr>
<tr>
<td>Lee, Wing, &amp; Fong, 1998</td>
<td>To examine the link between folate levels and depression in an Asian population.</td>
<td>Newly admitted Chinese patients with major depression and healthy hospital employee volunteers</td>
<td>The mean serum folate level was within the normal limits in the patient and control groups. Depressed patients had lower serum folate than control subjects. Authors note the Chinese diet, which is high in folate, could</td>
<td>Patients were not drug-free. Lack of detailed dietary analysis and longitudinal data on folate status and psychiatric outcome.</td>
</tr>
<tr>
<td>Study</td>
<td>Objective</td>
<td>Design</td>
<td>Findings</td>
<td>Limitations/Notes</td>
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<tr>
<td>Tiemeier et al. 2002</td>
<td>To examine associations of vitamin B12, folate, and homocysteine with depression</td>
<td>Participants with depressive symptoms and randomly selected non-depressed individuals over the age of 55 years in Rotterdam, Holland</td>
<td>Hyperhomocysteinemia, vitamin B12 deficiency, and folate deficiency were related to depressive disorders. After adjustment for functional disability and cardiovascular disease, the association between folate and deficiency and hyperhomocysteinemia with depressive disorders was reduced.</td>
<td>Cannot differentiate whether vitamin deficiency precedes or results from depression. Study not controlled for loss of appetite, a key symptom of depression affecting nutritional status. Prevalence of depression low in the study population.</td>
</tr>
<tr>
<td>Lerner et al. 2006</td>
<td>To compare cobalamin and folate levels in newly admitted psychiatric patients to assess correlation with psychiatric conditions.</td>
<td>Consecutively admitted psychiatric patients in Israel compared to age and gender matched healthy controls from a similar geographical and socioeconomic background.</td>
<td>Approximately 30 percent of admitted psychiatric patients had low folate levels whereas 2.5 percent of controls had low folate levels (P&lt;0.0001). Mean folate levels in the control group were significantly higher than in the patient group. A positive correlation was found between low folate levels and depression. Study shows a need for assessing folate levels in patients admitted to psychiatric wards.</td>
<td>Due to lack of resources, researchers unable to measure erythrocyte folate level, a better indicator of long-term folate status, and instead relied on circulating levels. Relatively small number of patients with depression disorder in the study population. Unable to determine the effect of patient medications on folate level. Psychiatric patients compared to non-psychiatric controls.</td>
</tr>
<tr>
<td>Tiemeier et al. 2006</td>
<td>To examine plasma pterins and folate in late life depression.</td>
<td>Participants with depressive symptoms and randomly</td>
<td>A significant relationship was found between depressive symptoms, folate and</td>
<td>With the study design it was not possible to conclude whether depression follows</td>
</tr>
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</table>
selected non-depressed individuals over the age of 60 years from Rotterdam, Holland. neopterin. In depressed individuals, the relationship between pterins and folate was different than in the non-depressed, where neopterin concentrations increased with folate levels in persons exhibiting symptoms of depression, but not in non-depressed persons.

Dimopoulos et al. 2007 To determine the correlation of folate, vitamin B12 and homocysteine plasma levels with depression in an elderly Greek population. Men and women over the age of 60 from a Greek population with depression and healthy controls from the same age group. Study subjects with depression had significantly lower levels of folate and vitamin B12 than the control group. Homocysteine was significantly higher in depressed individuals than in controls. The study had only one clinical and laboratory evaluation with no follow up. The number of participants was fairly small, making the study limited in power.

Table 2.2 summarizes the case control studies where the case was treated with folate and the control was treated with placebo.

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Purpose</th>
<th>Population</th>
<th>Conclusions</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Godfrey, &amp; Toone, 1990</td>
<td>To assess recovery from psychiatric illness by treatment with methylfolate</td>
<td>Patients from London, England with a DSM III clinical diagnosis of</td>
<td>In both depressive and schizophrenic patients methylfolate supplementation significantly</td>
<td>Study has a relatively small sample size. The cause of low folate status was not investigated. Unable to tell if the effect of</td>
</tr>
<tr>
<td>Coppen, &amp; Bailey, 2000</td>
<td>To investigate if the administration of folic acid would enhance the antidepressant action of fluoxetine</td>
<td>Patients from family practice clinics in England clinically diagnosed with major depression received 500mg folic acid or identical placebo in addition to 20mg fluoxetine daily.</td>
<td>Patients receiving folate had a significant increase in plasma folate over the course of the study. The increase was less in men than in women. Overall, there was significant improvement of depressive symptoms in the experimental group versus the placebo group.</td>
<td>Dose of folate may have been insufficient for treatment in men. Diet not controlled for folate intake. Results not adjusted for possible confounders.</td>
</tr>
</tbody>
</table>

Of the eight case control studies examining folate and depression, four found a significant relationship between low folate levels and depression. (Carney et al., 1990; Lee et al., 1998; Lerner et al., 2006; Dimopoulos et al. 2007). Two studies showed supplementation of folate compounds was effective in reducing depressive symptoms, increasing recovery (Godfrey, & Toone, 1990; Coppen, & Bailey, 2000). Additionally, two studies found a significant relationship between pterin compounds, depression, and folate status, offering further evidence linking depression to metabolic pathways in the central nervous system (Tiemeier et al., 2002; Tiemeier et al., 2006).
The 6 prospective and retrospective cohort studies were published between the years of 2004 and 2011 with the number of subjects ranging from 242 to 3996. Table 3.1 summarizes the retrospective studies.

### Table 3.1

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Purpose</th>
<th>Population</th>
<th>Conclusions</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Astorg et al. 2008</td>
<td>To investigate the association of folate intake with the occurrence of depressive episodes in middle aged French men and women</td>
<td>Dietary habits were measured at the onset of an eight year follow-up in 1864 middle aged French men and women.</td>
<td>There was no significant association found between risk of a depressive episode and folate intake during the follow-up period in men or women. Recurrence of depressive episodes was significantly reduced in men with a high folate intake, suggesting low folate intake may increase the recurrence rate of depression in men.</td>
<td>Limited to middle aged subjects. Study population was a sample selected from the initial study population. Antidepressant prescription was used as a proxy for depressive episodes, which could alter findings as depressed individuals often do not seek treatment.</td>
</tr>
<tr>
<td>Kamphuis, Geerlings, Grobbee, &amp; Kromhout, 2008</td>
<td>To examine Dietary intake of vitamins B6, B12, folate, serum homocysteine levels and their association with depressive symptoms</td>
<td>Three hundred thirty two men aged 70–90 years from the Netherlands.</td>
<td>Dietary intake of folate and vitamin B6 was associated lower levels of serum homocysteine. Intake of folate and vitamins B6 and B12 and serum homocysteine levels were not associated with depressive symptoms</td>
<td>Study limited only to elderly men. Serum homocysteine values were available in 1985 and information on depressive symptoms in 1990, the study had to assume those values from 1985 were representative for values in 1990.</td>
</tr>
<tr>
<td>Ginsberg,</td>
<td>To compare L-</td>
<td>Two hundred</td>
<td>L-methylfolate</td>
<td>Study was not</td>
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</table>
Oubre, & Daoud, 2011 methylfolate plus SSRI or SNRI treatment to SSRI or SNRI Monotherapy in a Major Depressive Episodes. forty two adults 18 to 70 years of age with a clinical diagnosis of a single or recurrent major depressive episode. supplementation in addition to prescribed SSRI/SNRI at treatment onset was more effective in improving depressive symptoms and function in patients within 60 days than SSRI/SNRI therapy alone. The addition of L-methylfolate led to major symptomatic improvement more rapidly than SSRI/SNRI monotherapy and had significantly fewer discontinuations due to adverse events. randomized. Baseline characteristics of the two groups were not similar. Folate levels of patients were not obtained at baseline and could have differed. The study lacked remission of depression as an endpoint.

Table 3.2 summarizes the prospective studies.

Table 3.2

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Purpose</th>
<th>Population</th>
<th>Conclusions</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Tolmunen et al 2004</td>
<td>To assess dietary folate and the risk of depression in Finnish middle-aged men</td>
<td>Two thousand three hundred thirteen men aged between 42 and 60 years from eastern Finland</td>
<td>Individuals with energy adjusted folate intake below the median had a higher risk of a discharge diagnosis of depression with a relative risk of 3.04 (95% CI: 1.58, 5.86) during the follow-up period than participants whose</td>
<td>Study limited only to middle aged men. The relationship between depression and dietary folate could be due to other health features of a folate-rich diet. Changes in eating habits during follow up could have biased results.</td>
</tr>
<tr>
<td></td>
<td>Study Objective</td>
<td>Sample Description</td>
<td>Results</td>
<td>Limitations</td>
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<td>Kendrick et al. 2008</td>
<td>To examine longitudinal blood folate levels and depressive symptoms among women in the Southampton Women’s Survey</td>
<td>Three thousand nine hundred ninety six women aged 20 to 34 years from Southampton, UK.</td>
<td>Lower blood folate levels were associated with depressive symptoms at baseline. Lower folate levels were not associated with depressive symptoms over a 2 year follow up period, which could suggest that low folate levels could be a consequence rather than a cause of depressive symptoms.</td>
<td>Study limited to young women. Incidence of depression determined by physician identifying and recording symptoms, which can be missed. Women followed for only two years, future cases of depression may have been missed.</td>
</tr>
<tr>
<td>Kim et al. 2008</td>
<td>To assess the predictive value of folate, vitamin B12, and homocysteine levels in late life depression.</td>
<td>Seven hundred thirty two male and female residents of Kwangju, Korean over the age of 65 years.</td>
<td>Lower levels of folate and vitamin B12 and higher levels of homocysteine were found at baseline in associated with a higher risk of incident depression on follow up. Lower folate, lower vitamin B12 and elevated homocysteine levels could be risk factors for late life depression.</td>
<td>Study limited to elderly age group. Data on folate supplementation was not available at baseline. During follow-up evaluation of participants, information concerning mental health was restricted to the previous month. Content of multivitamin preparations used by study subjects was not available.</td>
</tr>
</tbody>
</table>

Of the six cohort studies, there were three prospective and three retrospective studies. Two of the three prospective studies found a significant relationship between low folate levels and
higher incidence of depression on follow up (Tomlmunen et. al., 2004; Kim et al., 2008).
Kendrick et. al. found no link between folate status and risk of depression during a two year
follow up period, but did note low folate levels were associated with depressive symptoms at
baseline (2008). The two retrospective studies found no link between folate and risk of
depression (Astorg et al., 2008; Kamphuis et al., 2008). One retrospective study focused on
folate supplementation in combination with prescribed medication for depression was more
effective in improving depressive symptoms in patients than the prescribed medication alone
(Ginsberg et al., 2011).

Discussion

Cross Sectional Studies

In the cross sectional studies using serum as a marker for folate status, serum folate level was
used to assess folate status. This level is not a reliable indicator of overall folate status as it is
more of a snapshot of folate status which can be altered by high intake of folate in a previously
eaten meal. Red cell folate levels would be a more reliable indicator of long term folate status
but was not used. Also, in many of the studies, the plasma was frozen before analysis. This can
cause folate levels in the plasma to be artificially lowered, which is another issue contributing to
the reliability of folate levels in the studies. Of the cross sectional studies using serum markers
as assessment for folate status, only those studies by Beydoun et al. analyzed overall diet as well
as serum folate (2010A; 2010B). These studies are really the only examples in which intake of
folate can be controlled as a confounding factor. Interestingly, these studies found associations
between folate and depressive symptoms only in women, and included the widest age group of
participants studied.
The cross sectional studies using diet as a marker for folate status have a large limitation in that they assess folate by dietary measures and not a direct blood value. This introduces more potential error into the studies as folate intake is calculated from indirect methods and is not an optimal method of assessment. All of the dietary assessment methods used in the studies were validated prior to their use, which limits error somewhat. There are several advantages to using these tools, the opportunity to assess the diet as a whole gives a more long term indicator of folate status, and ease of use of these methods allow larger sample sizes across broader territory.

Across all cross sectional studies there are several findings of note. Most notably are gender differences. Of the cross sectional studies in populations including both men and women three found significant findings only in men (Murakami et al., 2008; Sanchez et al., 2009; Nanri et al., 2010). However, these studies included very specific populations, Murakami et al., 2008 and Nanri et al., 2010 studied only Japanese municipal workers. Sanchez studied only Spanish college graduates (2009). These samples are not representative of the entire population in the country they occurred, which makes it difficult to make any definitive statements.

Of the studies examined, almost half were cross sectional in nature. These studies definitely illustrate a link between folate statuses, whether measured through blood values or dietary analysis and depression. While this type of study allows examination of larger sections of the population, it is not without its limitations. The largest limitation is due to study design, while the studies can suggest a biological relationship between folate and depression, cross sectional design limits causal conclusions. Therefore, it is necessary to seek further evidence illustrating if
low folate levels is the cause rather than a consequence of depression through prospective follow-up and randomized control trials.

**Case-Control Studies**

All case control studies where the case was a depressed individual and the control was a euthymic individual linked low folate levels to depression. There are several flaws in the designs of the studies however. Two studies used consecutive admissions to inpatient psychiatric units as subject pools for cases (Carney et al., 1990; Lerner et al., 2006). While this is a convenient method, it does not lend itself well toward randomization of subjects or a sample representative of the normal population. Another limitation with the Carney et al. study is inpatient cases were compared to outpatient controls that were being treated for other psychiatric illnesses other than depression. Lee, Wing, and Fong’s study is also limited by the selection of controls from healthy hospital staff volunteers instead of a population matched to the cases (1998). The study by Dimopoulos et al. was greatly limited in power and by the fact there was only one clinical and laboratory evaluation with no follow up. A major limitation of these case control studies is that it is not possible to identify if depression preceded or followed folate deficiency, further study is needed to address this issue.

There are a limited number of studies where the case was being treated with a folate supplement and the control was treated with placebo. Overall, both studies showed improvement in depressive symptoms in the experimental group versus the placebo group. Godfrey and Toone examined the supplementation of methylfolate in combination with the patient’s previously prescribed psychiatric medication (1990). This limits the study as all patients were not on the
same treatment regimes. Also, the researchers were unable to illustrate whether the effect of methylfolate was due to a correction of folate deficiency, or is methylfolate had a direct pharmacological effect, which is important to differentiate, as methylfolate is a biologically active form of folate. Coppen and Bailey examined the effects of folic acid supplementation or identical placebo in combination with fluoxetine, a widely prescribed antidepressant medication. Patients in the experimental group showed increase in serum folate concentration over time with improvement of depressive symptoms. This study showed less improvement in men, suggesting supplementation levels may need to be higher in men in order for there to be a positive effect. A major limitation in this study was that it was not controlled for dietary folate intake, which could be responsible for some of the findings.

The number of significant case control studies examining the use of folate in the treatment of depression is greatly limited. It will certainly be necessary to focus future research on this deficit. A limitation in the current body of research is the lack of any significant studies examining the use of only folate in the treatment of depression, which could provide a great deal of information in order to better direct additional research.

**Cohort Studies**

The two retrospective examining the link between folate and depression did not show significant association. There were limitations in these studies. Astorg et al. focused only on middle aged individuals and used antidepressant prescription as a proxy for depressive episodes (2008). This could greatly confound results as medication could have been used for other causes than depression and not be representative of depressed patients, as often those who are depressed do
not seek treatment. The study by Kamphui et al. was limited in that it only included elderly men and poor design where blood levels of homocysteine was only available in 1985 and information on depressive symptoms in 1990, forcing researchers to assume the values from 1985 were representative for 1990 (2008). This greatly limits the study.

Ginsberg, Oubre, and Daoud compared L-methylfolate supplementation in combination with SNRI or SSRI treatment to SSRI or SNRI monotherapy for depression and found supplementation was more effective in improving depressive symptoms. This study was not randomized and was greatly limited because the baseline characteristics of the two groups were not similar, and folate levels were not measured at baseline.

All three prospective studies linked low levels of folate to higher risk of depression. Tolmunen et al. assessed dietary folate and found those with folate intake below the median had a higher risk of diagnosis of depression (2004). This study was limited to middle aged Finnish men and was not able to conclude if the relationship between depression and folate was due to health features of a folate rich diet. Kendrick et al. examined a large population of young women over two years and found low folate at baseline was associated with depression, but not over the follow up period (2008). This study illustrates that low folate levels could be a consequence rather than a cause of depression, but was limited in the fact that participants were only followed for two years and future cases of depression could have been missed. Kim et al. discovered low levels of folate and vitamin B12 and high levels of homocysteine were associated with higher risk of depression (2008). The population in this study was limited to an elderly age group and was missing data on folate supplementation, which could have altered results.
While there are several retrospective and prospective cohort studies examining links between folate and depression, these studies are somewhat limited. A main concern is the studies are generally in limited populations of specific age groups. In order to draw any definite conclusions, it will be necessary to focus research on prospective studies with sufficient length that include varying ages in the cohort and control for factors such as dietary and supplement intake of folate.

Variations in studies make it difficult to draw a definite conclusion from the body of research available. There is much evidence suggesting low folate status plays a major role in depression, but there has not been clear guidelines established indicating the benefits, risks, dose or indications for folate in depression.

**Benefits of Folate**

Depression and folate are directly linked to health problems outside the realm of psychiatric illness. Glassman and Shapiro showed changes in the central nervous system and platelets seen in depression could be linked to coronary artery disease (1998). Depression and mild forms of depressive mood were also shown to have a negative influence on coronary artery disease (Soderman, Lisspers, & Sundin, 2007). Several studies have proposed possible benefits of folate aside from those related to depression. Higher folate intake has been shown to have positive effects in vascular disease. An early study illustrated increasing intake may reduce total homocysteine levels in the body, preventing arteriosclerotic vascular disease (Boushey, Beresford, Omenn, & Motulsky, 1995). It was later found elevated folate status could have a
protective effect as those with high status had a greatly reduced incidence of acute coronary events (Voutilainen et al., 2004).

**Risks of Folate**

Folate is not a compound without risk. While meta-analysis of trials using folate did not find evidence suggesting folate is not safe, several studies have appeared since suggesting excess folate may lead to significant health problems (Taylor, Carney, Goodwin, & Geddes, 2004). In a study investigating if folic acid supplementation could reduce the risk of colorectal carcinoma, some evidence was found suggesting folic acid might increase the risk of colorectal neoplasia (Cole et al., 2007). In a study on ischemic heart disease in Norway, treatment with folic acid and vitamin B12 was associated with increased cancer outcomes and increased mortality (Ebbing et al., 2009). These studies do not show definite evidence linking folic acid supplementation to increased risks, but are worrisome enough to investigate the issue further.

**Dose of Folic Acid**

Dose of folate must also be examined in future studies. Original research suggested daily supplement of 300 to 400 picograms of folic acid could be beneficial to those on lithium prophylaxis (Coppen, Chaudhry, & Swade, 1986). More current data suggests 800 microgram folic acid supplementation should be tried in order to improve the treatment outcome in depression (Coppen, & Bolander-Gouaille, 2005). Doses of 2 milligrams of folic acid have been recommended during the acute, continuation, and maintenance treatment of depression (Abou-Saleh & Coppen, 2006).
There are several confounding factors to examine when considering the proper dose of folic acid. While psychiatric symptoms are associated with folate deficiency, not all folate deficient patients develop psychiatric symptoms; this makes it difficult to determine who will benefit from folate supplements (Young, & Ghadirian, 1989). Another study suggests men may require a significantly higher dose of folic acid than women for a therapeutic effect and more research is required to ascertain the dose (Coppen, & Bailey, 2000).

**Fortification of Folate**

Folate fortification in developed countries also complicated the issue of defining supplementation guideline for therapeutic effect. The practice of fortification has led to a decline in birth defects and an increase in serum and red blood cell folate levels (Eichholzer, Tönz, & Zimmermann, 2006). Fortification efforts have led researchers to conclude that 1 milligram supplementation may be sufficient in treating depression in those countries with fortification (Young 2007). Fortification efforts in the United States projected to raise the average folate intake by approximately 100 micrograms a day. Recent study has estimated the increased folate intake to be closer to 200 micrograms a day because of fortification efforts, and although there is some increase, it was found that the only group exceeding the upper limit of folate intake was those consuming supplements in addition to fortified foods (Choumenkovitch et. al 2002). Future investigations must focus on a proper dose for supplementation as there is yet to be a consensus made concerning this matter.

There are several small but specific bodies of research concerning folate and depression that will likely become more relevant as time goes on. These topics include folate and depression in
pregnancy, the use of L-methylfolate in supplementation for depression, and folate as an adjunct therapy for depression with traditional pharmacological approaches.

Folate and Pregnancy

In pregnancy nutrient reserves can be depleted. This can lead to increased risk of depression due to lack of folate throughout the pregnancy and through postpartum recovery, prospective studies are needed to examine the role of nutrients and the pathophysiology of depression (Bodner, & Wisner, 2005). A cross sectional study since did not find a link between folate levels and folate intake and the incidence of depression in the first trimester of pregnancy, nor did it identify a protective effect of folate in early pregnancy (Watanabe et al. 2010). Additional research is needed to examine if there is any benefit in folate supplementation in pregnancy.

L-methylfolate

L-methylfolate is the methylated, biologically active form of folic acid in the central nervous system. This form of supplementation may have special significance in those with genetic polymorphisms affecting methylation in the central nervous system. Evidence suggests L-methylfolate supplementation should be used in those who have documented low levels and folate and its metabolites, as well as those who do not respond to traditional antidepressant therapies (Stahl, 2007; Stahl 2008). The supplement is generally considered to be safe and tolerable, and may be an alternative to pharmacological agents, especially in those at risk for low folate levels (Farah, 2009). Much research is still needed to define the role of L-methylfolate in the treatment of depression. As of now there is no way to measure L-methylfolate specifically in
the body (Stahl 2010). Creation of an accurate assessment method would be a tool of great value and help direct the future course of research.

**Folate as an Adjunct in the Treatment of Depression**

Adjunctive treatment of depression with folate has been a topic of research for some time. Folate levels have been found to be a predictor of improvement in patients on SSRIs, which could make folic acid supplementation before the start of pharmacotherapy beneficial (Alpert, Silva, & Pogue, 2003). Additional analysis of research showed those with low folate levels could benefit from either a folic acid supplement or dietary increase of folate in order to maintain folate levels within the normal range before or during treatment with antidepressants in order to maximize efficacy (Morris, Tridevi, & Rush, 2008). Coppen et al. initially found supplementation of folic acid was useful in patients treated for psychiatric disorders including depression who were on lithium therapy (1986). As SSRI therapies for depression became popular in the 1990s, a need for research concerning the role of folate and antidepressant pharmacotherapy became apparent (Alpert, & Fava, 1997). Folic acid was found to greatly improve the antidepressant action of fluoxetine, and allowed the hypothesis that the effect might carryover in folate supplementation with other SSRI pharmacotherapies (Coppen, & Bailey, 2000). The effect of Fluoxetine was augmented by folate in another study, possibly by modifying the serotonergic system (Resler et al., 2008). One prospective study did show L-methylfolate supplementation given with SSRI/SNRI treatment was more effective in improving depressive symptoms and function in patients than the SSRI/SNRI alone (Ginsberg et. al., 2011). This research is very promising, but more prospective and controlled trials are needed.
**Strengths and Limitations**

There are several strengths of the studies included in this review. One of the largest strengths is the fact the studies are from international sources. This is important to consider because folate status differs in populations based on diet and can be altered in countries mandating fortification of folate in the food supply. There were also a wide range of age groups studied, as well as a suitable number including research on each gender. The main limitation of the studies included in this review is inconsistency in design. While the studies address a fairly wide range of issues centering on the role of folate in depression, there is not a central methodology by which the studies have been carried out. A specific difficulty in this is the wide variety and range of confounders present in the current research.

**Conclusions**

From purely a nutritional standpoint, it is necessary to correct folate deficiency in order to prevent illness in the population including major blood disorders such as megaloblastic anemia, leukopenia, and thrombocytopenia. Correcting folate deficiency in women of child bearing age is of the utmost importance in preventing birth defects such as spina bifida and anencephaly. There are established causal relationships between folate deficiency and these illnesses and birth defects. In the treatment of depression the relationship is yet to be proven, making the case for supplementation of folate in the treatment of depression complicated, and illustrating the need for further study.

There is a growing body of data illustrating that low folate level, whether found through blood chemistry or dietary analysis, is associated with depression. Review of evidence to 2009 by
Lazarou and Kapsou did not find enough data to justify the prescription of folic acid to those patients suffering from depression (2010). A larger problem with the current body of research is that much of the evidence comes from case-control and cross sectional studies. While these studies have been very useful in establishing a relationship between folate and depression, it is now necessary to move beyond that point. Future research should be focused on the root cause of folate deficiency, whether it is dietary, metabolic, genetic, or a combination of these factors. Once this is established it will then be possible to approach and examine possible treatment methodologies. As Gilbody et al. suggested in 2007, prospective studies and randomized-controlled trials examining therapeutic benefit of folate will be necessary to confirm or disprove a causal relationship. Only after discovery of such evidence can a definitive statement concerning the use of folate in depression be made.

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