One-carbon metabolism in psychiatric illness

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The cost of psychiatric illness to the UK economy was recently estimated at £77 billion annually. Despite years of research no firm aetiological explanation exists, and with no physiological or biochemical markers diagnosis is made entirely on a behavioural basis. All current pharmacological therapies are associated with serious long-term side effects. Substantial evidence supports the involvement of one-carbon cycle dysregulation in psychiatric illness, but this is not currently used as a basis for diagnosis or treatment. The present paper reviews the evidence for one-carbon cycle dysregulation in schizophrenic, bipolar, depressed and autistic patients. Also presented are novel findings from the field of epigenetics, which demonstrate how the one-carbon cycle-derived methyl donor S-adenosylmethionine influences the expression of key genes in the brain affecting memory, learning, cognition and behaviour, genes whose expression is reduced to varying degrees in these patient groups. Clinical evidence that nutritional supplements can rectify one-carbon cycle activity, and restore normal gene expression, suggests a novel approach to the development of biochemical tests and simple, non-harmful treatments for some psychiatric patients. Conversely, evidence from animal studies highlights the dangers of exposing the unborn fetus to very high dietary levels of folic acid, a one-carbon cycle cofactor. Fetal adaptations to a high-folate environment may interfere with folate metabolism postnatally, with serious consequences for the epigenetic regulation of gene expression. The public health implications of these diverse scenarios indicate an urgent need for further research in this field.

Introduction

One in four individuals experience an episode of psychiatric illness in their lifetime; the worldwide incidence of schizophrenia is 1% and that of bipolar disorder (BPD) is 1–2%. Due to the social stigma associated with psychiatric illness, and the side effects of neuroleptic treatments, the consequences for individuals can be devastating and lifelong. Despite years of research, no firm causal evidence exists, although the actions of symptom-modulating drugs have generated various hypotheses. Psychiatric diagnoses say nothing about aetiology, and are incapable of predicting the course of the illness or which treatments will be effective. Implementing drug therapy is thus a process of trial and error (Bentall, 2003).

Little attention is currently paid in psychiatry to the influence of nutrition on mental health, beyond concern that psychotropic medication is associated with weight gain, which may increase CVD risk in patients. The present paper explores the evidence that poor B vitamin or folate intake and/or disordered metabolism influences mental states by disrupting cellular methylation status, altering the expression and activity of key genes involved in brain development and function, and that nutritional therapy can improve or restore mental health in some patients.


Abbreviations: BHMT, betaine–homocysteine methyltransferase; BPD, bipolar disorder; CDP, cytidine diphosphate; CNS, central nervous system; COMT, catechol-O-methyltransferase; CPK_MMB, muscle-derived serum creatine phosphokinase; CSF, cerebral spinal fluid; DBPC, double-blind placebo-controlled; Dmnt, DNA methyltransferase; DSM, Diagnostic and Statistical Manual; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GNMT, glycine N-methyltransferase; Hey, homocysteine; HRM, heterozygous reeler mouse; LCPUFA, long-chain PUFA; MS, methionine synthase; MTHF, methylenetetrahydrofolate reductase; NMDA, N-methyl-D-aspartate; NTD, neural tube defect; OR, odds ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine methyltransferase; PG, prostaglandin; PLA, phospholipase A2; PLM, phospholipid methylation; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SANS, Scale for the Assessment of Negative Symptoms; SHMT, serine hydroxymethyltransferase; SNP, single nucleotide polymorphism; WTM, wild-type mice.

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A literature search of the databases ScienceDirect, High-Wire and Medline was conducted, using combinations of the search terms ‘schizophrenia’, ‘depression’, ‘bipolar disorder’ and ‘autism’ with each of the metabolites and enzymes of the one-carbon cycle. As the hypothesis developed, new search terms were added, including ‘methyltransferase’, ‘methylation’, and ‘reelin’. Papers were reviewed and relevant cited papers and books were also obtained. Recent studies were included if they were adequately controlled with sufficient numbers of subjects. Older studies not meeting these criteria were included if they tested associations that have not been re-examined more recently. The results of these searches are assembled in three sections. The first explores associations between diet- and/or genotype-induced disordered one-carbon metabolism and psychiatric illnesses. The second explains how methylation impacts on phospholipid structure and function, and how disordered one-carbon metabolism could produce the alterations in fatty acid profiles found in psychiatric patients. The third introduces the field of epigenetics, detailing animal and human studies that indicate mechanisms whereby pre- and postnatal environmental influences such as diet can influence gene expression, central nervous system (CNS) development and function, and psychiatric symptoms.

The one-carbon cycle and psychiatric illness

One-carbon metabolism

Fig. 1 illustrates the interaction between folate metabolism, the methionine cycle, and transmethylation reactions, collectively known as one-carbon metabolism. Vitamin B6-dependent serine hydroxymethyltransferase (SHMT) catalyses the conversion of serine to glycine, producing 5,10-methylenetetrahydrofolate (MTHF) from tetrahydrofolate. 5,10-MTHF can then be used: to synthesise purines; as the substrate for B2-dependent MTHF reductase producing 5-MTHF; as a substrate for thymidine synthase generating deoxythymidine-monophosphate from deoxyuridylic acid, the limiting reaction in DNA biosynthesis. 5-MTHF is the methyl-donor for the re-methylation of homocysteine (Hcy) by cobalamin-dependent methionine synthase (SAM), yielding methionine. Hepatic tissues have an alternate pathway for Hcy re-methylation via Zn-dependent betaine–homocysteine methyltransferase (BHMT) where betaine serves as the methyl-donor. Methionine is converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase. SAM is the methyl-donor in over 100 transmethylation reactions involving DNA, proteins, neurotransmitters, hormones and phospholipids. Donation of the methyl group leads to the formation of S-adenosylhomocysteine (SAH), which is hydrolysed to Hcy and adenosine by SAH hydrolase. The equilibrium of this reaction favours SAH production, hence Hcy and adenosine must be efficiently removed. In addition to re-methylation, Hcy is also metabolised via the trans-sulfuration pathway, where it combines with serine to form cysteine by two vitamin B6-dependent enzymes, cystathionine β-synthase and cystathionine γ-lyase.

Methylation regulation

SAM is responsible for all methylation reactions. Conversely, SAH inhibits such reactions by effectively competing with SAM for methyltransferase binding. The SAM:SAH ratio thus describes the ‘cellular methylation potential’. The major regulators of methylation potential are glycine N-methyltransferase (GNMT) and MTHF reductase. GNMT binds 5-MTHF and high 5-MTHF levels inhibit its

Fig. 1. The interaction between folate metabolism, the methionine cycle and methyl group metabolism. DHF, dihydrofolate; THF, tetrahydrofolate; SHMT, serine hydroxymethyltransferase; MAT, methionine adenosyltransferase; MSR, methionine synthase reductase; MS, methionine synthase; SAM, S-adenosylmethionine; BHMT, betaine–homocysteine methyltransferase; GNMT, glycine N-methyltransferase; MTHFR, methylenetetrahydrofolate reductase; PEMT, phosphatidylethanolamine; COMT, catechol-O-methyltransferase; Dnmt–1, DNA methyltransferase-1; 5-MTHF, 5-methyltetrahydrofolate; CBS, cystathionine β-synthase; SAHH, SAH hydrolase; SAH, S-adenosylhomocysteine.
activity. Conversely, high SAM concentrations inhibit MTHF reductase, reducing the concentration of 5-MTHF and releasing the inhibition of GNMT, which then reduces SAM levels. If SAM concentrations fall, MTHF reductase activity increases, producing more 5-MTHF, which inhibits GNMT, preserving SAM for other essential transmethylation reactions (Rowling et al. 2002) (Fig. 2). Hence, under normal circumstances the SAM:SAH ratio is tightly regulated. However, despite these and other regulatory mechanisms, perturbations in vivo are caused by dietary excess or deficiency of methionine or choline, or of cofactor vitamins B2, niacin, B6, B12 or folic acid, and/or enzyme polymorphisms.

Methylation and mental illness

Most studies correlating symptoms of psychiatric illness and disturbed one-carbon metabolism have examined individual aspects of the cycle; hence, the evidence is presented accordingly. However, disturbance in one part of the cycle affects the whole cycle.

Serine, glycine and serine hydroxymethyltransferase

Serine is the main one-carbon donor via the conversion of serine to glycine by SHMT (Cook, 2001; Davis et al. 2004). Serine also participates in the trans-sulfuration pathway as a cofactor for cysteine synthesis, and subsequently GSH, taurine and sulfate.

Glycine and β-serine are obligatory co-agonists at the N-methyl-D-aspartate (NMDA)-type glutamate receptors, which are believed to play a key role in the pathophysiology of schizophrenia (Heresco-Levy et al. 1999; Olney et al. 2004). The gluta
tamate hypothesis derives from observations that phencyclidine, ketamine and other non-competitive NMDA receptor antagonists produce a psychosis more closely matching schizophrenia than the dopamine agonist model. Disruptions of glutamatergic neurotransmission and glutamate receptor expression have been observed in post mortem studies (Tsai et al. 1998; Olney et al. 1999).

Early studies found significantly increased plasma serine in drug-free psychotics, with decreased SHMT activity (Waziri & Mott, 1986), and significantly increased serine and glycine concentrations in medial and temporal lobes of schizophrenic brains post mortem, and increased Km of SHMT (Waziri et al. 1992). A double-blind placebo-controlled (DBPC) cross-over trial of high-dose glycine in treatment-resistant schizophrenia found that 0.8 g glycine/kg per d (average 60 g/d) significantly reduced negative symptoms as measured by the Positive and Negative Syndrome Scale by 30 (SD 16) % (P < 0.001), and significantly improved Brief Psychiatric Rating Scale scores by 30 (SD 18) % (P < 0.001). (Positive symptoms denote visual, auditory and/or tactile hallucinations and delusions; negative symptoms denote social withdrawal.) Low, but within normal range, pre-treatment serum glycine significantly predicted treatment response (Heresco-Levy et al. 1999). This study followed two other DBPC trials in schizophrenics (15 and 30 g glycine/d), which both saw symptom improvements.

β-Serine (30 mg/kg per d) supplementation significantly improved positive, negative and cognitive symptoms of schizophrenia in a DBPC trial in Taiwanese patients (Tsai et al. 1998). β-Serine crosses the blood–brain barrier more easily than glycine, allowing a lower effective dose, but since glycine can be converted in vivo to β-serine and vice versa, the source of clinical effects is unclear. In a meta-analysis of eighteen DBPC trials of glycine (n 7), β-serine (n 2), D-cycloserine (n 7), and ampakine CX516 (n 2) in addition to anti-psychotics, treatment with glycine or β-serine modestly but significantly improved negative symptom scores (n 132; fixed effect model standardised effects)

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**Fig. 2.** Regulation of methyl-group metabolism. THF, tetrahydrofolate; SHMT, serine hydroxymethyltransferase; MAT, methionine adenosyltransferase; SAM, S-adenosylmethionine; MS, methionine synthase; BHMT, betaine–homocysteine methyltransferase; 5-MTHF, 5-methyltetrahydrofolate; SAH, S-adenosylhomocysteine; GNMT, glycine N-methyltransferase; MTHFR, methylenetetrahydrofolate reductase; CBS, cystathionine β-synthase; SAHH, SAH hydrolase; ↓, low; (−), inhibits; (+), stimulates; ↑, high.
mean difference $-0.66$; 95% CI $-1.02$, $-0.29$; $P = 0.0004$) (Tuominen et al. 2005).

More recently, a comparison of ninety-one US schizophrenic out-patients with subjects of the Framingham Offspring Study found a significant inverse correlation between serum glycine levels and the severity of negative symptoms as measured by the Scale for the Assessment of Negative Symptoms (SANS) in patients. Serum folate was significantly lower in patient smokers and non-smokers compared with matched controls. Low serum folate ($<3 \text{ ng/mL}$) was present in 16.4% of patients $v.$ 1.7% of controls, with no significant difference in Hcy levels. Multiple regression of sex, serum folate, glycine, serine, cobalamin and total Hcy concentrations, and cigarettes per day, revealed that only serum glycine concentration in smokers and non-smokers, and folate concentration in non-smokers, significantly predicted SANS total scores in patients ($P < 0.05$). No relationship was found between folate concentration and the serine:glycine ratio, suggesting no relationship with SHMT activity (Goff et al. 2004).

Sarcosine is a potent antagonist at the Gly-T-1 site, and may reduce glycine re-uptake. A DBPC trial of sarcosine (2 g/d) as an adjunct to normal therapy in thirty-eight schizophrenic patients revealed a significant effect of sarcosine on measures of positive, negative, and general psychiatric symptoms (Tsai et al. 2004).

**Methylenetetrahydrofolate reductase**

The most well-researched enzyme single nucleotide polymorphism (SNP) in the methionine cycle is the C677T variant of riboflavin-dependent MTHF reductase; the TT variant enzyme has about 35% of normal activity. TT homozygotes benefit from higher folate intake which prolongs enzyme-cofactor binding, increasing overall activity.

MTHF reductase TT and CC homozygotes metabolise folate differently, especially in the presence of riboflavin deficiency (Hustad et al. 2000; McNulty et al. 2002). Low MTHF reductase activity causes formylTHF accumulation at the expense of 5-MTHF, favouring nucleotide synthesis over Hcy re-methylation and ensuring optimum DNA synthesis and repair during adequate folate intake. Conversely, there is some evidence that folate deficiency further reduces 5-MTHF supply for re-methylation, which may lead to global DNA hypomethylation which is associated with carcinogenesis (Ueland et al. 2001). Carrying the TT genotype appears to modulate the risk of developing cancer, with varying effects depending on the cancer site. Preliminary evidence suggests that TT homozygotes may have an increased risk of breast cancer, cervical neoplasia and endometrial cancer, and a decreased risk of acute adult leukaemia and colorectal cancer (Kim, 2000). Curiously, schizophrenics appear to have a reduced cancer risk, in spite of very high smoking rates, low exercise, and poorer diet (Cohen et al. 2002; Grinspoon et al. 2005).

In a Japanese psychiatric population (297 schizophrenic, thirty-two major depression, forty BPD; 419 controls), the TT frequency was significantly greater amongst patients with schizophrenia (odds ratio (OR) 1.9; $P = 0.0006$) and major depression (OR 2.8; $P = 0.005$) (Arinami et al. 1997). In a Canadian population (forty-three treatment-responsive schizophrenics, sixty-two non-responders) the TT frequency was significantly greater in the treatment-responsive group (Joober et al. 2000), but no difference in T allele distribution or TT genotype was found between 210 Spanish schizophrenics and 218 controls (Virgos et al. 1999). However, a recent meta-analysis of six studies (1119 patients, 1308 controls) found that TT homozygotes had a significantly increased risk of developing schizophrenia (OR 1.48; 95% CI 1.18, 1.86) (Lewis et al. 2005).

Another MTHF reductase SNP, A1298C, reduces enzyme activity by 60% in CC genotypes; double homozygotes (677TT/1298CC) are very rare. Found in the SAM-binding region, the A1298C variant enhances SAM-mediated enzyme inhibition. In Canadians heterozygous for both SNP, lymphocyte MTHF reductase activity was 50–60% of CC/AA individuals (Weisberg et al. 1998). Two studies examined the combined MTHF reductase genotypes in schizophrenia. 677CT and TT genotypes were more frequent among ninety-nine male and 135 female Korean schizophrenic patients than controls (OR 2.25; $P < 0.05$), but no difference was found in the frequency of the A1298C SNP (Na & Lee, 2004). Likewise, in a Turkish population of Diagnostic and Statistical Manual (DSM)-IV schizophrenic and schizoaffective neuroleptic-responders (130 patients vs. 226 matched controls), the TT genotype was more frequent in patients (OR 2.504; 95% CI 1.276, 4.915; $P = 0.006$), but in this population the compound TT/AA genotype further increased schizophrenia risk (OR 3.157; 95% CI 1.522, 6.545; $P = 0.001$) (Szaci et al. 2003).

**Folic acid**

5-MTHF is actively transported into the nervous system at the choroid plexus, and its entry is limited by an efficient blood–brain barrier mechanism (Reynolds, 2002). Cerebral spinal fluid (CSF) folate levels are about 3-fold higher than serum folate, indicating the importance of folate to the CNS, yet folate deficiency is the most common vitamin deficiency in the developed world.

Plasma folate $\leq 2.5 \text{ ng/mL}$ or erythrocyte folate $<200 \text{ ng/mL}$ was found in 15–38% of depressed patients from several patient cohorts (Alpert & Fava, 1997). Dietary intake of folate and depressive symptoms were also associated in a cross-sectional study of Finnish middle-aged men. Those in the lowest third of energy-adjusted folate intake had a higher risk of being depressed (OR 1.67; 95% CI 1.19, 2.35; $P = 0.003$) (Tolmunen et al. 2003). The recent draft report from the Scientific Advisory Committee on Nutrition on folate and disease prevention (available at http://www.sacn.gov.uk) cited a prospective study of 2313 Finnish men (42–60 years) followed-up for an average of 13 years (Tolmunen et al. 2004). A higher risk of a discharge diagnosis of depression (relative risk 3.04; 95% CI 1.58, 5.86) was found in those with an energy-adjusted folate intake below the median vs. those above the median (mean energy-adjusted folate intake 256 $\mu$g/d; median intake not stated).

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Of forty-six patients with severe DSM-III depression, twenty-four had significantly higher plasma total Hcy levels than normal (P < 0·01), or neurologically diseased (P < 0·001), control groups. Those with high plasma total Hcy had the lowest erythrocyte, serum and CSF folate levels. The low folate–high total Hcy group also had significantly lower CSF concentrations of the monoamine metabolites 5-hydroxyindole acetic acid, homovanillic acid and 3-methoxy-4-hydroxyphenyl glycol (derived from serotonin, dopamine and noradrenaline respectively). An interesting observation was that CSF folate correlated with serum, but not erythrocyte, folate in both the whole depressed group (r = 0·424; P < 0·02), and in the neurological controls (r = 0·747; P < 0·001), suggesting that serum folate is a reliable marker for CSF folate (Bottiglieri et al. 2000).

Folate status also modulates anti-depressant treatment response; among 213 out-patients with major depressive disorder, those with low serum folate at baseline were less likely to respond to fluoxetine (20 mg/d for 8 weeks) than those with normal folate, as assessed by investigators blind to status. Furthermore, folate therapy alone has proved an effective treatment for depression; an open-label trial of 50 mg methylfolate/d in twenty elderly patients with depressive disorders, of whom only two were deemed folate deficient, was associated with an 81 % response rate over 6 weeks (Alpert & Fava, 1997).

Of 123 patients with major depression or schizophrenia, 33 % had borderline or explicit folate deficiency. In a DBPC trial of 15 mg methylfolate/d for 6 months in deficient patients (twenty-four depressed, seventeen schizophrenic), combined with psychotropic medication, methylfolate significantly improved clinical and social recovery in both patient groups v. placebo (Godfrey et al. 1990). Although there were no significant group differences in the distribution of psychotropic drugs taken, the use of a variety of drugs is a confounding factor in this study.

When thirty-five Dutch schizophrenics were compared with 104 controls, serum folate < 10th percentile (n = 10), and erythrocyte folate > 95th percentile (n = 13) of control levels increased the adjusted risk of schizophrenia (OR 6·9, 95 % CI 2·1, 23·1, P = 0·001; OR 10·8, 95 % CI 2·8, 41·3, P < 0·001 respectively) (Muntjewerff et al. 2003). This folate profile is consistent with reduced MTHF reductase activity, but no increase of the C677T SNP was found; however, the TT frequency in controls (18 %) was higher than in another Dutch population (8 %), and the number of patients was small.

An American study (post-folate fortification) found significantly lower serum folate in ninety-one schizophrenic out-patients compared with controls from the Framingham Offspring Study (5·74 v. 10·00 ng/ml; P < 0·001). Folate levels of smokers (4·87 ng/ml) and non-smokers (7·21 ng/ml) were both significantly lower than controls. Furthermore, folate concentration inversely correlated with SANS total score, significantly in non-smokers but not in smokers (Goff et al. 2004).

A study of Israeli schizophrenic in-patients from a socially deprived area found 75 % of male and 65 % of female patients had low plasma folate levels (< 3·7 ng/ml) (Stahl et al. 2005). Interestingly, only 6·4 % of the male patients displayed abnormal mean corpuscular volume, suggesting that folate supply for DNA synthesis was maintained at the expense of the re-methylation pathway. The authors comment that a similar study in a less disadvantaged area of Israel found less folate deficiency (5·6 % < 2·5 ng/ml v. 41 % < 2·5 ng/ml), and that low folate is therefore not a constant feature of schizophrenia. However, the latter patients had low cobalamin, suggesting that the common denominator could have been reduced Hcy re-methylation due to cofactor deficiency.

It is interesting that depression is more frequently associated with folate deficiency than cobalamin deficiency, when both reduce MS activity. This could simply be because folate deficiency is more common, but may be mediated by GNMT. In cobalamin deficiency, 5-MTHF is trapped and levels increase, inhibiting GNMT and conserving SAM for other methyltransferase activities. However, when cobalamin is adequate and folate is deficient, GNMT remains active, competing with other methyltransferases for a reduced SAM supply. The mechanism by which folate influences mood is currently unknown, but the effects on SAM supply may be a contributing factor.

Folate supplementation above a therapeutic window may actually worsen depressive symptoms in patients (Alpert & Fava, 1997). Doses of 15 mg/d have been associated with mental changes in healthy volunteers. Sleep alterations, malaise, hyperactivity and irritability have been reported; symptoms similar to those of deficiency (Hunter et al. 1970). An animal study also suggests that folate exerts a U-shaped pattern of effect; when low CSF 5-hydroxyindole acetic acid levels were found in patients with folate-responsive depression, brain serotonin was determined in rats fed folate-deficient or excess-folate diets; both had reduced serotonin (Botez et al. 1979).

Methionine synthase and cobalamin

Only two human enzymes use cobalamin as a cofactor; MS and methylmalonyl mutase. MS re-methylates Hcy, using 5-MTHF as the methyl donor, and requires niacin-dependent MS reductase to reductively activate MS, since the enzyme–vitamin complex is susceptible to oxidation. When MS activity is impaired, THF cannot be regenerated from 5-MTHF, resulting in reduced nucleotide biosynthesis and macrocytosis. The brain has an absolute requirement for cobalamin for Hcy re-methylation since the BHMT pathway is absent. Although brain re-methylation has not been quantified, an animal study suggests that it must be substantial: when MS was inactivated by placing pigs in an atmosphere containing 15 % nitrous oxide for 7 d, SAH increased 10-fold in the brain and spinal cord. Furthermore, MS recovery (via new enzyme synthesis) took several days in liver, kidney and brain, and > 2 weeks in spinal cord. Changes in MS activity were closely correlated with the SAM:SAH ratio in the brain, but not the liver, confirming the importance of MS to brain function (Molloy & Weir, 2001). Hypoxia also inactivates MS, and mountain climbers suffering from hypoxia report hallucinations, a prominent symptom of psychosis.

Interestingly, an in vitro study using radiolabelling techniques demonstrated that supplementary cobalamin
increases MS activity 2–3·5-fold, even when no deficiency exists, enhancing Hcy flux through the transmethylation pathway, without affecting methylmalonyl mutase activity (Oltean & Banerjee, 2003).

Severe cobalamin deficiency causes upper spinal cord degeneration, and eventually death. Neuropsychiatric symptoms often precede haematological symptoms of deficiency (Lindenbaum et al. 1988; Weir & Scott, 1995; Gullette et al. 2003). A review of the literature showed that psychiatric symptoms might be the initial or only sign of deficiency; most common signs are depression, mania, psychosis, cognitive impairment and obsessive–compulsive disorder (Durand et al. 2003).

In the Rotterdam Study, 3884 elderly individuals were screened for depressive symptoms. Of these subjects, 112 fulfilled the DSM-IV criteria for depressive disorders. Cobalamin-deficient subjects were significantly more likely to have a depressive disorder than controls (adjusted OR 1·69; 95 % CI 1·10, 2·56) (Tiemeier et al. 2002). It should be noted that serum holotranscobalamin or methylmalonate are better markers of active cobalamin status than serum cobalamin.

**Methionine**

The finding that a methionine load exacerbates symptoms of psychosis in schizophrenic patients is frequently cited in the literature. However, the original studies used methionine in combination with monoamine oxidase inhibitors (for a review, see Wyatt et al. 1971), which are now known to exacerbate psychosis.

In a DBPC cross-over study comparing methionine (without monoamine oxidase inhibitors) and glycine treatment in schizophrenic patients, seven out of eleven patients showed a marked exacerbation of psychotic symptoms when fed 20 g methionine/d for 1 week, followed by 10 g/d for 1 week. No patients reacted negatively to glycine, whilst one patient showed significant improvement with methionine (Antun et al. 1971). The improved sociability of four patients noted by ward staff may have been due to the effect of glycine on negative symptoms, which were unknown at the time, and hence confounded this study.

However, CSF methionine was significantly higher in thirty-six (sixteen female, twelve male) psychotic patients v. twenty-five controls. CSF methionine levels in drug-naive patients (4·3 (SD 1·3) μmol/l; n 14) were similar to other patients (4·5 (SD 1·4) μmol/l; n 22), and all patients had significantly higher CSF methionine than controls (2·8 (SD 1·0) μmol/l; P < 0·0001). Ten patients had levels higher than any controls (Regland et al. 2005).

**Methionine adenosyltransferase**

When ninety psychiatric patients were investigated on admission to hospital, the $V_{max}$ of erythrocyte methionine adenosyltransferase activity was low in depressed and schizophrenic patients, and over-active in manic patients. After pharmacological treatment the $V_{max}$ values were normalised in all patient groups (Tolbert et al. 1988).

**S-adenosylmethionine**

SAM is the methyl-donor in the rate-limiting step in the synthesis of serotonin, dopamine and noradrenaline. Studies in Alzheimer’s, HIV and depressed patients have shown that orally administered SAM crosses the blood–brain barrier and increases CSF SAM concentrations. The efficacy of SAM in treating depression has been demonstrated in more than forty clinical trials, and two reviews of the evidence concluded that SAM is as effective as standard anti-depressants (imipramine, chlorimipramine, nomifensine, and minaprine) with significantly fewer side effects (Bottiglieri, 2002; Mischoulon & Fava, 2002).

Two multicentre, randomised DBPC studies (double dummy design) compared SAM treatment with the tricyclic anti-depressant imipramine in major depression. In the first, 143 patients received up to 1600 mg SAM/d and six tablets per d of imipramine placebo, whilst 138 received 150 mg imipramine/d and SAM placebo, for 6 weeks. In the second, imipramine (n 148) was compared with 400 mg SAM/d, injected intramuscularly (n 147) (the placebo SAM injection contained mannitol). In both studies, SAM treatment was as effective as imipramine but the safety and tolerability of SAM was significantly superior to imipramine ($P = 0·001$) (Chiaie et al. 2002).

SAM is also reported to improve cognitive deficits in dementia, and is effective for treating depression associated with Parkinson’s disease, alcoholism and the puerperium. However, SAM supplementation mediates a switch from depression to mania in some patients with BPD (Bottiglieri, 2002; Mischoulon & Fava, 2002). A small study found a 50 % decrease in blood levels of SAM in acute, but not chronic, schizophrenics v. controls ($P < 0·001$) (Andreoli & Maffei, 1975), but no recent data are available.

**Methyltransferase activity**

Methyltransferases catalyse the transfer of a methyl group from SAM to a recipient molecule, producing SAH and the methylated product. SAH competitively binds to SAM, reducing phosphatidylethanolamine (PE) methyltransferase (PEMT) activity by 53 % (in rat liver), and catechol-O-methyltransferase (COMT) activity by 69 % (in human brain). Other methyltransferases with CNS effects include histamine N-methyltransferase (inactivates histamine), phenylethanolamine N-methyltransferase (synthesises adrenaline), arginine methyltransferase (methylates myelin basic protein), and hydroxy-indole-O-methyltransferase (synthesises melatonin) (Clarke & Banfield, 2001). Interestingly insomnia, a common symptom of psychiatric illness, is associated with melatonin depletion. In rats, folate deficiency decreased pineal melatonin concentration, and nocturnal urinary excretion of melatonin metabolites (Fournier et al. 2002).

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COMT inactivates circulating catechol hormones, catechol neurotransmitters (for example, dopamine, adrenalin, noradrenaline), and xenobiotic catecholamines (for example, caffeeic acid, t-dopa) by methylating their catechol moieties. An SNP of the COMT gene (val<sup>158</sup>met or H/L), accounts for a 4-fold variation in enzyme activity, the val allele having four times the activity of the met allele. The population distribution is about 33% val,val, 46% val/met, and 21% met/met. COMT genotype distribution did not vary between 181 Alzheimer’s patients and 208 age-matched controls; however, among patients with psychosis (32·6%), 88·1% were COMT H carriers (P < 0·01). The adjusted OR for psychosis risk in COMT H carriers was 2·66 (95% CI 1·6, 6·62) (Borroni et al. 2004). The H allele was also significantly associated with phobic anxiety development in a population of 1234 women. The OR for scoring ≥ 6 ≥ 0 or 1 on the phobic anxiety scale of the Crown-Crisp Experimental Index was 1·99 (95% CI 1·17, 3·40) for the HH genotype (McGrath et al. 2004). Conversely, a meta-analysis found only a weak association between the SNP and schizophrenia in European, but not Asian, patients (Glatt et al. 2003). Most recently, a large association study in a Han Chinese population found no effect of COMT polymorphism on schizophrenia risk, and a meta-analysis of all studies to date came to the same conclusion (Fan et al. 2005).

However, increased COMT activity does negatively influence the performance of frontally mediated cognitive tasks. A higher number of met alleles was associated with fewer perseverative errors in the Wisconsin Card Sorting Task in healthy individuals, but their schizophrenic siblings had fewer perseverative errors in the Wisconsin Card Sorting tasks. A higher number of met alleles was associated with better performance in the Wisconsin Card Sorting tasks. The working memory improvements in met carriers were confirmed in schizophrenics taking a range of anti-psychotics in another small study (Weickert et al. 2004). This has been demonstrated in L-dopa-treated Parkinson’s patients, and confirmed in animal studies. t-Dopa is a COMT substrate, and t-dopa-treated patients have increased plasma total Hcy (Miller et al. 2003; Muller et al. 2004). Interestingly, symptoms of psychosis and mood changes indistinguishable from those seen in schizophrenia often develop in Parkinson’s patients after long-term t-dopa treatment, or following an increase in dose (Doraiswamy et al. 2003). Furthermore, when thirty schizophrenics were assessed before and after anti-psychotic treatment (olanzapine for 8 weeks), met allele load significantly predicted improvements in working memory, prefrontal cortex activation, and negative symptoms (Bertolino et al. 2004). The working memory improvements in met carriers were confirmed in schizophrenics taking a range of anti-psychotics in another small study (Weickert et al. 2004).

The high activity COMT val/met genotype consumes four times as much SAM as the met/met genotype. This high methylation demand may detrimentally affect memory by impacting on other SAM-dependent neurotransmitter pathways. Decreased SAM availability for the de-activation of neurotransmitters may even lead to increased endogenous hallucinogens, as proposed by the transmethylation hypothesis (Hoffer et al. 1954); the hallucinogen adrenochrome is a by-product of adrenaline which is inactivated by COMT. Lastly, stress causes the production of large amounts of biogenic amines, which also increases methylation demand (Levi & Waxman, 1975).

A kinetic study of the transfer rate of the radio-tagged methyl group of methionine to CO₂ in breath showed that the rate was three times slower in seven schizophrenic patients, with no overlap of data points (Sargent et al. 1992). Since surplus methyl groups are oxidised to CO₂ via the methylation of glycin by GNMT, this study suggests that reduced SAM synthesis or an increased methylation demand is associated with schizophrenia.

**Homocysteine**

The betaine pathway of Hcy re-methylation is absent in the brain, and the activity of cystathionine β-synthase is about 20% of hepatic cystathionine β-synthase activity, whilst cystathionine γ-lyase is believed to be absent (Weir & Molloy, 2000). Hence, brain tissue has only three ways of metabolising Hcy: recycling via MS; catabolism to cystathionine; export into the circulation.

Recent studies found significantly increased plasma total Hcy levels in DSM-III depression (Bottiglieri et al. 2000), BPD (Osher et al. 2004) and schizophrenia (Susser et al. 1998; Levine et al. 2002; Na & Lee, 2004; Applebaum et al. 2004), with one negative study in schizophrenics (Muntjewerff et al. 2003). However, none of the studies gave full data on the many factors known to increase plasma total Hcy, for example, smoking, coffee intake, and low folate or cobalamin status. Nevertheless, a DBPC cross-over trial of Hcy-lowering nutrients (2 mg folic acid, 25 mg pyridoxine and 400 µg B₁₂ once per d for 3 months) in highly symptomatic schizophrenics with plasma total Hcy > 15 µmol/l (seventeen male, one female) had a significant effect on positive (+2·4 points), negative (+1·6 points) and general psychopathology (+3·4 points) subscales of Positive and Negative Syndrome Scale scores (Shumeiko et al. 2003).

Several mechanisms of Hcy neurotoxicity have been proposed including NMDA receptor activation or hypofunction, enhanced oxidative stress, DNA damage and apoptosis. Hcy is a partial agonist at the NMDA glutamate site, and a partial antagonist at the glycine co-agonist site. However, whereas Hcy was neurotoxic at concentrations of 10–100 µmol/l, causing over-stimulation of the NMDA receptor when glycine was elevated (as in stroke or head trauma), under normal conditions Hcy was not toxic until in the millimolar range (Lipton et al. 1997). Furthermore, a recent study found no difference in CSF Hcy levels between forty-one schizophrenic patients and twenty-nine controls (Levine et al. 2005). Thus, it seems likely that elevated plasma total Hcy is a biomarker of disturbed methylation status in neuropsychiatric disorders, rather than the cause of
psychiatric disturbance, although high levels should clearly be treated to reduce the high risk of CVD in schizophrenics.

Restoring normal methylation

A recent study of methylation factors in twenty autistic children v. thirty-three controls found significantly reduced plasma levels of methionine, SAM, SAM:SAH, total Hcy, cystathionine, cysteine, total glutathione and total glutathionine:GSSG, along with significantly increased levels of SAH, adenosine and GSSG (Table 1). This profile describes reduced methylation capacity and increased oxidative stress. An intervention in eight children (800 μg folic acid and 1000 mg betaine twice daily for 3 months), brought the methionine cycle metabolites within the normal range, and the addition of twice-weekly injections of 75 μg methylcobalamin/kg for a further 1 month normalised the trans-sulfuration pathway metabolites. The researchers also noted behavioural improvements, but these details have not yet been published (James et al. 2004).

Discussion

Whilst most aspects of one-carbon metabolism have been associated with psychiatric symptoms, the most replicated biomarkers to date appear to be low serum folate and high plasma total Hcy. However, psychiatric complications do not occur in most folate-deficient–high total Hcy subjects. Hence a combination of factors may be necessary to elicit psychiatric symptoms, for example, a genetic pre-disposition mediated by one or more SNP, plus poor diet, and a stress- or psychiatric symptoms, for example, a genetic pre-disposition contributes to plasma total Hcy levels. Observations in Pemt-/- mice found a 50% reduction in plasma total Hcy compared with controls. Hepatic methylation enzyme activities did not differ from Pemt +/- mice, and SAM was not depleted in Pemt-/- cells, hence the PEMT pathway appears to be responsible for about 50% of plasma total Hcy load in mice (Noga et al. 2003).

Significantly, a stable-isotope study demonstrated a profound distinction in PC profiles between those produced via the PEMT and CDP-choline pathways. PC molecules produced from the CDP-choline pathway were mainly comprised of medium-chain, saturated fatty acids, whilst those derived from the PEMT pathway contained significantly more long-chain PUFA (LCPUFA) (DeLong et al. 1999). Furthermore, the quantitative profiling of lipid metabolites in Pemt-/- mice demonstrated a key role for the PEMT pathway in regulating the distribution of arachidonic acid and DHA to peripheral tissues (Watkins et al. 2003). PEMT deficiency significantly reduced: hepatic and plasma PC DHA content; plasma PC arachidonic acid and stearic acid; plasma cholesteryl ester DHA and arachidonic acid. These studies suggest that PEMT activity is almost solely responsible for the mobilisation of DHA and arachidonic acid into plasma, and hence to peripheral

Methylation and phospholipids

Methylation and phospholipid metabolism

Dysregulation of phospholipid metabolism in schizophrenia, major depression and BPD is well documented (Horrobin, 1999; Peet & Bennett, 1999; Sarmiento et al. 1999). Schizophrenia is associated with reduced erythrocyte membrane arachidonic acid and DHA, hypothesised to be due to the over-activity of phospholipase A2 (PLA2) and PLC that release arachidonic acid from the Sn-2 position of phosphatidylcholine (PC).

PC is produced in the liver via three pathways: transfer of phosphocholine to 1,2-diacylglycerol via cytidine diphosphate (CDP)-choline (the Kennedy pathway) which is choline dependent; sequential methylation of PE catalysed by PEMT, consuming three molecules of SAM; acylation of lysophosphocholine. The pool of PC produced via transmethylation, although much smaller, is more biologically active than the CDP-choline-derived pool (Hirata & Axelrod, 1980; Watkins et al. 2003).

The PEMT pathway is responsible for about 30% of hepatic PC production and, since the synthesis of each PC molecule produces three molecules of SAH, significantly contributes to plasma total Hcy levels. Observations in Pemt-/- mice found a 50% reduction in plasma total Hcy

<p>| Table 1. Comparison of plasma one-carbon cycle metabolites in control children and autistic children, pre- and post-treatment with vitamin cofactors (adapted from James et al. 2004) |
|-------------------------------------------|-------------------------------------------|-------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Mean SD Range</th>
<th>Mean SD Range</th>
<th>Mean SD</th>
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<tbody>
<tr>
<td>Methionine (μmol/l)</td>
<td>31.5 5.7 23–48</td>
<td>19.3*** 9.7 15–25</td>
</tr>
<tr>
<td>SAM (nmol/l)</td>
<td>96.9 12 77–127</td>
<td>75.8** 16.2 68–100</td>
</tr>
<tr>
<td>SAH (nmol/l)</td>
<td>19.4 3.4 16–27</td>
<td>28.9*** 7.2 14–41</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>5.2 1.3 4–8</td>
<td>2.9*** 0.8 2–4</td>
</tr>
<tr>
<td>Adenosine (μmol/l)</td>
<td>0.27 0.1 0.1–0.4</td>
<td>0.39* 0.2 0.17–0.83</td>
</tr>
<tr>
<td>Total homocysteine (μmol/l)</td>
<td>6.4 1.3 4.3–9.0</td>
<td>5.8* 1 4.0–5.8</td>
</tr>
<tr>
<td>Cystathionine (μmol/l)</td>
<td>0.17 0.05 0.1–0.27</td>
<td>0.14* 0.06 0.04–0.2</td>
</tr>
<tr>
<td>Cysteine (μmol/l)</td>
<td>202 17 172–252</td>
<td>163*** 15 133–189</td>
</tr>
<tr>
<td>Total glutathione (μmol/l)</td>
<td>7.6 1.4 3.8–9.2</td>
<td>4.1*** 0.5 3.3–5.2</td>
</tr>
<tr>
<td>GSSG (nmol/l)</td>
<td>32.9 2.1 20–43</td>
<td>0.29 0.07</td>
</tr>
<tr>
<td>Total glutathione:GSSG</td>
<td>25.8 8.9 13–49</td>
<td>8.6*** 3.5 4–11</td>
</tr>
</tbody>
</table>

SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

Mean value was significantly different from controls: *P < 0.05, **P < 0.01, ***P < 0.002, ****P < 0.0001.
tissues. Consequently, any factor that inhibits PEMT activity (which is regulated by the concentration of PE, SAM, and SAH) also modifies plasma and membrane FA composition. Three animal studies illustrate this effect. SAM:SAH fell from 5.6 to 0.3 in rat livers perfused with Hcy and adenosine, and the incorporation of labelled methionine into PC was decreased >99% (Vance et al. 1997). Rats fed a folate-deficient diet for 6 weeks showed a significant reduction of plasma EPA (P < 0.01), DHA (P < 0.0001) and arachidonic acid (P < 0.01), whilst platelets had reduced EPA and DHA (P < 0.05) and increased arachidonic acid (P < 0.01). Incorporation of [14C]arachidonic acid into platelets from folate-deficient rats was significantly greater than controls (P < 0.01), and thrombin-stimulated mobilisation of arachidonic acid was also greater (P < 0.002) (Durand et al. 1995). The PC:PE ratio, a marker of membrane fluidity, was lowest in rat livers between 24.00 hours and 02.00 hours when SAH was high, whereas maximum PC:PE correlated with one of the SAM:SAH maxima (Chagoya de Sanchez et al. 1991).

Of interest in the context of FA supplementation trials in schizophrenia is that plasma and tissue EPA was not depleted in Pent -/- mice, suggesting that EPA transport may not be PEMT-dependent. If the PEMT pathway is compromised in schizophrenia this may explain why, in a DBPC trial, EPA supplementation (2 g/d) was associated with improved scores on the Positive and Negative Syndrome Scale, whilst DHA treatment was ineffective (Peet et al. 2000).

**Choline deficiency**

Choline plays a dual role in phospholipid metabolism: directly as a precursor of PC in the Kennedy pathway; indirectly as a precursor of betaine which produces methionine, and hence SAM, via BHMT. Choline-derived SAM can then be used to synthesise PC via the PEMT pathway (DeLong et al. 2002). Choline deficiency therefore impacts on both major pathways of PC synthesis.

Dietary choline makes a significant contribution to methylation status via BHMT, which has been overlooked in many studies examining diet and Hcy levels (Zeisel et al. 2003). Although this pathway is not present in the brain, the effects of oral SAM on mood demonstrate that it crosses the blood-brain barrier; hence, choline-derived SAM also impacts on cerebral methylation.

High PLA2 activity is associated with schizophrenia (Ross et al. 1997; Glen et al. 2003a). Interestingly, choline deficiency increases PLA2 activity; a study in mice fed a choline-deficient diet found PLA2 activity increased by 50% after 1 week and by 300% after 4 weeks (Singh et al. 1990).

Highly elevated skeletal muscle-derived serum creatine phosphokinase (CPK_M) is frequently found in schizophrenic and bipolar patients during episodes of acute psychosis (Manot et al. 1998). It was assumed that this was due to muscle damage caused by tension or agitation during psychosis. However, many patients are hypoxic at such times, and no evidence of myoglobinuria or pigmenturia accompanying CPK-emia was found in a study of twenty-five acute psychotic patients, which might be expected if muscle damage were the cause (Hermesh et al. 2001). Interestingly, in three of four human volunteers fed a choline-deficient diet for up to 42 d, CPK_M levels were increased up to 66-fold (P < 0.01). In addition, mouse myocytes grown in a choline-deficient medium leaked 3.5-fold more CPK_M than controls (P < 0.01); PC concentrations were 43% of control levels, as were intracellular choline, phosphocholine, and glycerophosphocholine. The deficient cells had greater osmotic fragility, resulting in increased CPK_M leakage (da Costa et al. 2004). These findings suggest a role for choline or PC deficiency in a previously unexplained physiological symptom of psychosis.

Human hippocampal choline, measured by proton magnetic resonance spectroscopy, decreased in major depressive episodes and normalised in parallel with treatment response to electroconvulsive therapy (Ende et al. 2000). Choline increase was also found in electroconvulsive-therapy-treated rats (Sartorius et al. 2003).

**Membrane fluidity**

Much has been written about the influence of LCPUFA on membrane fluidity and hence receptor function; however, the degree of phospholipid methylation (PLM) is also a significant determinant of fluidity. Whilst diet influences membrane composition in the long term, PLM provides short-term local regulation of phospholipid packing density by increasing the spacing between head groups (Hirata & Axelrod, 1980; Zhao et al. 2001).

PLM plays an important role in transduction of receptor-mediated signals through cell membranes. As phospholipids are successively methylated, they are translocated from inside the cell membrane to the exterior (Hirata & Axelrod, 1980). For example, MET313 of the dopamine D4 receptor is on the inner surface of the cell membrane and supplies a methyl group for local methylation of PE to PC in response to dopamine stimulation. 5-MTHF is then required for the re-methylation of D4 receptor key to D4 receptor Met. In vitro studies showed that 14 d after 5-MTHF supply was compounded, basal PLM was reduced, whilst dopamine-stimulated PLM increased by 200%. However, after 28 and 42 d basal PLM recovered and dopamine-stimulated PLM fell below initial levels (Zhao et al. 2001). The authors suggest that reduced basal PLM may alter the D4 receptor membrane environment, increasing agonist responsiveness. A study examining PLM in lymphocytes of male schizophrenics aged 27–70 years found basal PLM reduced by 3-5-fold compared with controls, indicating a profound defect in this pathway. Furthermore, dopamine stimulated methylation by 30% in controls, v. 165% in patients (Deth et al. 1996).

**Niacin skin flush test**

A reduced skin-flush reaction in response to topical niacin has been replicated in forty-one schizophrenic populations worldwide (I Glen, personal communication; Maclean et al. 2003).

As Fig. 3 illustrates, niacin stimulates the synthesis of newly derived PC from arachidonic acid-rich PE via the
PEMT pathway, and activates PLA2 to release free arachidonic acid. Prostaglandin (PG)D synthase, cyclo-oxygenase-1 and cyclo-oxygenase-2 then produce PGD2 from free arachidonic acid. PGD2 acts upon capillary endothelial cells causing vasodilatation, producing the skin-flush response (Bennett & Horrobin, 2003).

Although the increased PLA2 activity found in schizophrenia implies an increased flush response, it is hypothesised that increased activity leads to desensitisation after over-stimulation of the pathway, or that reduced membrane arachidonic acid reduces the substrate available for PGD synthase.

Alternatively, since low cellular methylation potential reduces PEMT activity, this pathway may be disrupted by inadequate formation of newly derived PC from PE due to high SAH and/or low SAM.

Sex differences and effects of diet

In mice fed a high-fat high-cholesterol diet, PC production increased to support hepatic export of bile and lipoprotein. Cytidyldyl transferase activity, the rate-limiting enzyme of the Kennedy pathway, was unchanged; hence the extra PC was produced via the PEMT pathway (Drouva et al. 1987; Noga & Vance, 2003). Some consequences of a high-fat high-cholesterol diet, therefore, are an increased methylation demand, and higher SAH and Hcy production.

Sex differences exist in the phospholipid metabolism of mice; hepatic PEMT activity in females is double that in males, whilst males have more cytidyldyl transferase activity. In rats, oestrogen stimulated PE methylation in pituitary membranes, and elevated plasma oestradiol was associated with increased PEMT activity (Drouva et al. 1987). Oestrogen also stimulates the desaturases needed for the production of LCPUFA from their parent EFA; thus females may be better equipped to both synthesise and transport LCPUFA to the periphery.

Interestingly, puerperal psychosis has been linked to oestrogen levels. Psychosis risk is twenty-two times higher in the first month post-partum than pre-pregnancy. At term, serum oestradiol is very high and of placental origin; the concentration falls precipitously within a few days of parturition and recovery of ovarian production can be slow (Ahokas et al. 2000). In some patients, further psychotic episodes occur pre-menstrually (Brockington & Meakin, 1994).

Lithium and valproate

Both Li and valproate are effective mood stabilisers in BPD, despite their structural dissimilarity and differing biochemical effects. A study was conducted in yeast cells to determine common drug targets. Both drugs increased the rate of PC synthesis, and decreased phosphatidylinositol synthesis. The authors suggest that restoration of the phosphatidylinositol:PC ratio required for secretory vesicle formation is therapeutic (Ding & Greenberg, 2003). If cellular methylation potential is disturbed in BPD, reduced PEMT activity would also disturb the phosphatidylinositol:PC ratio; restoration of normal methylation might therefore stabilise mood.

Finally, significantly reduced PC methylation was found in Li-responsive patients. Ex vivo evidence demonstrated that Li increased PLM in responders, but not non-responders (Kingsbury & Garver, 1998).

Oxidative stress

Increased oxidative stress may be a causative factor in the phospholipid disturbances found in schizophrenic and bipolar patients. The brain is under higher oxidative stress than other organs since it produces high reactive oxygen species levels and enzymic antioxidant defence is relatively poor. LCPUFA-rich plasma membranes are the preferred target for reactive oxygen species-mediated injury, hence neural membrane lipids are especially sensitive to peroxidation.

Reduced membrane LCPUFA and increased plasma lipid peroxides as measured by thiobarbituric acid-reactive substances have been reported in both medicated and never-medicated schizophrenics (Arvindakshan et al. 2003), and in first-episode psychotic patients (Khan et al. 2002). Furthermore, analysis of expired ethane levels, a specific marker of n-3 fatty acid peroxidation, showed significantly greater median levels in schizophrenics than controls (16 (50% interquartile range 0·6) v. 0·2 (50% interquartile range 0·4) parts per billion); the difference was still significant between control and schizophrenic smokers (Glen et al. 2003b).

Glutathione

GSH is constantly exported from the liver, but plasma levels are kept relatively low by the activity of the cell surface enzyme γ-glutamyl transpeptidase, which degrades GSH to cys-gly and then cysteine. Since the CNS lacks the full trans-sulfuration pathway, this process is essential for transporting the building blocks of GSH to the CNS. Thus, hepatic thiol status is directly related to CNS antioxidant defence.

In a study of twenty-one drug-naïve, and five drug-free schizophrenics, CSF GSH was significantly reduced by 27% (P < 0·05) compared with controls, whereas levels of dopamine and serotonin metabolites were similar.

Fig. 3. The biochemical sequence of the skin-flush response. PE, phosphatidylethanolamine; PC, phosphatidylcholine; AA, arachidonic acid; PG, prostaglandin; PEMT, phosphatidylethanolamine methyltransferase; PLA2, phospholipase A2; COX, cyclo-oxygenase.
Furthermore, when magnetic resonance spectroscopy was used to determine GSH levels in the pre-frontal cortex of fourteen male schizophrenic in-patients, five previously treated with neuroleptics, five drug-naïve, and four drug-free for at least 6 months, GSH was reduced by 52% compared with controls ($P = 0.0012$) (Do et al. 2000). Since dopamine metabolism contributes to oxidative stress, the authors suggest that a GSH deficit would result in degenerative processes surrounding dopaminergic terminals. Also, since GSH potentiates the NMDA receptor response to glutamate, low GSH may result in hypoaactivation of NMDA receptors. A follow-up study investigated the effects of dopamine in cultured cortical neurons with low GSH; dopamine decreased GSH by 40%, and a 24 h application of dopamine significantly decreased neuronal processes (Grima et al. 2003).

The previously cited study in autism demonstrated that disruption of the one-carbon cycle can be associated with diminished GSH and hence reduced antioxidant capacity (James et al. 2004). It is therefore feasible that lipid peroxidation in schizophrenia could be mediated by low GSH, resulting from one-carbon cycle disruption. Conversely, oxidative stress impinges directly on the one-carbon cycle, since the cobalamin–MS complex is easily oxidised; methionine adenosyltransferase and BHMT are also sensitive to oxidation. Consequently, any endogenous or exogenous insult that increases cellular oxidative stress would impinge on both phospholipid and methylation status.

Methylation and epigenetics

Epigenetic modification of gene expression

Epigenetics is defined as the modification of gene expression with no alteration in DNA base sequence. Methylation is one of several known epigenetic mechanisms that facilitate short-term adaptation of genomic DNA to the local environment. DNA methylation occurs when a methyl group is transferred from SAM to cytosine residues in the dinucleotide sequence CpG. Dense methylation of CpG ‘islands’ found predominantly in the gene promoter region is generally associated with irreversible gene silencing, whilst the more common partial methylation is associated with genes that can be reactivated. Methylation silences gene expression by two different mechanisms. Methylation of transcription factor binding sites directly inhibits transcription without necessarily altering chromatin structure. Alternatively, methylated DNA binding proteins are recruited to a densely methylated region and target histone deacetylases to the chromatin, resulting in histone deacetylation and chromatin inactivation (Abdolmaleky et al. 2003; Weaver et al. 2005).

Valproate, a drug commonly used for treating epilepsy, mood disorders and schizophrenia, is an histone deacetylases inhibitor which induced DNA demethylation (Detich et al. 2003) and increased gene expression in vitro (Chen et al. 2002), and re-activated cerebral genes in vivo whose activity had been down regulated by supplementing mice with methionine (Tremolizzo et al. 2002).

Dysregulation of the methylation cycle may have different effects on gene methylation and expression depending on the tissue, the specific gene and region of the gene involved. Hence, it is difficult to predict the effect that dietary methyl source insufficiency (or excess), and consequent DNA methylation dysregulation, will have on individual gene expression. However, the finding that the expression of key genes associated with psychiatric illnesses can be manipulated by diet (see p. 129 of proof) suggests that this is an area worthy of more research.

Epigenetics and schizophrenia

Despite a decade of genetic research, no DNA mutations have yet been identified that suggest a cause of, or genetic predisposition to, the development of psychiatric illness. The concordance rate for psychiatric phenotypes in monozygotic twins is well below 100%: 41–65% in schizophrenia; 62–79% in BPD; 31% for males and 48% for females in major depression.

However, preliminary findings suggest that epigenetic DNA modifications resulting in altered gene expression may underlie the phenotypic differences between monozygotic twins. A study comparing the content and distribution of methylated DNA and histone acetylation in peripheral lymphocytes between monozygotic twins aged 3–74 years found that young twins were epigenetically indistinguishable, whilst older twins exhibited significant differences affecting their gene expression portrait (Fraga et al. 2005). In a study comparing the 5′-regulatory regions of the dopamine D2 receptor gene in two sets of monozygotic twins (aged 24 years), one concordant and one discordant for schizophrenia, the patient from the discordant pair was found to be epigenetically ‘closer’ to the concordant pair than to his unaffected twin (Petronis et al. 2003).

Reelin and glutamic acid decarboxylase67

Reelin is a glycoprotein that regulates neuronal migration in the developing embryonic brain, and is also expressed in the adult brain. In adults, reelin is secreted from γ-aminobutyric acid (GABA)ergic interneurons into the extra-cellular matrix (Carboni et al. 2004; Fatemi et al. 2005), and is currently known to bind to two apo receptors, ApoER2 and VLDL receptor, and to the integrin receptor. Reelin potently enhances synaptic transmission, and strengthens between-neuron synaptic contacts, believed to be one of the processes underlying memory. Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the conversion of glutamate to GABA, and hence regulates glutamate and GABA levels in the brain. Deficits in brain reelin levels affect memory processing, learning, synaptic organisation and cognition (Fatemi et al. 2005).

In all post mortem schizophrenic brains examined to date, a reduction in reelin and GAD67 gene expression and about 50% reduction in reelin protein levels have been found in all brain areas examined, including multiple cortical regions, hippocampi and cerebella (Impagnatiello et al. 1998; Guidotti et al. 2000). Down regulation of reelin and GAD67 was also found in the brains of bipolar patients, and a non-significant trend for
reduced reelin was observed in depression. Analysis of post mortem markers in pre-frontal cortices showed that GAD$_{67}$ protein was the most useful marker for discriminating between schizophrenia, BPD and depression. Significant reductions in GAD and reelin were also observed in autistic subjects; the reelin deficit was greater than in schizophrenia and BPD (Fatemi et al. 2005).

Although little is known about the regulation of reelin expression in mature neurons, incorporation of the methylation inhibitor 5-aza-deoxycytidine activated reelin expression >50-fold in vitro, whilst methylation of the reelin promoter silenced expression. Furthermore, the histone deacetylase inhibitors trichostatin A and valproate significantly increased reelin mRNA levels (Chen et al. 2002).

Heterozygous reeler mice

An animal model of reelin insufficiency, the heterozygous reeler mouse (HRM), exhibits some of the neuropathology detected in schizophrenia including: decreased extracellular reelin, with a marked decrease in dendritic spine expression density in pyramidal neurons of the cortex and hippocampus, and decreased cortical thickness; decreased GAD$_{67}$ expression in frontal cortex, and an alteration in the mesolimbic dopamine system, but not the nigro-striatal system; a deficit in sensorimotor gating or pre-pulse inhibition of startle, and an exaggerated response to fear; significantly poorer performance on an olfactory discrimination task. The similarities between this model and autism have also been noted (Carboni et al. 2004; Fatemi et al. 2005).

Dizocilpine is a non-competitive NMDA receptor antagonist. Such antagonists cause cognitive impairment and psychotic symptoms in normal human subjects indistinguishable from those observed in schizophrenia, and exacerbate psychosis in schizophrenics. A recent study suggested that Dnmt-1 mRNA-positive neurons was inversely correlated with the number of reelin mRNA-positive neurons in cortical GABAergic interneurons, independent of neuroleptic type. Dnmt-1 activity was significantly greater in schizophrenic than in non-psychiatric controls. Furthermore, one patient with no history of neuroleptic use had results well within the range of medicated patients (Veldic et al. 2004a). Other groups using different patient cohorts have confirmed these findings. A follow-up study found that Dnmt-1 expression was increased in psychotic patients taking valproate alone (n 4) or anti-psychotics alone (n 15) but not in those taking both (n 9) (Veldic et al. 2004b).

In an in vitro study of mouse embryonic cortical neurons, Dnmt-1 knockdown blocked the increased methylation of the reelin promoter and down regulation of reelin and GAD$_{67}$ mRNA expression induced by methionine administration. This study supports the hypothesis that the reduction of reelin and GAD$_{67}$ mRNA found in post mortem schizophrenic brains is due to hypermethylation of the respective promoters by Dnmt-1 (Noh et al. 2004). Furthermore, in the HRM model of schizophrenic neuropathologies, the biochemical (reelin promoter methylation) and behavioural (pre-pulse inhibition of startle and social interaction deficits) alterations elicited by methionine administration were normalised by valproate (Tremolizzo et al. 2005).

No explanation for increased Dnmt-1 expression in schizophrenia has been suggested in the literature. Clearly excess dietary methionine is unlikely to be the cause; however, the finding of elevated CSF methionine in schizophrenics cited earlier (Regland et al. 2004) may be relevant if confirmed in further studies. Conversely, animal studies demonstrate that methyl-deficient diets also increase Dnmt-1 activity (Christman et al. 1993; Slack et al. 1999). Rats fed a methyl-deficient diet for 1–4 weeks developed alterations in gene expression; RNA and Dnmt activity increased 2–3-fold. At 1–2 weeks after normal diet restoration, gene expression and methyltransferase activity was normalised. Thus, it appears that the response of Dnmt-1 to dietary methyl-group sources is biphasic; both dietary excess and deficiency increase enzyme activity and alter gene expression in animals. However, whether methyl-group deficiency reduces reelin expression has not yet been tested.

DNA methyltransferase-1 and schizophrenia

DNA methyltransferase (Dnmt)-1 is responsible for the maintenance methylation of hemi-methylated DNA post-replication. In vitro evidence suggests that Dnmt-1 expression is regulated by negative feedback whereby methylation of a regulatory element of Dnmt-1 is sensitive to cellular methylation capacity. An eight-fold increase in Dnmt-1 mRNA induction was observed when cells were challenged by 5-azacytidine, a methylation inhibitor. Moreover, rodents fed methionine-deficient diets exhibited both global hypomethylation and increased Dnmt-1 expression and activity (Slack et al. 1999).

A recent post mortem study of schizophrenic brains found that the number of Dnmt-1 mRNA-positive neurons was inversely correlated with the number of reelin mRNA-positive neurons in cortical GABAergic interneurons, independent of neuroleptic type. Dnmt-1 activity was significantly greater in schizophrenic than in non-psychiatric controls. Furthermore, one patient with no history of neuroleptic use had results well within the range of medicated patients (Veldic et al. 2004a). Other groups using different patient cohorts have confirmed these findings. A follow-up study found that Dnmt-1 expression was increased in psychotic patients taking valproate alone (n 4) or anti-psychotics alone (n 15) but not in those taking both (n 9) (Veldic et al. 2004b).

In an in vitro study of mouse embryonic cortical neurons, Dnmt-1 knockdown blocked the increased methylation of the reelin promoter and down regulation of reelin and GAD$_{67}$ mRNA expression induced by methionine administration. This study supports the hypothesis that the reduction of reelin and GAD$_{67}$ mRNA found in post mortem schizophrenic brains is due to hypermethylation of the respective promoters by Dnmt-1 (Noh et al. 2004). Furthermore, in the HRM model of schizophrenic neuropathologies, the biochemical (reelin promoter methylation) and behavioural (pre-pulse inhibition of startle and social interaction deficits) alterations elicited by methionine administration were normalised by valproate (Tremolizzo et al. 2005).

No explanation for increased Dnmt-1 expression in schizophrenia has been suggested in the literature. Clearly excess dietary methionine is unlikely to be the cause; however, the finding of elevated CSF methionine in schizophrenics cited earlier (Regland et al. 2004) may be relevant if confirmed in further studies. Conversely, animal studies demonstrate that methyl-deficient diets also increase Dnmt-1 activity (Christman et al. 1993; Slack et al. 1999). Rats fed a methyl-deficient diet for 1–4 weeks developed alterations in gene expression; RNA and Dnmt activity increased 2–3-fold. At 1–2 weeks after normal diet restoration, gene expression and methyltransferase activity was normalised. Thus, it appears that the response of Dnmt-1 to dietary methyl-group sources is biphasic; both dietary excess and deficiency increase enzyme activity and alter gene expression in animals. However, whether methyl-group deficiency reduces reelin expression has not yet been tested.

Diet and gene expression

Whilst dietary methyl donor deficiency has been the subject of many studies, the effects of excess SAM precursor supplementation are less well known. Three studies in mice demonstrate the effects of such supplementation in pregnant mice.
Epigenetic variation in the expression of the agouti gene is evident in the coat colour of mice with the viable yellow allele (A\textsuperscript{vy}) of agouti. The A\textsuperscript{vy} gene is expressed to different degrees in genetically identical mice depending on the degree of gene methylation. The failure to methylate and suppress the A\textsuperscript{vy} gene during development leads to agouti expression in all tissues, as opposed to cyclic expression limited to hair follicles. Epigenetic variations in A\textsuperscript{vy} expression produce phenotypes ranging from pure yellow through slightly mottled, mottled, heavily mottled to the normal (pseudoagouti) mouse. The pure yellow phenotype is obese, and has an increased risk of diabetes and cancer.

Two levels of SAM precursor supplements were compared with a control diet in pregnant A\textsuperscript{vy}/a mice. The mid-range diet contained choline, betaine and folic acid (each 5 g/kg), and 0.5 g cobalamin/kg. The high-level diet contained three times the level of choline, betaine, folate and cobalamin plus 7.7 g L-methionine/kg and 150 mg Zn/kg. Although a range of coat colour variation remained in the offspring, the distribution was significantly shifted towards the normal coat colour with increasing supplement levels (P < 0.001) (Cooney et al. 2002).

In a second study, a/a females were supplemented with folic acid, cobalamin, choline chloride and anhydrous betaine, and mated with A\textsuperscript{vy}/a males. Once again, coat colour distribution in A\textsuperscript{vy}/a offspring was significantly shifted towards the pseudoagouti phenotype compared with controls (P = 0.008). The highly significant effect of supplementation on coat colour vanished when A\textsuperscript{vy} methylation was included in the model, indicating that the A\textsuperscript{vy} CpG methylation mediates the effect of supplementation on coat colour. Furthermore, A\textsuperscript{vy} gene methylation from the tail tip was increased at all seven examined sites, and the average percentage methylation was highly correlated with samples from the liver, kidney and brain (Waterland & Jirtle, 2003).

In the third study, the effect of maternal high-dose folic acid supplementation was examined in folate-binding protein-1 knock-out mice. Of note was that supplementation caused global DNA hypomethylation in the folate-binding protein-1 deficient embryos compared with controls. The authors proposed that folinic acid inhibited GNMT, leading to alterations in the levels of SAM and SAH. SAM:SAH was increased in the liver but reduced in the brain (Finnell et al. 2002).

A fourth study, in 60 d old male WTM and HRM, examined the effects of t-methionine supplementation (6-6 mmol/kg twice daily for 15 d). The treatment down regulated the expression of both reelin and GAD\textsubscript{67} mRNA by about 40 % in both WTM and HRM (despite the lower reelin production in this strain) compared with vehicle-treated mice. Similar treatment with glycine produced no effects. Furthermore, the down regulation was associated with a significant increase (about 2-fold) in the number of methylated cytosines in the reelin promoter region, and the mean of cytosine residues correlated with levels of reelin mRNA (R = -0.67; P < 0.001). Valproate administration normalised the induced down regulation of reelin and GAD\textsubscript{67} mRNA in both WTM and HRM (Tremolizzo et al. 2002).

Although the first two of these studies imply that maternal supplementation of methylyating factors improves offspring health, the third study suggests that such interference with epigenetic gene-regulatory mechanisms may have unintended consequences. The gene expression pattern of multiple genes may be altered, in a positive or negative direction, and if such changes occur in the gametes, they may be heritable. Last, the fourth study demonstrates that exposure to excess SAM precursors postnatally can reduce reelin and GAD\textsubscript{67} production by increasing gene promoter methylation.

### Folate supplements in pregnancy

In 1982, the Spanish NHS recommended early folate treatment for all women intending to conceive to prevent neural tube defects (NTD). In fact, physicians began prescribing multivitamins and folate some years earlier. In 1976 3 % of pregnant women received multivitamins, rising to 10 % in 1977, 35 % in 1982 and 55 % in 1986. Two studies found that the MTHF reductase TT genotype frequency in Spain rose from 13 % to 26 % over the same period, hypothesised to be a direct result of folate supplementation (Munoz-Moran et al. 1998). The authors suggest that the increase in TT genotype frequency after folate supplementation was because extra folate prevented the spontaneous abortions that occur fairly frequently in TT homozygous fetuses under normal conditions.

What might be the short- and long-term health effects on infants programmed in utero to be dependent on a high-folate diet, of a substantial drop in folate intake postnatally? Other less well-studied polymorphisms may have increased in frequency and more subtle effects might be found at the cellular level, such as reduced folate-binding protein due to folate ‘flooding’ in utero. Indeed, isolated cells can show a permanent change in function due to fetal programming, for example, pancreatic islets from animals born to protein-deficient mothers release less insulin (Rees et al. 2000; Petry et al. 2001; Gluckman & Hanson, 2005).

In 1991, the Medical Research Council published a study examining folic acid supplementation in pregnant women at high risk of an NTD pregnancy (Anonymous, 1991). The double-blind trial compared 4 mg folate with supplements of seven other vitamins, combined folate and vitamins or neither, and showed a 72 % protective effect of folate against NTD in utero. However, the authors noted the inability of the study to detect rare or slight adverse effects.

Only one study examined the effects of multivitamins containing 800 μg folate in women not known to be at risk of an NTD pregnancy. Although the NTD rate was reduced, post hoc analysis appeared to indicate increased fertility and a significantly increased risk of early abortion in supplemented women (P < 0.04) (Hook & Czeizel, 1997).

Following the Medical Research Council study, all women intending to conceive in the UK and the USA are encouraged to take folic acid supplements and, since January 1998, all grain products in the USA have been fortified with 140 μg folic acid/100 g grain product. A study examining the effects of folate fortification noted that fortified foods typically contain 160–175 % of the labelled content. In addition, many ‘super-fortified’ breakfast...
cereals, labelled as containing 400 μg/30 g serving are typically consumed in 60 g portions; thus an individual could easily consume more than the safe upper limit of folate (1 mg/d) in one serving of cereal (Quinlivan & Gregory, 2003). The authors estimate that > 5% of adults consume > 1 mg folate/d, and point out that an intake > 200 μg exceeds the capacity of the body to convert it to 5-MTHF, leading to unmetabolised folic acid in the bloodstream. Since this is readily absorbed and retained by cells, without the need for conversion to THF by MS, this may disturb cellular folate homeostasis.

Despite fortification, US women intending to conceive are still encouraged to take folate. Consequently, a woman consuming a typical high-protein US diet may have a more than adequate intake of methionine, choline and cobalamin, combined with a high folate intake derived from her diet, fortification, multivitamin and folate supplements.

**Excess folate, reelin and autism?**

Data collected in California showed that new cases of level 1 DSM-IV autism from 1971 to 1980 were consistent at 1–200 annually. Since 1980, the number of new cases annually has increased dramatically and continues to rise, a situation that is repeated across the USA (Figs. 4 and 5). Although the UK incidence of autism also appears to have increased, the lack of a central database makes verification difficult.

A post mortem study found reductions of 43–44% in reelin 410 and its isoforms in autistic cerebella (Fatemi et al. 2001). A polymorphism in the reelin gene that reduces reelin production by 25% was recently identified in 20% of a group of US autistic subjects (Persico et al. 2001), but was not replicated in other studies. Clearly, an 8- to 10-fold increase in autism cases in 20 years must be due to one or more environmental factors. Viral infections, either in utero or during infancy, and multiple vaccinations at sensitive times for brain development, are suggested triggers. Viral infection mid-gestation reduced reelin levels in the brains of neonatal mice (Fatemi et al. 2005). Although multiple vaccinations ceased in Japan a decade ago with no reduction in autism incidence, many parents report an adverse reaction to vaccination shortly before a dramatic regression through developmental milestones before diagnosis. Thimerosal, a Hg-based preservative used in vaccines for many years, has recently been banned in the USA after suggestions that Hg may be the environmental trigger. Autistic children have a reduced ability to excrete Hg, possibly due to reduced functioning of the trans-sulfuration pathway. It is too early to tell whether the Thimerosal ban will have a significant effect on autism incidence.

A possible connection between infections and/or vaccinations and autism is suggested by the fact that immune stimulation draws on the folate pool for clonal expansion. Thus, immune stimulation due to infection(s) or multiple vaccinations could further reduce an insufficient folate pool to a critical level, reducing the cellular methylation potential and up regulating Dnmt-1. The effect of folate deficiency and increased Dnmt-1 activity in carriers of the RELN polymorphism, or in children ‘programmed’ in utero to be dependent on a high-folate diet, may be to further reduce reelin production and GABA turnover to levels too low to sustain normal neuronal metabolism at a critical time for brain development.

It is notable that the two periods of selective apoptosis of neuronal synapses in the brain coincide with the appearance of symptoms of autism (at 2–3 years) and schizophrenia (during adolescence). Perhaps we will witness a transition from the currently increasing rates of schizophrenia and BPD, to an ‘explosion’ as this generation of highly pre-natally supplemented children reach adolescence.

**Folate supplements and gene expression**

Dietary manipulation can restore normal gene expression following disruption caused by inadequate methylation. A study in uraemic patients with hyperhomocysteinaemia demonstrated the effects of folate supplementation on gene

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**Fig. 4.** The incidence of autism in California from 1992 to 2003 in ages 6–22 years (●) and ages 3–22 years (▲) (no. of cases in the US school years 1992–2003). (Reproduced with permission from www.fightingautism.org; data are from www.ideadata.org and www.cdc.gov/nchs/)
expression in vivo. The expression of two tightly linked genes, H19 and IGF2, is inversely regulated by epigenetic mechanisms including methylation. After 8 weeks of folate washout, patients with plasma total Hcy levels > 62 µmol/l were found to have biallelic expression of H19 in peripheral mononuclear cells, whereas expression in controls and in patients with lower plasma total Hcy was monoallelic. Biallelic expression of H19 was associated with negligible expression of IGF2. Treatment with 15 mg methylfolate/d reduced plasma total Hcy levels, restored monoallelic expression of H19, and significantly increased IGF2 expression (Ingrosso et al. 2003).

Discussion and conclusion

The evidence presented demonstrates that biomarkers of disturbed one-carbon metabolism are a common feature of psychiatric illness, but does not establish a causal relationship. One-carbon dysregulation could be a cause or a consequence of psychiatric illness, or an epiphenomenon. Illness-induced poor appetite or diet could create nutrient deficiencies causing biochemical dysregulation. However, if dysregulation were solely a by-product of psychiatric illness, then nutrient supplements would be expected to restore normal one-carbon metabolism but not to impact on psychiatric symptoms, yet clinical trials demonstrate significant symptom improvements.

A major problem in the study of biochemical imbalances in psychiatric patients is the lack of access to brain tissue in vivo; peripheral cell and plasma metabolites may not replicate the situation in the brain. The findings that plasma folate correlates with CSF folate in human subjects, and that MS activity is closely correlated with the cerebral SAM:SAH ratio in pigs, are promising but inconclusive. However, a number of clinical trials have demonstrated that alleviating deficiencies of various methylating factors has a significant effect on psychiatric symptoms, supporting the hypothesis that disturbed plasma levels of one-carbon metabolites reflect disturbed cerebral methyl metabolism, which presents as psychiatric symptoms. The unanswered question of whether plasma or peripheral cell metabolites replicate the situation in the brain may be of academic interest, but should not delay further studies exploring the association between methylation metabolites and clinical symptoms, both before and after nutrient supplementation.

The SAM:SAH ratio has not yet been determined in schizophrenia, BPD and depression, but if disturbed, the downstream disruption of methyltransferase activities could account for much disease-associated pathology, including altered gene expression, membrane fatty acid composition, memory and cognitive function, and sleep patterns.

Novel findings from the field of epigenetics provide a plausible link between poor methylation status and psychiatric symptoms. The expression of RELN and GAD genes are reduced in depressed, bipolar, schizophrenic and autistic brains, and GAD protein levels can differentiate between the conditions. In schizophrenic brains, reduced reelin is associated with increased Dnmt-1 expression and activity. Both low and high cellular methylation status increases Dnmt-1 expression, but whether either is causal in schizophrenia has not been determined. Dietary manipulation altered the expression of other methyl-sensitive genes in uraemic patients, and RELN and GAD expression have responded to nutrient supplementation in mice.

The prevalence of low serum folate and/or cobalamin levels, and high plasma or serum total Hcy, in the psychiatric population strongly suggests that all patients should be appropriately tested and treated, before pharmacological therapy. For a sub-group of depressed patients such treatment might be sufficient to restore health, and for others it may improve their response to anti-depressants. However, optimum supplementation levels must be determined, since over-methylation is also detrimental. The high rate of CVD in the schizophrenic population confirms the importance of monitoring plasma or serum total Hcy levels, and Hcy-lowering nutrient therapy has improved psychiatric symptoms in this patient group. Given the proven benefits and lack of side effects of SAM in treating depression, it is surprising that SAM is not part of current treatment protocols.

In view of the enormous costs of psychiatric illness, both personal and social, and the detrimental long-term effects of neuroleptic medications, further research is urgently needed to elucidate the role of nutrients in the prevention and treatment of psychiatric illness.
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One-carbon metabolism in psychiatric illness


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