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METHYLATION

Methylation is an enormously important metabolic process in the body. Disturbances in methylation have widespread influences on numerous body systems, and serve as risk factors for the development of a number of conditions. Supporting methylation is therefore an incredibly important consideration for many clinicians whether they see patients who require endocrine support, mental health support, liver and gastrointestinal support or cancer support, among others.

Methylation is the transfer of a methyl group to another compound. Phosphorylation is probably the most common reaction in the body. Methylation is second only to phosphorylation in frequency.

Methylation is a major metabolic process within the body. Essentially, methylation refers to the transfer of a methyl group from a methyl donor, usually S-adenosylmethionine (SAMe), to a molecule. The diversity of molecules to which a methyl group can be transferred is what makes methylation such an important and ubiquitous metabolic process.

Some examples of methylation include:

- The methylation of drugs to support their metabolism and excretion;
- The methylation of neurotransmitters and hormones, such as dopamine, histamine, melatonin and oestrogen, to facilitate their excretion;
- The synthesis and repair of myelin proteins;
- Produce energy (CoQ10, carnitine, ATP)
- Helps mobilize fats, cholesterol and their elimination, which reduces atherosclerosis, cholestatic and regulates weight; and
- The methylation of DNA to modify the transcription of genes.

Illustrating methylation’s central importance to health and wellbeing, SAMe has been considered second only to ATP in the number of enzymes for which it is a substrate. Ensuring that methylation is optimal is therefore an important consideration for nearly every practitioner.

It is involved in hundreds of reactions in the body (two major donors are SAMe and BH4). One of the most important methylation reactions in the body is the conversation from homocysteine to methionine.
The endogenous synthesis of SAMe involves an intricate and tightly controlled metabolic cycle. In short, when SAMe donates a methyl group it becomes S-adenosylhomocysteine (SAH); SAH then becomes homocysteine; homocysteine then becomes methionine; and methionine then becomes SAMe again.

While many of the reactions involved in the synthesis of SAMe are, from a nutritional perspective, relatively straight-forward, the metabolic conversion of homocysteine to methionine is far more complex. To be ‘re-methylated’ to methionine, homocysteine requires a methyl group. This methyl group is sourced from either 5-methyltetrahydrofolate (5MTHF) or trimethylglycine (TMG). Although TMG plays an incredibly important role in the re-methylation of homocysteine to methionine, the enzyme that transfers the methyl group from TMG to homocysteine is largely expressed only in liver and kidney cells. The enzyme that 5MTHF uses to transfer the methyl group from itself to homocysteine, on the other hand, is expressed in nearly every body cell. This makes the folate metabolic cycle (which produces 5MTHF an extremely important cycle to support when managing methylation cycle disorders.

In addition to re-methylating homocysteine, 5MTHF is also the most common form of folate found in the body. This is because enterocytes and hepatocytes contain specialised enzymes to convert other forms of folate, including folic acid and folinic acid, into 5MTHF. Because 5MTHF rapidly elevates serum 5MTHF concentrations, supplemental 5MTHF supplementation is ideal for raising serum 5MTHF concentrations and re-methylating homocysteine.

The administration of 5MTHF alone is not sufficient to reduce homocysteine, however. An adequate supply of vitamin B12 is also essential for transmethylation to take place.

Vitamin B12 participates in two main reactions in the body. Each of these reactions requires a slightly different coenzyme form of vitamin B12: either methylcobalamin or adenosylcobalamin. Methylcobalamin is the most common form of vitamin B12 found in the serum, while adenosylcobalamin is the storage form of vitamin B12 found in the mitochondria.

Given that methylcobalamin is the main form of cobalamin found in the serum and the form found in the methionine synthase enzyme, it makes sense to supplement with this form of cobalamin for the management of methylation cycle disorders. Other forms of supplemental cobalamin include cyanocobalamin and hydroxocobalamin. Both of these forms have the capacity to be metabolised to either methylcobalamin or adenosylcobalamin.

Without sufficient vitamin B12, cells within the body can enter what is termed the ‘methylfolate trap.’

The methylfolate trap is a hypothetical metabolic scenario characterised by vitamin B12 deficiency, raised homocysteine, raised 5MTHF and reduced intracellular folate concentrations.
Because of the methylfolate trap, it is recommended that vitamin B12 be taken with any coenzyme form of folate, especially in populations liable to vitamin B12 deficiency, such as the elderly and vegetarians. Supplemental B12 may not be entirely sufficient for some patients, however. A variety of patients can have difficulty absorbing vitamin B12, which also places them at high risk of developing vitamin B12 deficiency. Usually, folic acid and folinic acid supplementation can mask the development of vitamin B12 deficiency in these individuals. SMTHF, however, does not.

Balancing methylation is required through lifestyle, diet, environment, mental outlook, nutrition. This can be demanding, difficult to achieve, hard to maintain and takes time.

Cofactors need to complete methylation and to turn off methylation. Excess substrates may turn off methylation (negative feedback).

Methylation is regulated by:

- Many enzymes require cofactors (derived from vitamins/minerals) to activate
- Cofactors required to complete methylation
- Cofactors required to turn off methylation
- Excessive substrates may turn off methylation via feedback inhibition

Methylation is disturbed by:

- Diet - excess animal proteins, saturated fats, sugars, coffee, alcohol
- Lack of cofactors driving methylation forward (Low Zn, Mg, B6)
- Medications e.g. Antacids (lowers B12), oral contraceptive pill, diuretics
- Poor digestion, leaky gut
- Smoking (depletes folate, SAMe)
- Niacin (depletes methyl groups)
- Environmental toxins e.g. acetaldehyde (alcohol, yeasts), heavy metals - As, Hg
- Genetic mutations (MTHFR, CBS, COMT, MS/MTR)
- High SAMe leads to reduced endogenous levels (via negative feedback)
- High cysteine leads to reduced endogenous levels (via negative feedback)
- High oral/IV GSH leads to reduced endogenous levels (via negative feedback)
The re-methylation of homocysteine is not the only option available to reduce serum homocysteine concentrations; another pathway, termed the transsulfuration pathway, is responsible for reducing homocysteine concentrations via the metabolic conversion of homocysteine to cysteine. The first enzyme in the transsulfuration pathway, cystathionine β-synthase (CBS), is a vitamin B6-dependent enzyme, making pyridoxine an essential component for the reduction of serum homocysteine concentrations.

The majority of folates in the serum appear to be 5MTHF which correlates with homocysteine levels. Supplemental 5MTHF reduces homocysteine, as folinic acid does.

Natural folates are destroyed by UV rays and cooking, so dietary provision of folate can be insufficient. Natural folates are degraded easily by gastric acid, however, gastric acid also contains vitamin C which can help protect the folate molecule.

The healthy human liver can only metabolise around 400mcg folic acid within a one hour period. Extra folic acid may appear as unmetabolised folic acid (UMFA). Folic acid may compete with natural folates for their place on folate receptors and folate enzymes.

**S-adenosylmethionine (SAMe)** donates a methyl group in reactions such as:

- Production of melatonin.
- Conversion of noradrenaline to adrenaline.
- Production of phosphatidylcholine.
- Metabolism of oestrogen and other catechols.
- It is also involved in DNA methylation.
- SAMe main methyl donor; GSH master antioxidant. SAMe is second only to ATP for enzymes involved in (donates methyl group to >200 enzymes).

A low value for SAMe represents a low methylation capacity.

**S-adenosylhomocysteine (SAH)** inhibits many methyltransferase reactions (meaning SAMe won’t work as effectively). As the conversion from SAH to homocysteine only occurs when adenosine and homocysteine are low, we want to reduce homocysteine if it is too high.
Upon transfer of its methyl group, SAMe is rapidly converted to SAH and hydrolysed to homocysteine and adenosine. This is a reversible reaction that favours SAH synthesis. If homocysteine is allowed to accumulate, it will be rapidly metabolised to SAH which is a strong inhibitor of all methylation reactions. The activities of the methyltransferase enzymes are governed by the ratio of SAMe:SAH. A ratio of SAMe:SAH <4.5 represents low methylation capacity.

If SMTHF is within reference range but either SAMe or SAMe:SAH ratio is low, then the methylation cycle block may be more relative to insufficient B12 rather than insufficient folate. This can be confirmed with elevated levels of methylmalonate as seen in ORGANIC ACIDS – METHYLATION COFACTORS profile.

**Glutathione (GSH)** – the level of GSH in the plasma is likely to be more reflective of tissue intracellular glutathione status than the more commonly and more easily measure red blood cell or whole blood glutathione levels because red blood cells are normally net producers of glutathione. Also the reduced form (as distinguished from total glutathione = reduced + oxidised) is more diagnostic of the status of glutathione function.

Note glutathione is involved in the intracellular metabolism of vitamin B12. Adequate glutathione levels are needed to avoid B12 deficiency and insufficient methylcobalamin to support methionine synthase in the methylation cycle.

Normally oxidised glutathione (GSSG) is recycled back to reduced glutathione (GSH) by glutathione reductase which requires B2. If GSSG is elevated above normal range, this represents oxidative stress.
INTEGRATIVE & FUNCTIONAL TESTS THAT ASSESS METHYLATION CAPACITY

- **Homocysteine** is an amino acid associated with atherosclerosis that can become elevated when there is need for folate, vitamin B6, and/or vitamin B12. Excess homocysteine levels have been correlated with many cardiovascular disorders. It also decreases insulin sensitivity and increases oxidative stress.

- **Formiminoglutamate (FIGLU)** is a functional marker of folate need (measured in ORGANIC ACIDS – METHYLATION COFACTORS profile).

- **Methylmalonate (MMA)** is a functional marker of vitamin B12 need (measured in ORGANIC ACIDS – METHYLATION COFACTORS profile).

- **Amino acids** are precursors for methylation; plus, many amino acids undergo methylation during metabolism, e.g. methionine is the precursor to homocysteine, while glycine and serine are both methyl donors. Using amino acids, a clinician can peer even more deeply into a patient’s methylation status. This might be appropriate in more complicated or refractory cases of neuropsychiatric disorders, infertility, or autism. For example, if methionine or glycine is abnormal, it could indicate aberrations in a patient’s methylation pathway, requiring either more amino acids or more methylation cofactors.

- **Vanilmandelate (VMA) and homovanillate (HVA)** are urinary neurotransmitter metabolites. When low, VMA and HVA may indicate impaired methylation (clearance) especially if present in conjunction with certain symptoms (extreme anxiety, stress or mania). These are measured in ORGANIC ACIDS – METABOLIC profile which also include FIGLU and MMA. Low levels could also indicate a COMT SNP leading to an inability to transfer methyl groups.

- **Methylenetetrahydrofolate reductase (MTHFR)** is a genetic test that looks for compromised MTHFR-enzyme activity that may lower methylation capacity and cause elevated homocysteine.

- **Oestrogen methylation ratio (2OHE1:2OMeE1)** evaluates a person’s ability to methylate oestrogens, a critical step in oestrogen metabolism. The 2OHE1/2OMeE1 ratio assesses whether a patient can transform 2-hydroxyestrone into 2-methoxyestrone via methylation (measured in ESTROGEN METABOLITES – LEVEL 3 profile). The 2-hydroxyestrone (2OHE1) /2-methoxyestrone (2OMeE1) ratio indicates how efficiently a patient can turn 2-hydroxyestrone into 2-methoxyestrone using the enzyme catechol-o-methyl transferase (COMT). Methylation is an important mechanism for detoxifying estrogens and clearing them from the body. Some research suggests that a lower 2OHE1/2OMeE1 ratio may be protective against breast cancer.
Be mindful that a single test doesn’t tell a practitioner all they need. One test that evaluates a methylation marker may not be enough to determine that all biochemical processes are running smoothly. For example, while homocysteine is a widely recognised methylation marker, other markers can examine a patient’s methylation capacity from a different angle and gives a fuller picture.
# NUTRIPATH METHYLATION PROFILES

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Code</th>
<th>Analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylation Profile</td>
<td>5101</td>
<td>S-Adenosyl Methionine (SAMe), S-Adenosyl Homocysteine (SAH), SAMe:SAH ratio; 5-methyl tetrahydrofolate (5MTHF), Folinic acid, Tetrahydrofolate (THF)</td>
</tr>
<tr>
<td>Folate Metabolism Profile</td>
<td>5102</td>
<td>S-methyl tetrahydrofolate (SMTHF), Folinic acid, Tetrahydrofolate (THF); Vitamin B12, red cell Folate, Homocysteine</td>
</tr>
<tr>
<td>Methionine Metabolism Profile</td>
<td>5103</td>
<td>S-Adenosyl Methionine (SAMe), S-Adenosyl Homocysteine (SAH), SAMe:SAH ratio; Vitamin B12, red cell Folate, Homocysteine; Methionine</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>5104</td>
<td>Vitamin B6</td>
</tr>
<tr>
<td>Vitamin B12 &amp; Red Cell Folate</td>
<td>6014</td>
<td>Vitamin B12, Red Cell Folate</td>
</tr>
<tr>
<td>Glutathione, Oxidised</td>
<td>5107</td>
<td>Glutathione, oxidised</td>
</tr>
<tr>
<td>Glutathione, Reduced</td>
<td>5012</td>
<td>Glutathione, reduced</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5109</td>
<td>Ammonia</td>
</tr>
<tr>
<td>MTHFR</td>
<td>5018</td>
<td>MTHFR (Methylenetetrahydrofolate reductase) gene mutation</td>
</tr>
<tr>
<td>Histamine</td>
<td>4006</td>
<td>Histamine</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>4007</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>Amino Acids (Urinary)</td>
<td>5004</td>
<td>Alanine, Arginine, Asparagine, Aspartic Acid, Citrulline, Cysteine, GABA, Glutamate, Glutamine, Glycine, Histidine, 1-Methyl Histidine, 3- Methyl Histidine, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, hydroxy Proline, Serine, Taurine, Threonine, Tryptophan, Tyrosine, Valine</td>
</tr>
<tr>
<td>Oestrogen Metabolites - Level 3</td>
<td>1209</td>
<td>2OHE1, 16αOHE1, 2:16 ratio, 4OHE1; 2OMeE1, 4MeOE1, 2OHE1:2OMeE1, E1:4OMeE1 ratios</td>
</tr>
<tr>
<td>Organic Acids – Methylation Cofactors</td>
<td>4018</td>
<td>Formiminoglutamic acid (FIGLU), Methylmalonic acid (MMA)</td>
</tr>
</tbody>
</table>
# COMMON TEST RESULTS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>COMMON RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Elevated</td>
</tr>
<tr>
<td>Methylmalonic acid (MMA)</td>
<td>Normal – High</td>
</tr>
<tr>
<td>Formiminoglutamic acid (FIGLU)</td>
<td>Normal – High (low folic acid)</td>
</tr>
<tr>
<td>B12</td>
<td>Elevated</td>
</tr>
<tr>
<td>Folic acid, Folinic acid</td>
<td>Elevated</td>
</tr>
<tr>
<td>Active Folate (rbc)</td>
<td>Low</td>
</tr>
<tr>
<td>5MTHF</td>
<td>Low</td>
</tr>
<tr>
<td>THF</td>
<td>Low</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Normal – High</td>
</tr>
<tr>
<td>Amino acids (24hr urine)</td>
<td>Normal - High Taurine</td>
</tr>
<tr>
<td></td>
<td>Low Histidine (low folic acid)</td>
</tr>
<tr>
<td>Glutathione reduced and oxidised</td>
<td>Low</td>
</tr>
<tr>
<td>SAMe</td>
<td>Low</td>
</tr>
<tr>
<td>SAH</td>
<td>Normal – High</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Low - Normal – High</td>
</tr>
<tr>
<td>Urea breath test</td>
<td>Normal – High</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Low SHIAA, Low HVA; High FIGLU, High MMA</td>
</tr>
<tr>
<td>Mauve Factor (Pyrroluria)</td>
<td>Elevated</td>
</tr>
<tr>
<td>Oestrogen metabolites</td>
<td>Elevated</td>
</tr>
</tbody>
</table>

NOTE: High methionine foods can falsely elevate Homocysteine. Limit them 24 hours prior. Fast 12 hours before blood draw.

MTHFR mutations may show as increased rbc folate and low plasma folate levels.

## SAMe:SAH ratio

<table>
<thead>
<tr>
<th>SAMe:SAH ratio (N = 2:1)</th>
<th>CONSIDERATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low SAMe / Low SAH</td>
<td>Methionine intake</td>
</tr>
<tr>
<td>Low SAMe / High SAH</td>
<td>Copper toxicity, folate intake</td>
</tr>
<tr>
<td>High SAMe / Low SAH</td>
<td>Overmethylator; Methionine intake</td>
</tr>
<tr>
<td>High SAMe / High SAH</td>
<td>Methionine intake</td>
</tr>
</tbody>
</table>
Ways to Lower Homocysteine

Homocysteine → Methionine (via TMG, with Zn) OR Homocysteine → Methionine (via methylB12)
Homocysteine → Cystathionine (via B6)

Homocysteine → Tetrahydrofolate → 5,10-MTHF → 5-MTHF (via B2)
SYMPTOMS OF OVER / UNDER METHYLATION

As described by Dr. Carl Pfeiffer and the Pfeiffer Treatment Centre, these symptom lists are generalisations and are not suitable for diagnosis due to the wide ranging influences of these variables. Note, pyrroluria may overlap with methylation/histamine imbalances.

<table>
<thead>
<tr>
<th>Methylation/histamine imbalances</th>
<th>Under-methylated (histadolin)</th>
<th>Over-methylated (histamin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergies or sensitivities</td>
<td>Inhalant/seasonal (e.g Hay Fever)</td>
<td>Neither Chemical (e.g perfume)</td>
</tr>
<tr>
<td>Prone to hives (urticaria)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Salivary and tear flow</td>
<td>High</td>
<td>Average</td>
</tr>
<tr>
<td>Dry eyes</td>
<td>Never</td>
<td>Occasional</td>
</tr>
<tr>
<td>Pain tolerance</td>
<td>Low</td>
<td>Average</td>
</tr>
<tr>
<td>Ease in reaching orgasm</td>
<td>High</td>
<td>Average</td>
</tr>
<tr>
<td>Libido when in relationship</td>
<td>High</td>
<td>Average</td>
</tr>
<tr>
<td>Body hair</td>
<td>Sparse</td>
<td>Average</td>
</tr>
<tr>
<td>Anxiety tendencies</td>
<td>Outwardly calm with internal anxiety</td>
<td>None</td>
</tr>
<tr>
<td>Theobias</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hyperactive tendencies</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nervous/restless legs</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Obsessive compulsive tendency</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Perfectionist</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Competitiveness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Motivation</td>
<td>High (self-motivated)</td>
<td>Medium</td>
</tr>
<tr>
<td>Academic/science accomplishment</td>
<td>High (good student)</td>
<td>Medium</td>
</tr>
<tr>
<td>Artistic/musical ability</td>
<td>Average or low</td>
<td>Very high</td>
</tr>
<tr>
<td>Religion</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Additional personality or psychiatric tendencies</td>
<td>Oppositional or defiant</td>
<td>None applicable</td>
</tr>
<tr>
<td></td>
<td>Social isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anorexia or Bulimia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shopping/gambling disorders</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Catatonic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delusional thinking</td>
<td></td>
</tr>
<tr>
<td>Response to E-vitamins or folate</td>
<td>Negative</td>
<td>Positive or nil or unknown</td>
</tr>
<tr>
<td>Reaction to anti-histamin</td>
<td>Positive</td>
<td>Unknown or nil.</td>
</tr>
<tr>
<td>Mood response to anti-depressants</td>
<td>Positive</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Response to valium/xanax/avisa</td>
<td>No effect or negative effect</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Response to benzodiazepines</td>
<td>Negative</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Response to SAMe</td>
<td>Positive</td>
<td>Unknown or nil.</td>
</tr>
</tbody>
</table>
TREATMENT OF METHYLATION ISSUES

There are currently a number of treatments available for methylation problems.

If you have inflammation taking 5-MTHF can make it worse so supplements such as curcumin and Krill oil may be necessary to help overcome the inflammation.

A methyl donor such as methionine, TMG or SAMe should also be used however start with lower doses and ramp it up gradually to avoid over-methylating which can make things worse in some people.

Glutathione may also be supplemented to help increase low levels.

Protocols developed by Prof. Carl Pfeiffer and Dr William Walsh involve a methyl donor such as the amino acid methionine. In addition calcium helps release the body’s store of histamine, while zinc aids the calcium-methionine program and provide sufficient relief. Typical treatment includes the following nutrients in high doses: calcium, magnesium, B6, vitamin C, zinc and methionine.

Undermethylation induced depression suffers should avoid folic acid as it can further reduce serotonin levels even though it can assist with methylation. Manganese, copper and choline may also worsen depression symptoms and thus should also be avoided in depressive cases.

If undermethylators cooperate with treatment and works to give up detrimental addictions, the prognosis is good with improvement usually noted in 4-8 weeks with about 3-6 months needed to correct this chemical imbalance.

Undermethylators respond to methionine, SAMe, Mg, Ca, Zn, B6 inositol but may react poorly to folic acid, B12, choline. Overmethylators have poor reactions to SSRI medications and chemical sensitivities; can have low histamine levels, elevated copper. Supplementation with folinic acid, B12, B5 and B3 can help these individuals.

To find out more about this protocol see Mental Illness: The Nutrition Connection, a book by Prof. Carl Pfeiffer.
TREATMENT TIPS

- Use TMG, not DMG to decrease Homocysteine.
- Limit folic acid from fortified foods.
- Cease folic acid. Folic acid is synthetic. Elevated serum folic acid does not necessarily mean good levels of 5MTHF.
- Use folinic acid and/or 5MTHF, and use with methylB12.
- Need B6 and B2 to make 5MTHF. Need B6 for GSH.
- SAMe needs magnesium as cofactor. TMG needs zinc as cofactor.
- Methylfolate can increase nitric oxide (NO). NO and superoxide lead to increased nitrotyrosine/peroxynitrite which is oxidative.
- Elevated Niacin - can lead to elevated Serotonin in those with a MAO problem.
- Elevated Folate and B12 in blood does not necessarily mean good cellular or tissue levels.
- Elevated B12 – possible transcobalamin problem, low MTHF or yeast overgrowth
- Elevated Histamine - leads to reduced SAMe as it is broken down by SAMe. Therefore, low SAMe leads to increased Histamine and high SAMe leads to reduced Histamine.
- Low Glutathione - can lead to decreased SAMe.
- Low Homocysteine – chronic illness due to using to make GSH and quench oxidative stress.
- Low Homocysteine - may mean CBS SNP mutation or high B6 supplementation.
- High Homocysteine may be from down regulated CBS (B6) problem.
- Elevated Homocysteine is associated with increased risk for CVD. Best recommendation for primary prevention is levels < 8 umol/L.
- Best to use TMG, folinic acid, B6 and B12 to reduce Homocysteine.
- Fish oil, particularly DHA, has been found to raise the expression of numerous enzymes involved in folate mediated one-carbon metabolism.
- Reduce oxidative stress/inflammation before 5MTHF supplementation.
- As soon as patient feels good you tell them to STOP SUPPLEMENTS. Do not want to over-supplement and over-stimulate. Re-start when feeling bad again.
## METHYLATION IN PRACTICE

### FOLATE METABOLISM

<table>
<thead>
<tr>
<th>Coenzyme nutrients</th>
<th>Folic acid, Vitamin C, 5MTHF, B6, B2, B12, Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supportive nutrients</td>
<td>TMG, NAC, Histadine, Choline, Methionine</td>
</tr>
<tr>
<td>Pathology tests</td>
<td>Folate Metabolism profile; MTHFR; Organic Acids – Methylation Cofactors; Zinc, heavy metals</td>
</tr>
<tr>
<td>Medications that may effect</td>
<td>ACE inhibitors, Antacids, Antibiotics, Anticonvulsants, Antivirals, Aiguaniides, Bile acid sequesterants, Cortisosteroids, Diuretics, H2 receptor antagonists, OCP, Salicylates, Tricyclic antidepressants, Thyroxine</td>
</tr>
<tr>
<td>Nutrients that may effect</td>
<td>Cu, Pb, Co, Hg, Ni, Cd; excess Ca, Fe, Mg</td>
</tr>
<tr>
<td>Diet/Lifestyle factors that may effect</td>
<td>Alcohol, elderly, athletes, pregnancy and lactation, smoking; Diary-free, gluten-free, low vegetable intake, vegan, vegetarian, excess sugar</td>
</tr>
</tbody>
</table>

### METHIONINE METABOLISM

<table>
<thead>
<tr>
<th>Coenzyme nutrients</th>
<th>Mg, Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supportive nutrients</td>
<td>Folinic acid, Vitamin C, B12, B2, Zinc, Taurine, protein, SAMe, NAC</td>
</tr>
<tr>
<td>Pathology tests</td>
<td>Methionine Metabolism profile; CRP, heavy metals, Liver Detoxification profile</td>
</tr>
<tr>
<td>Medications that may effect</td>
<td>Antibiotics, B-adrenergic agonists, caffeine, Cortisosteroids, Diuretics, HRT, OCP, Laxatives, Thyroxine</td>
</tr>
<tr>
<td>Nutrients that may effect</td>
<td>Pb, Cd; elevated Ca, P, Fe, Mn</td>
</tr>
<tr>
<td>Diet/Lifestyle factors that may effect</td>
<td>Excess coffee intake, excess sugar/refined carbs, smoking; Gluten-free, low protein, high carb</td>
</tr>
</tbody>
</table>
### SAMe Pathway

<table>
<thead>
<tr>
<th>Coenzyme nutrients</th>
<th>SAMe</th>
</tr>
</thead>
</table>
| Supportive nutrients | SAMe low – Mg, Methionine, Histidine, B3, protein  
                          SAMe excess – Glycine, Nicotinamide |
| Pathology tests | Methylation profile; Methionine Metabolism profile |
| Medications that may effect | Acetaminophen, Antidepressants, L-dopa, oestrogen, Calcium channel blockers |
| Diet/Lifestyle factors that may effect | Excess coffee intake, smoking; Low protein |

### Transulphation Pathway

<table>
<thead>
<tr>
<th>Coenzyme nutrients</th>
<th>B6, NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supportive nutrients</td>
<td>Taurine, Folinic acxid, Vitamin C, Zinc, B12, B2</td>
</tr>
<tr>
<td>Pathology tests</td>
<td>Homocysteine, Glutathione</td>
</tr>
<tr>
<td>Medications that may effect</td>
<td>Aspirin, Antibiotics, Diuretics, Insulin, oestrogen, OCP, MSG, steroids</td>
</tr>
<tr>
<td>Nutrients that may effect</td>
<td>Pb</td>
</tr>
<tr>
<td>Diet/Lifestyle factors that may effect</td>
<td>Ageing, alcohol, drug taking, pregnancy, smoking; Excess coffee intake, high carbs</td>
</tr>
</tbody>
</table>

### Betaine Pathway

<table>
<thead>
<tr>
<th>Coenzyme nutrients</th>
<th>TMG, Zinc, Choline, Phosphatidylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supportive nutrients</td>
<td>Folinic acid, Vitamin C, B6, B12, B2, NAC, SAMe</td>
</tr>
<tr>
<td>Pathology tests</td>
<td>Homocysteine, Zinc</td>
</tr>
<tr>
<td>Medications that may effect</td>
<td>ACE inhibitors, Corticosteroids, Diuretics, OCP, H2 receptor antagonists, Antibiotics, Fibrates</td>
</tr>
<tr>
<td>Nutrients that may effect</td>
<td>Cu, Pb, Co, Hg, Ni, Cd, excess Ca</td>
</tr>
<tr>
<td>Diet/Lifestyle factors that may effect</td>
<td>Athletes, alcohol, elderly, pregnancy and lactation; Gluten-free, high fat, low protein</td>
</tr>
</tbody>
</table>
USE OF METHYLFOLATE IN DEPRESSION

The brain uses 5MTHF to regulate the synthesis or production of neurotransmitters and thus can help to regulate mood.

5MTHF acts as an enzymatic cofactor in the re-methylation of BH2 to BH4 and homocysteine to methionine and thus the production of SAMe which also seems to regulate mood.

5MTHF, B12, SAMe can be used to augment antidepressants in depressed patients who do not respond adequately to treatment or potentially as a standalone treatment.

BH4 is critically important in production of neurotransmitters starting from cofactor (MTHF). Remember, BH4 recycling is performed by MTHF. Therefore, if no MTHF then there is low BH4 and a deficiency of neurotransmitters.

High MTHF supplementation leads to elevated SAMe which then inhibits MTHF (feedback) which then lowers BH4.

<table>
<thead>
<tr>
<th>FOLIC ACID</th>
<th>METHYLFOLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Must be processed by the body</td>
<td>Immediately effective</td>
</tr>
<tr>
<td>Increases UMFA</td>
<td>Does NOT increase UMFA</td>
</tr>
<tr>
<td>Masks B12 deficiency</td>
<td>Does NOT mask B12 deficiency</td>
</tr>
<tr>
<td>Raises plasma folate slowly</td>
<td>Raises plasma folate quickly</td>
</tr>
<tr>
<td>Reduces neural tube defects</td>
<td>Reduces neural tube defects</td>
</tr>
<tr>
<td>Readily available</td>
<td>Readily available</td>
</tr>
<tr>
<td>Competes with active folate binding</td>
<td>This is active folate</td>
</tr>
<tr>
<td>May depress NK cells, down regulate receptors</td>
<td>Not studied</td>
</tr>
</tbody>
</table>

Please note 5MTHF is not a TGA approved supplement.
GLUTATHIONE

Biochemistry and Metabolism

Reduced Glutathione (GSH) is a linear tripeptide of L-glutamine, L-cysteine, and glycine. Technically N-L-gamma-glutamyl-cysteinyl glycine or L-Glutathione, the molecule has a sulfhydryl (SH) group on the cysteinyl portion, which accounts for its strong electron-donating character.

As electrons are lost, the molecule becomes oxidised, and two such molecules become linked (dimerised) by a disulfide bridge to form Glutathione disulfide or oxidised Glutathione (GSSG). This linkage is reversible upon re-reduction.

Glutathione is under tight homeostatic control both intracellularly and extracellularly. A dynamic balance is maintained between GSH synthesis, its recycling from GSSG/oxidized Glutathione, and its utilisation.

Glutathione synthesis involves two closely linked, enzymatically-controlled reactions that utilize ATP. First, cysteine and glutamate are combined by gamma-glutamyl cysteinyl synthetase. Second, GSH synthetase combines gamma-glutamylcysteine with glycine to generate Glutathione. As Glutathione levels rise, they self-limit further GSH synthesis; otherwise, cysteine availability is usually rate-limiting. Fasting, protein-energy malnutrition, or other dietary amino acid deficiencies limit Glutathione synthesis.

Glutathione recycling is catalyzed by Glutathione disulfide reductase, which uses reducing equivalents from NADPH to reconvert GSSG to 2GSH. The reducing power of ascorbate helps conserve systemic Glutathione.

Glutathione is used as a cofactor by:

- Multiple peroxidase enzymes, to detoxify peroxides generated from oxygen radical attack on biological molecules;
- Transhydrogenases, to reduce oxidized centers on DNA, proteins, and other biomolecules; and
- Glutathione S-transferases (GST) to conjugate Glutathione with endogenous substances (e.g., estrogens), exogenous electrophiles (e.g., arene oxides, unsaturated carbonyls, organic halides), and diverse xenobiotics. Low GST activity may increase risk for disease—but paradoxically, some Glutathione conjugates can also be toxic.

Direct attack by free radicals and other oxidative agents can also deplete Glutathione. The homeostatic Glutathione redox cycle attempts to keep Glutathione repleted as it is being consumed. Amounts available from foods are limited (less that 150 mg/day), and oxidative depletion can outpace synthesis.
The liver is the largest Glutathione reservoir. The parenchymal cells synthesize GSH for P450 conjugation and numerous other metabolic requirements—then export GSH as a systemic source of SH-reducing power. Glutathione is carried in the bile to the intestinal luminal compartment. Epithelial tissues of the kidney tubules, intestinal lining and lung have substantial P450 activity—and modest capacity to export Glutathione.

Glutathione equivalents circulate in the blood predominantly as cystine, the oxidised and more stable form of cysteine. Cells import cystine from the blood, reconvert it to cysteine (likely using ascorbate as cofactor), and from it synthesize GSH. Conversely, inside the cell, Glutathione helps re-reduce oxidised forms of other antioxidants—such as ascorbate and alpha-tocopherol.

**Mechanism of Action**

Glutathione is an extremely important cell protectant. It directly quenches reactive hydroxyl free radicals, other oxygen-centered free radicals, and radical centers on DNA and other biomolecules. Glutathione is a primary protectant of skin, lens, cornea, and retina against radiation damage and other biochemical foundations of P450 detoxification in the liver, kidneys, lungs, intestinal, epithelia and other organs.

Glutathione is the essential cofactor for many enzymes that require thiol-reducing equivalents, and helps keep redox-sensitive active sites on enzyme in the necessary reduced state. Higher-order thiol cell systems, the metallothioneins, thioredoxins and other redox regulator proteins are ultimately regulated by Glutathione levels—and the GSH/GSSG redox ratio. GSH/GSSG balance is crucial to homeostasis—stabilizing the cellular biomolecular spectrum, and facilitating cellular performance and survival.

Glutathione and its metabolites also interface with energetics and neurotransmitter syntheses through several prominent metabolic pathways. Glutathione availability down-regulates the pro-inflammatory potential of leukotrienes and other eicosanoids. Recently discovered S-nitroso metabolites, generated in vivo from Glutathione and NO (nitric oxide), further diversify Glutathione’s impact on metabolism.

**ASSESSMENT OF GSH STATUS: GGT**

Gamma glutamyl transferase (GGT) is located on the outer surface of all cell membranes and plays a key role in glutathione homeostasis in all cells, but is particularly important within hepatocytes. GGT is responsible for breaking down extracellular GSH and providing cysteine, the rate-limiting substrate for intracellular synthesis of GSH.22,23
GGT is a sensitive indicator of hepatic inflammation and oxidative damage, and has been widely used as an indicator of heavy or chronic alcohol use. However, recent research indicates that it can also be a good surrogate marker for increased need for detoxification.\textsuperscript{24,25} This is because GGT plays a key role in the synthesis and degradation of glutathione and therefore is a marker of glutathione requirement. Given that glutathione is the main intracellular antioxidant and is critical for drug and xenobiotic detoxification, GGT can be used as a marker for detoxification capacity. Essentially, increased toxin exposure, oxidative stress or damage to hepatocytes increases the demand for GGT, raising levels.

If there has been xenotoxic exposure, GGT levels will be raised but alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) will often remain normal. In adult women, GGT levels should be <30 units/L, whilst in men they should be <50 units/L (men have higher levels of GGT due to normal healthy prostatic production).\textsuperscript{26} Any increase in GGT levels above this indicates an increased need for cellular glutathione, most commonly due to toxicity.

Cellular GGT is involved in the recycling of extracellular glutathione, known as the gamma glutamyl cycle. GGT releases glutamate and cysteiny1 glycine, which is broken into cysteine and glycine. Cellular GGT is necessary for the metabolism and exportation of toxins conjugated by glutathionation. This exportation of conjugated toxins for excretion via bile or urine is known as Phase II detoxification.

GGT has a reciprocal relationship with glutathione: a decrease in intracellular glutathione is associated with a rise in GGT levels, as GGT attempts to import more amino acids into the cell for glutathione manufacture.

This inverse relationship has been confirmed in animal studies; in one study, mice were fed a proteindeﬁcient diet which resulted in reduced glutathione levels. It was noted that during this diet, GGT levels rose.

Once substituted on a protein-rich diet, glutathione levels increased, which correlated with a decrease in GGT. Other studies in knockout mice have also demonstrated that glutathione-deﬁcient mice have increased GGT levels.\textsuperscript{28}

Supplements, including N-acetylcysteine,\textsuperscript{33} S-adenosylmethionine (SAMe)\textsuperscript{34,35,36} and whey protein\textsuperscript{37,38,39,40} have also been shown to increase cellular glutathione content in persons suffering from a disease-related glutathione deﬁciency.

Zinc has been shown to have some influence over de novo glutathione synthesis.\textsuperscript{41,42,43} Furthermore, magnesium has signiﬁcant inﬂuence on glutathione synthesis, it appears to be an essential cofactor.\textsuperscript{44,45,46,47,48}
# IMBALANCES IN HISTAMINE & PYRROLURIA

<table>
<thead>
<tr>
<th>Common nutritional imbalances</th>
<th>HIGH HISTAMINE (under-methylator)</th>
<th>LOW HISTAMINE (over-methylator)</th>
<th>Pyrroluria</th>
<th>High Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW calcium, methionine, B6</td>
<td>LOW folate, B3, B12</td>
<td>Low zinc, B6, manganese and arachidonic acid (omega-6)</td>
<td>Low Zinc</td>
<td>Excess copper</td>
</tr>
<tr>
<td>Excess folic acid</td>
<td>Excess copper and methionine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common neurotransmitter imbalances</th>
<th>HIGH HISTAMINE</th>
<th>LOW HISTAMINE</th>
<th>Pyrroluria</th>
<th>High Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low serotonin, dopamine and noradrenaline</td>
<td>HIGH histamine</td>
<td>LOW histamine</td>
<td>LOW serotonin</td>
<td>HIGH noradrenaline and adrenaline</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory tests*</th>
<th>Whole blood histamine &gt; 70 ng/ml</th>
<th>Whole blood histamine &lt; 40 ng/ml</th>
<th>Elevated urine Kryptopyroles (Mauve Factor)</th>
<th>Serum copper &gt; 140 mcg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ceruloplasmin</td>
<td>High serum copper</td>
<td></td>
<td>Low ceruloplasmin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cause of imbalances</th>
<th>Genetic tendency for under-methylation</th>
<th>Genetic tendency for over-methylation</th>
<th>Abnormal haemoglobin synthesis</th>
<th>High copper</th>
</tr>
</thead>
</table>

| Beneficial supplements | Calcium, methionine, magnesium, zinc, TMG, omega-3s, B6, SAMe, inositol, vitamins A, C & E | B3, B12, folate, choline, manganese, zinc, omega-3, vitamins C & E | B6, zinc, Evening Primrose Oil | Zinc, manganese, vitamin C & B6 |

| Potentially harmful supplements | Folate, choline, copper, histidine | Methionine, SAMe, inositol, tryptophan, phenylalanine, St. John’s wort, tyrosine, copper, TMG and DMG. | Histidine, copper, omega-3 EFAs | Copper containing supplements, oestrogen |

*Reference ranges vary with laboratory techniques.
MTHFR SNP

Increasingly investigated is the methylenetetrahydrofolate reductase single nucleotide polymorphism (MTHFR SNP). The MTHFR enzyme leads to the production of methyl-folate (5-MTHF or 5-methyltetrahydrofolate), the predominant form of folate circulating in the body. Methyl-folate is needed to recycle homocysteine to methionine.

Genetic variants in MTHFR can affect susceptibility to neural tube defects, occlusive vascular disease, acute leukaemia, and colon cancer. This genetic test can reveal a variation that affects the MTHFR enzyme and can ultimately impair methylation.

Patients with cardiovascular disease, neuropsychiatric disorders, schizophrenia, or suicidal tendencies may benefit from this test. A chronically high homocysteine that does not normalise with B vitamins is a clue that MTHFR may be impaired. There are a number of enzymes involved, so we can’t necessarily limit the investigation to genetic variation of MTHFR only.

Because of the variety of possible genetic mutations affecting enzyme function, it is important to assess functional markers of methylation, not just genetic markers. Homocysteine may be elevated while FIGLU and MMA are normal in a patient with the MTHFR SNP.

Variations of MTHFR polymorphisms will affect blood levels of different form of folates.

Even with MTHFR polymorphisms, supplementation of folic/folinic acid leads to good serum levels especially if taken with vitamin B2.

MTHFR Variants & Effects

<table>
<thead>
<tr>
<th>MTHFR C677T</th>
<th>MTHFR A1298C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A222V, rs1801133)</td>
<td>(E429A, rs1801131)</td>
</tr>
<tr>
<td><strong>Cardiovascular effects</strong></td>
<td><strong>Neurological effects</strong></td>
</tr>
<tr>
<td>Homocysteine (possibly elevated)</td>
<td>Neurotransmitters (possibly low levels)</td>
</tr>
<tr>
<td>DNA regulation</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Glutathione production</td>
<td>Low BH4 (tetrahydrobiopterin) levels</td>
</tr>
<tr>
<td>MTHFR C677T HETEROZYGOUS = 40% loss of function</td>
<td>A1298C HETEROZYGOUS = 20% loss of function</td>
</tr>
<tr>
<td>MTHFR C677T HOMOZYGOUS = 70% loss of function</td>
<td>A1298C HOMOZYGOUS = 40% loss of function</td>
</tr>
</tbody>
</table>

MTHFR C677T HETEROZYGOUS + A1298C HETEROZYGOUS = 40% loss of function
Who to Screen For MTHFR Mutations?

<table>
<thead>
<tr>
<th>ADD/ADHD</th>
<th>Cancer</th>
<th>Insomnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addictive behaviours</td>
<td>CFS, Fibromyalgia</td>
<td>MS</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>Chronic pain</td>
<td>Neuropathy, neurological disorders</td>
</tr>
<tr>
<td>Allergies, chemical sensitivity</td>
<td>Chronic viral infection</td>
<td>Parkinson’s</td>
</tr>
<tr>
<td>Alzheimer’s, dementia</td>
<td>Congenital heart defects</td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Depression, Bipolar</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Diabetes</td>
<td>Spina bifida, neural tube defects</td>
</tr>
<tr>
<td>Autism</td>
<td>Immune deficiency</td>
<td>Thyroid dysfunction</td>
</tr>
<tr>
<td>Birth defects</td>
<td>Infertility, recurrent miscarriages</td>
<td></td>
</tr>
</tbody>
</table>

Also consider for those: preconception care, cervical dysplasia, drug sensitivities, supplement sensitivities, high Histamine, High B12.

Having two MTHFR variants is more common than having high Homocysteine or any health condition linked to high Homocysteine.

Therefore MTHFR is not all about high Homocysteine.

MTHFR 1298 associated with neurological (due to low biopterin).

BH4 needed to convert Tyrosine to Dopamine, Tryptophan to Serotonin, and Arginine to Nitric oxide.

MTHFR 1298 is not seen as severe as the 677 polymorphism.
SUPPORTING NUTRIENTS FOR MTHFR

- Folinic acid; or L-methylfolate (5MTHF) – take with Vitamin C 300-900mg
- Sublingual B12; or methylcobalamin and/or hydroxycobalamin
- Vitamin E (mixed tocopherols), D3, C, B2, B6 (P5P)
- Krill oil, fish oil (EPA/DHA)
- Curcumin; Silymarin; Withania, other adaptagens
- Se, Zn, Mg, K
- NAC, MSM, SAMe, Methionine, Inositol, TMG, CoQ10, ALA, L-Carnitine, Ribose
- Glutathione
- Probiotics
- Multivitamin/minerals

Diet alone can affect changes in methylation. Increase dietary methylation via:

- Animal based protein – creatine, choline, B12, carnitine
- Methionine – eggs, soy protein, spirulina
- Vitamin B6 – grains, soy protein, nuts, seeds
- Vegetarians need to be supplemented - creatine, choline, B12 and carnitine.

Affected by:

- Lifestyles – meat & potatoes, vegan/vegetarian, obesity, athletes, Type As
- Addictions – alcohol, smoking
- Environment – heavy metals, pesticides/herbicides, xenobiotics
- Medications – acetaminophen, antacids, nitrous oxide
MTHFR C677T BASIC PROTOCOL (www.mthfr.net)

When starting a MTHFR protocol, do not begin treatment until you have considered patients:

- History, lifestyle, diet. Start with foundational health first ideally. If not, then side effects are likely.
- Dietary intake - switch to GAPS or Paleo, low reactive.
- Toxin and chemical exposure? Reduce it.
- Digestion? Improve it. Look at CDSA/parasitology.
- Medications - look for folate antagonists.
- Supplements - which make them worse? Reduce.
- Remove all folic acid from diet.
- Consider B2, B12, B6; Mg.
- Homocysteine levels.
- Oxidative stress.
- Thyroid and adrenals health.
- Fatty acids levels.

The outcome of supplementing with various nutrients can vary tremendously due to other genetic defects, dietary and lifestyle choices and environmental exposures.

IF HETEROZYGOUS OR HOMOZYGOUS MTHFR C677T:

The biggest differences in recommendations between these two types of mutations are:

- Folic acid needs to be avoided more seriously by homozygous individuals;
- The amount of methylfolate required for homozygous mutations is greater;
- The blood thinning requirement is greater for homozygous individuals.
Common recommendations for supporting those with C677T MTHFR mutations:

- Limit ingestion of folic acid in fortified foods as they cannot process folic acid well.
- Limit or cease taking supplements or drugs with folic acid in them.
- Avoid folic acid blocking drugs such as birth control or Methotrexate.
- Avoid drugs which increase homocysteine such as nitrous oxide (used in dentistry).
- Avoid antacids as they block absorption of vitamin B12 and other nutrients.
- Eliminate gluten from the diet – especially wheat.
- Eliminate or reduce dairy from the diet.
- Limit intake of processed foods
- Increase intake of whole foods and home-prepared meals.
- Eat a ‘rainbow’ of colours from fruits and vegetables daily.
- Limit intake of high methionine-containing foods if homocysteine elevated.
- Eat smaller, but more frequent meals, throughout the day with some form of protein.
- Avoid cooking, drinking, storing and heating in any type of plastic container.

General Supplement Recommendations for MTHFR C677T

While these are the general supplements recommended by Dr Lynch, he never recommends taking them all right away.

*Begin by taking the most important nutrient first* (depending on the individual) in a *small amount* for at least a few days to see patient response. If patient responds well, continue and add in another supplement.

This way you can easily identify if a specific supplement or nutrient is causing problems.

**NOTE:** If patient is *not* sensitive to supplements in general, then it is recommended to start with a comprehensive multivitamin and multimineral as this supports numerous biochemical functions within your body. It also provides a fast testing ground to see if you respond well to numerous nutrients.

If a patient does not tolerate a multivitamin well, this is a sign that you must proceed more slowly and work on healing gut/digestion and dietary intake and lifestyle habits first.
NOTE: Many people think that once a supplement is recommended, it is needed to take every day, sometimes multiple times a day and forever. WRONG. This is a COMMON MISTAKE made by patients and practitioners.

A supplement is defined as ‘something that completes or enhances something else when added to it’. This means that a supplement enhances your biochemistry and physiology – and once complete – stop. If you continue to supplement beyond completing or enhancing your patient’s biochemistry, you are going to push it beyond where it needs to be.

Is your patient already taking Methylfolate and feeling good?
Excellent! However, it may be a ‘honeymoon’ period and in a few days or weeks, side effects may appear.

“I’ve made people go down the ‘other side’ by not pulsing methylfolate and by not preparing their biochemistry for it. It is much harder getting them back where they were than it is preventing them from getting side effects in the first place. I’ve seen it happen way too much – caused by me, caused by other doctors and caused by over-excited people feeling amazing and pushing their system too hard with methylfolate. Methylfolate is powerful.”

These two recommendations may make a significant difference in how people respond to methylfolate and methylation in general.

1. Electrolytes
2. Glutathione

1. Electrolytes.
Methylfolate supports methylation. Methylation supports cell growth and division.

Cells contain magnesium and potassium – and glutathione. If any of these are deficient, then the cell does not function properly.

As the cells malfunction, patients can experience greater side effects and a flare of their immune system.

Solution: Take electrolytes BEFORE taking any form of methylfolate OR methylcobalamin.

Who needs electrolytes? Given that a significant number of people are potassium deficient – most.
Key signs that you need electrolytes:

- Nausea
- Dizzy
- Frequent urination
- Drinking water and then having to go to the bathroom quite quickly
- Muscle aches/spasms
- Frequent thirst which is insatiable
- Dry skin

Key issues increasing need for electrolytes:

- Stress
- Exercise
- Sweating
- Diet high in sodium / low in potassium (MOST of us)
- Caffeine intake
- Processed foods (due to high sodium/low potassium)
- High protein diet (GAPS and Paleo – high protein depleting magnesium and potassium – especially if not eating greens/veges)

How to take electrolytes: Sip or drink one serving 20 minutes prior to exercise and possibly another serving during or after – depending on the duration of activity.

If you are not active due to fatigue, simply add ½ to 1 serving in a tall glass of filtered water and drink over a longer time period.

2. Glutathione

As methylfolate supports methylation, cells divide. As cells divide, the amount of glutathione they have reduces. If one is already deficient in glutathione – and many people with MTHFR are deficient in glutathione – then there is going to be a flare of side effects.

Foods which increase glutathione are those which contain cysteine, glutamine and glycine. Also requires magnesium, ATP, amino acid transport across the cell membrane and also the outer mitochondrial membrane. Then these components work together to form the glutathione.

Then, once the glutathione is formed, it gets used up quickly IF there is adequate selenium. After it gets used, it is damaged and has to get repaired and this requires vitamin B2 as active riboflavin. This active form of riboflavin is FAD and needs T4 thyroid hormone to form it.
Solution: Easiest way to increase glutathione levels is with liposomal glutathione. This allows the glutathione to ‘slip’ inside the cell with tiny liposomes. In fact, this is more effective at raising red blood cell levels of glutathione than IV glutathione — and significantly less expensive. You can also obtain oral glutathione capsules and glutathione precursors.

Who needs glutathione? Most people, especially those with any chronic condition as it is likely they will be low in glutathione.

How to take glutathione: Start very slowly with a small amount. If you are sensitive in general to things, start with just a few drops.

Pulsing glutathione is also likely recommended. This means taking it every other day or every few days initially. As you continue to improve or feel better, you may increase the frequency or the dosage, slowly.

NOTE: If your patient feels worse, have them stop taking it. You may need to ‘open up their sulfite pathway with vitamin B1 (Thiamin) and molybdenum first. If you know you do not tolerate sulfites – wine, dried fruits – or sulfur-containing foods like eggs, cruciferous vegetables or your flatulence smells like sulfur, then you should also support sulfite pathway first with B1 and molybdenum before taking glutathione. Avoiding sulfur-containing foods and supplements for a few days is also recommended to help clear out the sulfite pathway.

Vitamins & Adrenals

Of course, there are other factors at play – such as a generalised deficiency in various B vitamins and other minerals.

If you suspect that your patient may be deficient in various minerals or vitamins, then it is important that you replenish them prior to supporting with methylfolate or methylcobalamin. Because if you support with these two powerful methyl donor nutrients, it can cause a ‘clog’ in their biochemistry. This ‘clog’ may occur in how your brain chemicals (neurotransmitters) get formed and/or eliminated.

If you feel your patient has okay mineral status and vitamins except B vitamins, supplement without methylfolate and methylcobalamin first. This helps prime a lot of biochemical pathways without stimulating methylation. Most people respond very well to this.

Also reducing patient’s stress and supporting their adrenals gives best results. Stress is a direct stimulator of methylation. If reducing stress, then the demand on methylation goes down. Therefore, dependence upon nutrients such as methylfolate and methylcobalamin go down.

If one is stressed, they are using up a variety of nutrients – and their cortisol is likely low – especially if they have been stressed or anxious for some time. This leads to autoimmunity, fatigue, hypothyroidism, wasting, poor memory, hard to get out of bed in the morning, frequent urination (loss of K and retention of Na).
Supporting adrenals can be quite rapid if:

- Eating properly
- Avoiding caffeine and stimulants
- Supplementing with adaptogens
- Sleeping before 11 PM
- Getting at least 7 hrs of rest nightly
- Increasing activities which you really enjoy.

**SUMMARY**

Introduce one nutrient or one change at a time – every few days – so you are assured it is either helping, doing nothing or hindering. If you add many things or change many things at once, it becomes frustrating trying to pinpoint what is going on.

It is important to prepare your cells and other biochemical pathways before stimulating them with methylfolate and methylcobalamin.

Following the lifestyle, dietary and environmental recommendations as above in MTHFR BASIC PROTOCOL is a very important first step.

The next step is to begin with some basics – and these basics are as explained above:

- electrolytes
- glutathione
- B vitamins (no B12 and no methylfolate)
- multivitamin/multimineral (no B12 and no methylfolate – plus no Iron or Copper)
- reducing stress by supporting lifestyle, diet and adrenals.

**AVOID**

- Prescribing high dose 5MTHF without titrating up – potential massive side effects, even hospitalization.
- Giving 5MTHF first instead of methylB12 as can lead to methyl trapping.
- Prescribing multiple supplements to have them start the day.
- Performing potent therapy such as coffee enemas, saunas, Epsom salt baths before foundation work completed.
- Measuring homocysteine as a guide to ‘therapeutic guide post’ – totally inaccurate marker as has four other routes to reduce its levels.
SIDE EFFECTS WITH METHYLFOLATE

If your patient is feeling improvement consistently, then you are on the right track.

If your patient begins to feel heavy, tired, dry mouth, irritable, “toxic”, or otherwise “not right”, then something in your protocol needs to change.

These are all signs that one must be aware to otherwise you are potentially increasing the circulation of toxins and not eliminating them properly.

Common undesirable side effects of methylfolate must be identified, such as:

- Muscle / Joint pain
- Stomach pain
- Nausea / Vomiting
- Headache
- Irritability
- Anxiety
- Depression
- Insomnia
- Palpitations
- Rash
- Herxheimer reaction
- Seizures
- Sweating

If methylation becomes excessive, side effects will occur as noted above. This requires adjustment in your patient’s protocol.

If side effects occur, then the amount of supplementation likely needs to be reduced. There are other steps that must be taken prior to supplementing with methylfolate if these side effects occur.

For example, these adjustments may range from:

- Stopping all methylation-supportive nutrients;
- Taking these nutrients 4 days on and 3 days off;
- Taking them every other day;
- Taking them only in the morning;
- Decreasing the amount taken every other week.
TO NEUTRALISE SIDE EFFECTS

- 50-100mg time-released niacin – Niacin is broken down via methylation by SAMe. Niacin also helps breakdown glutamate and increase GABA. (Caution flushing for 20-30 minutes due to histamine release)

- Curcumin – reduce inflammation. If give 5MTHF before inflammation controlled, patients symptoms will become worse.

- Evaluate potassium levels. Commonly low in ‘detox’ reactions. Supplement with high potassium foods – apricots, avocados, dates, carrot juice, almonds, backed beans, lima beans, potatoes

THEORY

Excess 5MTHF and BH4 increase noradrenaline and adrenaline levels causing increased anxiety, palpitations.

Excess 5MTHF may also utilise BH4 thereby causing an increase in peroxynitrite and nitric oxide. These cause pain, headaches and soreness.

If NO tolerance to methylfolate: Cease. Heal gut, change diet, check for Helicobacter pylori, gene test (COMT, CBS, and MAO), and other labs.

If NO tolerance to methylB12: Switch to HYDROXYCOBALAMIN – start low and titrate up. Heal gut, change diet, check for Helicobacter pylori, gene test (COMT, CBS, and MAO), and other labs.
# DRUGS TO AVOID WITH MTHFR

<table>
<thead>
<tr>
<th>MEDICATION</th>
<th>AFFECT</th>
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<tbody>
<tr>
<td>Antacids</td>
<td>Depletes B12</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>Deplete cobalamin and folate absorption – commonly used with gallbladder issues in pregnancy</td>
</tr>
<tr>
<td>Colestipol</td>
<td>Decreases cobalamin and folate absorption</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Inhibits DHFR (dihydrofolate reductase)</td>
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<tr>
<td>Nitrous oxide</td>
<td>Inactivates MS (methionine synthase)</td>
</tr>
<tr>
<td>Niacin</td>
<td>Depletes SAMe and limits pyridoxal kinase = active B6 – useful during times of over-methylation</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Limits pyridoxal kinase = active B6)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>Decreases renal function and increases Homocysteine</td>
</tr>
<tr>
<td>Metformin</td>
<td>Decreases cobalamin absorption</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Folate antagonist</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Folate antagonist</td>
</tr>
<tr>
<td>Oral Contraceptive Pill</td>
<td>Deplete folate</td>
</tr>
<tr>
<td>Antimalarial e.g. JPC 2056, Pyrimethamine, Proguanil</td>
<td>Inhibits DHFR (dihydrofolate reductase)</td>
</tr>
<tr>
<td>Antibiotic e.g. Trimethoprim, Bactrim</td>
<td>Inhibits DHFR (dihydrofolate reductase)</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Inhibits DHFR (dihydrofolate reductase)</td>
</tr>
<tr>
<td>Triamterene</td>
<td>Inhibits DHFR (dihydrofolate reductase)</td>
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http://heart.bmj.com/content/83/2/127/T1.expansion.html

OTHER GENE MUTATIONS TO CONSIDER

Consider the following SNPs:

MTHFR; COMT, MAO A, CBS, MTR/MTRR, GSTM1, SOD, GAD, HNMT, QDPR, NOS, SUOX.

COMT, MAO – processes neurotransmitter catabolism and estrogens
CBS – processes Homocysteine and if unregulated, depletes CH3, increases taurine
MTR/MTRR – recycles B12 and processes B12 for methionine production
GST1, SOD – major detox.
GAD – transforms glutamate to GABA
HNMT – processes Histamine (secondary enzyme; DAO is primary)
QDPR – recycles BH4
NOS – processes ammonia, forms NO from arginine
SUOX – processes sulphites/sulphur and this SNP is made worse from CBS up regulation

DAO inhibited by alcohol, therefore wine causes histamine reaction.

Caution using 5MTHF in those with COMT gene mutation as can lead to increased dopamine converting to high noradrenaline and adrenaline levels.

Caution glutathione intake leading to down regulating CBS pathway. Use Se, cysteine, B6 and Vitamin C.

See increased ammonia in CBS mutation or lowered BH4.

See www.smartdna.com.au or www.fitgenes.com for further SNP testing.
CONCLUSION

Methylation can be considered the crossroads of metabolism. Disruption of methylation can therefore be conceptualised as either a cause of various symptoms and pathologies or as a consequence of various symptoms, lifestyle and dietary choices.

It is well known that various metabolic pathways involved in methylation are driven by specific coenzyme nutrients, which when depleted or deficient can lead to dysfunction of the pathways on which they work. It is perhaps less known that various genetic polymorphisms, diagnosed conditions, medications, hormones, nutrients, diets and lifestyle circumstances can contribute to the availability of these essential coenzyme nutrients.

When considering the influence of methylation (and the various pathways involved) it is therefore valuable to utilise ad integrate your knowledge of all of these factors in the interpretation of your patient’s biochemistry – aim to integrate pathology and gene tests with your knowledge of your patient’s diagnosed conditions, medications, hormone profiles, dietary requirements and lifestyle choices.

Through adapting this integrative and personalised approach, methylation need not necessarily be a phenomenon to treat but rather one that can enrich your understanding of you patient’s current physiology and presenting concerns.

While it might appear to be a simple carbon and hydrogen group getting passed around between chemicals, methylation is actually very important in the world of nutritional biochemistry. It can be the difference between life and death or health and disease. Patients struggling with infertility, autism, cancer, and stroke or cardiac events may all greatly benefit from assessment of methylation markers.

As discussed, no one test is enough to make definitive conclusions about a patient’s methylation capacity. While homocysteine is a widely recognized methylation marker, other markers can help the clinician view a patient’s methylation capacity from different angles. The genetic test, MTHFR, and markers such as FIGLU, MMA and 5-MTHF can be used with homocysteine to assess a patient’s methylation status. Finally, amino acids, oestrogen metabolism, and neurotransmitter metabolites can give additional information about a patient’s methylation pathways.
RESOURCES

www.mthfr.net
www.mthfrsupport.com.au
www.mthfrsupport.com

GLOSSARY

SAMe  S-Adenosylmethionine
SAH  S-Adenosylhomocysteine
TMG  Trimethylglycine (also known as Betaine)
Folic acid  Folate (synthetic form)
Folinic acid  Also known as calcium folinate, 5-formyl-THF.
SMTHF  5-methyltetrahydrofolate (common form of folate in nature, though unstable). Also known as methylfolate
FIGLU  Formiminoglutamic Acid
MMA  Methylmalonic Acid
PSP  Pyridoxal-5-phosphate
NAC  N-Acetyl Cysteine
GSH  Glutathione
ALA  Alpha Lipoic Acid
EFA  Essential Fatty Acid
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www.neurosciencemyths.com/Methylation.htm


http://www.glutathioneexperts.com/what-is-glutathione.html


