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a **phytotherapist's** perspective

Nutrients & Green Tea to Support Methylation

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Key Points at a Glance

Methylation

- Biochemical process of transferring methyl groups among compounds in the body
- Considered an important determinant in many chronic diseases due to its role in the regulation of DNA, protein structure, and the production of phospholipids, certain hormones and neurotransmitters
- Our ability to maintain adequate levels of methylation declines with age
- Necessary nutrients include folate, choline, vitamins B6 and B12
- Homocysteine is metabolised via remethylation and trans-sulfuration pathways
- Lowering homocysteine and/or having adequate methylation nutrients may have an impact on many diseases and may, for example, reduce risk of cardiovascular disease (CVD), cancer and dementia

B Vitamins

- B2, B6, B12 and folic acid can be taken in the form of their metabolites (known as activated B vitamins)
- Activated B vitamins may be important in those with DNA polymorphisms that encode less active forms of enzymes involved in B vitamin metabolism
- Epidemiological studies:
  - high homocysteine, low B vitamins associated with increased risk of cancer
  - low folate and B12, increased homocysteine can occur in depressed patients
- B6, folate and B12 are particularly critical to haematopoiesis (blood-building activity)
- Increased risk of B-vitamin deficiency in the elderly
- Clinically demonstrated to:
  - improve response to antidepressant fluoxetine in female patients (0.5 mg/day folic acid)
  - decrease the risk of stroke and overall CVD
  - improve endothelial dysfunction as assessed by flow-mediated vasodilation
  - increase blood flow in coronary artery disease (0.8 mg/day folic acid + 0.4 mg/day B12)
  - improve memory and decrease brain atrophy in mild cognitive impairment patients with high homocysteine levels (0.8 mg/day folic acid + 20 mg/day B6 + 0.5 mg/day B12; taken for 2 years)
  - improve memory in depressed older adults, particularly in those with high homocysteine levels (0.4 mg/day folic acid + 0.1 mg/day B12; taken for 2 years)
  - decrease oxidation-induced DNA damage in a susceptible population, particularly in those with hypercholesterolaemia (0.4-0.8 mg/day folic acid)
  - increase glutathione concentrations in autistic children (0.8 g/day oral folinic acid + B12 by injection)
- Safety: folate and B12 should be consumed together; B6 should not exceed doses above 400 mg/day

Green Tea

- Contains catechins
- Epidemiological studies:
  - difficult to assess 'dosage' in terms of dried herb equivalent or catechins, but likely to be high
  - association of green tea consumption and cancer risk have produced mixed results, or have required high intake
  - inverse associations found between intake of green tea and the DNA methylation of two tumour-related genes (high dose, probably 300-400 mg/day of catechins)
- Clinically demonstrated to:
  - decrease oxidation-induced DNA damage in lymphocytes of healthy volunteers and type 2 diabetics (1.5 g/day of dried leaf)
  - provide an antioxidant effect in healthy volunteers (1.75 g/day dried leaf) and chemical workers (3 g/day of dried leaf)
- Safety:
  - unlikely to have clinically-relevant antifolate activity or adverse foetal effects, although small addition of folate, or use of activated forms of folate, may be advisable if green tea alone is given to pregnant women
  - effect on iron absorption is unclear
  - small number of liver damage cases reported with use of ethanol extracts or highly concentrated water extract; effect on liver enzymes not expected when EGCG dose is below 600 mg/day

Indications

- Reduce risk of cardiovascular disease, particularly stroke
- May reduce cognitive decline by reducing age-related brain atrophy
- To support genomic stability and to help prevent cancer
- May support patients with depression, particularly those with low folate and/or high homocysteine levels

The Importance of Methylation

Methylation is the biochemical process of transferring methyl groups (CH_3) among various compounds in the body. Methylation is required to ensure the proper functioning of metabolic pathways, efficient enzyme activity and the regulation of gene expression. One example of methylation that has wide ranging consequences is the methylation cycle of homocysteine. Beyond homocysteine, methylation is considered an important determinant in many chronic diseases due to its role in the regulation of DNA, protein structure, production of phospholipids, and production of certain hormones and neurotransmitters. DNA methylation is an important regulator of gene transcription. Aberrant DNA methylation has been implicated in a range of degenerative diseases associated with ageing, including cardiovascular disease (CVD), cancer and neurological diseases.[1-3]

Our endogenous ability to maintain adequate levels of methylation declines with age. Hypomethylation has a wide spectrum of effects that include genetic, epigenetic and metabolic alterations, and is considered a major determinant of how quickly we age.[4] For example, defective somatic cell methylation and accumulated genetic instability have been proposed as key mechanisms contributing to ageing.[5]

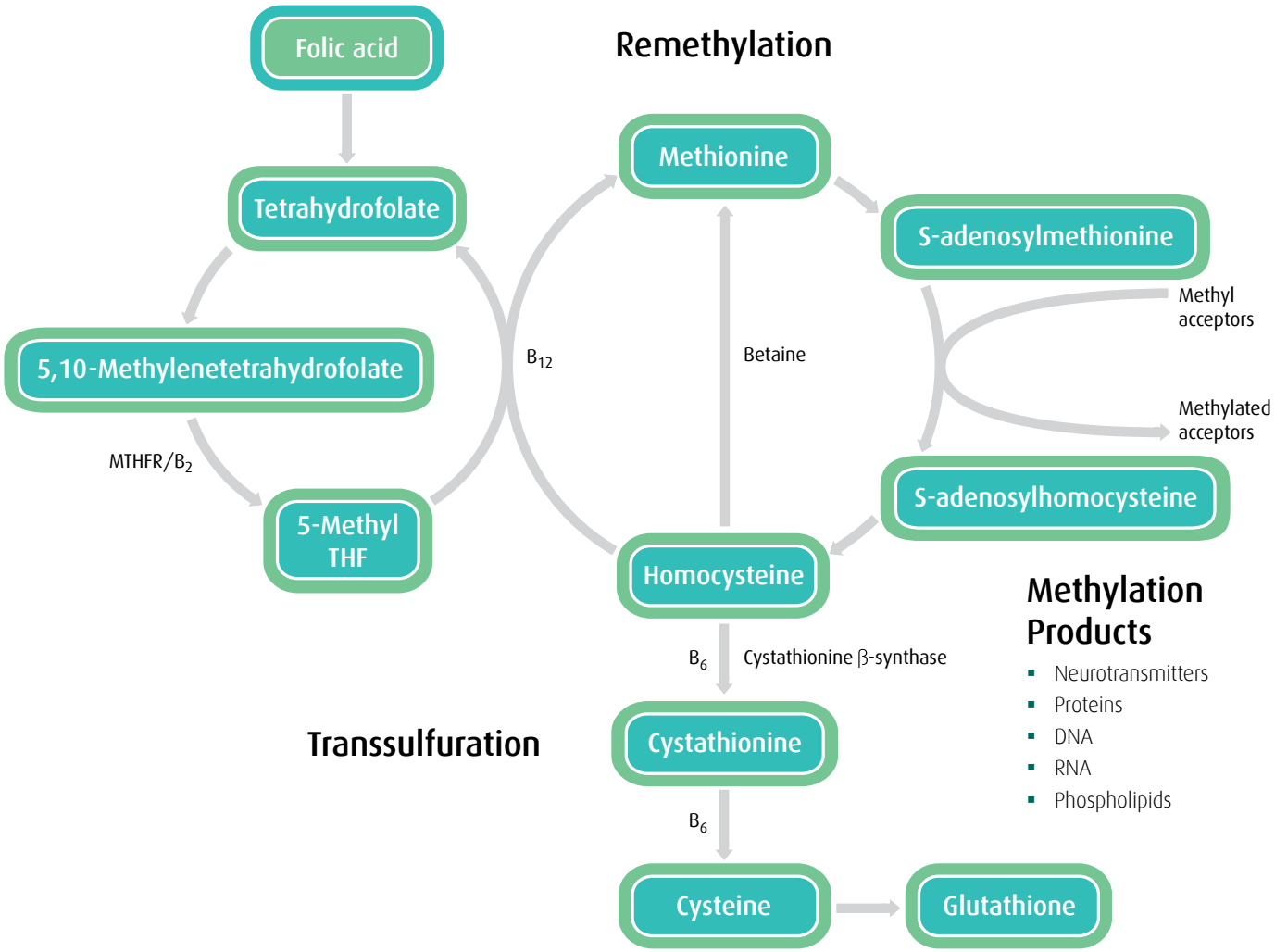
Methylation can be impaired by poor nutritional habits. The regular intake of nutrients involved in methylation, such as folate, vitamin B6, vitamin B12 and choline, can slow down the gradual hypomethylation observed during the aging process.[6]

Inadequate methylating nutrients may also result in impaired DNA methylation status leading to increased cancer risk.[7] These methylating factors play key roles in one-carbon metabolism and DNA methylation because they influence the supply of methyl groups and consequently the biochemical pathways of methylation processes.[8]

Homocysteine: What is it?

Homocysteine is a naturally occurring sulfur-containing amino acid, created when the body metabolises methionine. Homocysteine is a key determinant of the methylation cycle, and has profound importance in health and diseases. Under normal circumstances, homocysteine is metabolised by one of two divergent pathways: either irreversibly degraded via the trans-sulfuration pathway to cysteine, or remethylated back to methionine (*see Figure 1*).[9] When these metabolic conversions become impaired that homocysteine can rise to dangerous levels. Genetic and lifestyle factors, including inadequate nutrient status, systemic disease and various drugs have also been implicated as primary causes of elevated plasma homocysteine.[10]

Figure 1. Mechanisms of homocysteine detoxification.[15,16]



Remethylation

The remethylation of homocysteine to methionine involves two intersecting biochemical pathways. In the first instance, homocysteine is recycled to methionine by taking on a methyl group provided by methylcobalamin (vitamin B12), a reaction catalysed by the enzyme methionine synthase and dependent on riboflavin (vitamin B2) and 5-methyltetrahydrofolate (5-MTHF).[11,12]

In the second instance, homocysteine is recycled to methionine via the addition of a methyl group supplied by betaine (also known as trimethylglycine or TMG), a reaction catalysed by the enzyme betaine-homocysteine methyltransferase.[9,11] Betaine is not approved as an active ingredient in listable therapeutic goods in Australia. However, choline is a precursor for betaine and is one of the primary food sources of methyl groups.[12]

It should also be noted that sufficient supply of 5-MTHF for folate-dependent remethylation of homocysteine is dependent on the enzymatic reduction of 5,10-methylene-THF and the catalytic activity of methylenetetrahydrofolate reductase (MTHFR), a physiologically irreversible reaction. A single nucleotide polymorphism of the MTHFR gene can result in inadequate folate-dependent remethylation and subsequent elevations in homocysteine concentrations.[9]

The interrelationship between choline, methionine and folate are apparent when knockout mice are studied. MTHFR knockout mice, which have impaired availability of methyl groups from 5-MTHF, deplete choline and betaine in order to maintain homocysteine remethylation. Methionine adenosyltransferase knockout mice, which have impaired formation of s-adenosylmethionine (SAM), activate the gene expressing betaine:homocysteine methyltransferase and have increased dietary choline requirements. Further, cystathionine beta-synthase (CBS) knockout mice, which accumulate homocysteine and must convert it to methionine to remove it, deplete choline and betaine pools in the liver. Each of these genetic variations highlight the importance of adequate dietary intake and stores of choline in the body.[13]

Trans-sulfuration

The trans-sulfuration of homocysteine to cysteine (and eventually glutathione) is catalysed by cystathionine-ß-synthase (CBS), a process that is heavily dependent on pyridoxal 5-phosphate (vitamin B6 or PLP) as a cofactor. Elevated homocysteine can be caused by a genetic defect that blocks the trans-sulfuration pathway by inducing a deficiency of the vitamin B6-dependent enzyme CBS.[14]

Benefits of Supporting Methylation

Cardiovascular Health

Initial interest in methylation and its role in controlling homocysteine related primarily to its role in CVD. Research continues to demonstrate that people with high homocysteine levels are at greater risk of cardiovascular incidents, including high blood pressure, stroke, metabolic syndrome, type 2 diabetes, inflammation and CVD in general.[1,17,18] High levels of homocysteine have been shown to cause damage to endothelial cells,[19] oxidative stress,[20] and stimulation of a pro-coagulant and pro-inflammatory reaction between blood components.[21,22]

Results from observational and genetic epidemiological studies suggest lower serum homocysteine levels are associated with lower incidence of CVD.[23] Genetic studies are also providing stronger evidence for the potential role of folate and the related B vitamins in CVD, primarily through the investigation of the common C677T polymorphism in the gene encoding the folate-metabolising enzyme MTHFR. Epidemiological evidence suggests this common polymorphism increases the risk of CVD, especially stroke, by up to 40% overall.[24,25]

In a prospective cohort study of 1,823 stroke patients, increased homocysteine was significantly associated with all-cause mortality. Results showed an increased risk for stroke recurrence and all-cause mortality ($p < 0.0001$) when the highest and lowest categories were compared.[26] Studies have also shown that a 3 µmol/L lowering of homocysteine concentration resulted in a 10% reduced risk of recurrent stroke. Stroke mortality also decreased significantly following the introduction of folic acid fortification in the USA and Canada.[26]

In a prospective, case-control study of 3,090 patients with pre-existing, chronic, coronary heart disease, elevated homocysteine levels were associated with 2.5 times increased risk of subsequent coronary events. The effect was dose-dependent, with each 5 µmol/L increase in homocysteine concentration conferring a 25% higher risk.[27]

In a high-quality meta-analysis which included 12 studies and a total of 23,623 members of the general population, those with high homocysteine levels had increased risk of coronary heart disease mortality ($p = 0.012$), cardiovascular mortality ($p = 0.033$), and all-cause mortality ($p < 0.001$), compared to those with low homocysteine levels.[17]

A cross-sectional study of 156 Spanish adolescents also found that fasting homocysteine levels were negatively correlated with cardiovascular fitness in females, even after adjusting for potential confounders.[28]

Cancer

A meta-analysis of studies involving 15,046 cases and 20,712 controls examined the association of serum levels of homocysteine, and its metabolising factors, on overall risk of cancer.
A high serum level of homocysteine and folate deficiency was consistently associated with increased risk of cancer, with little effect by type of cancer or ethnicity. In addition, MTHFR C677T, A1298C and G1793A polymorphisms were related to elevated serum level of homocysteine, and folate and vitamin B12 deficiency.
However, only the MTHFR C677T polymorphism was positively associated with overall risk of cancer.[29]

There is epidemiological, clinical and animal evidence to support the hypothesis that insufficient folate supply favours the genesis of colorectal tumours. The chemopreventative effect of folate has been highlighted because of its role in DNA synthesis and mitosis, as well as DNA methylation and regulation of gene expression.[30-32]

Higher folate levels and lower homocysteine levels have also been shown to reduce the risk of colorectal adenoma recurrence.
In individuals who did not take multivitamins, those with the highest homocysteine levels were at a significantly greater risk of adenoma recurrence compared to those with the lowest homocysteine levels.[33] Another study found a strong association between elevated homocysteine and colorectal cancer.[34]

A large, population level case-control study found that blood donors with high plasma levels of homocysteine were at significantly increased risk of developing squamous cell carcinoma of the head and neck compared to controls ($p = 0.009$).[35] Another case-control study of breast cancer patients found significantly higher homocysteine levels in breast cancer patients compared to controls.[36] One study with 35 patients noted that chemotherapy may also lower folate and increase homocysteine levels.[37]

Research has provided evidence for the role of folate in preventing tobacco-related cancers. Individuals with the MTHFR C677T variant were at increased risk of lung cancer and those who were homozygous for the C677T variant were shown to have increased plasma homocysteine levels.[38]

Cognitive Function & Dementias

A meta-analysis of 14 cohort studies found high plasma levels of homocysteine was associated with increased risk of cognitive decline. The community-based studies (1999-2013) were of high methodological quality and included 15,908 healthy individuals with a follow-up period of 2.7 to 8 years.[39]

A meta-analysis of 8 publications (mostly retrospective case-control studies; 1998-2007) found that when Alzheimer's disease patients were compared with normal controls, the Mini-Mental State Examination (MMSE) score was lower and the plasma homocysteine level was higher (with both results were statistically significant).[40]

In the elderly, elevated homocysteine has been correlated with regional and whole brain atrophy. Low B12 may also be associated with progressive brain atrophy.[41]

A meta-analysis of 15 studies investigated the possible correlations between cognitive function, homocysteine, folate and vitamin B12 levels in Parkinson's disease (PD) patients. The results suggested that PD patients with cognitive dysfunction were likely to have higher homocysteine levels, lower folate and lower vitamin B12 levels.[42]

Pregnancy

A recent study of homocysteine levels measured in the first trimester of pregnancy (8-12 weeks' gestation) indicated that high serum homocysteine was associated with history of pregnancy losses, hypertensive disorders of pregnancy and preterm birth. High homocysteine levels were also found in women who went on to have pregnancy-related hypertension, pregnancy loss and low birth weight babies later in their pregnancy. Women who had a pregnancy loss showed homocysteine levels significantly higher than those who did not (24.65 vs. 13.51 µmol/L; $p = 0.0002$).[43]

Neural tube defects (NTDs) are common complex congenital malformations resulting from failure of the neural tube closure during embryogenesis. Increased homocysteine has been found in plasma[44,45] and in amniotic fluid[46] of women with NTD-affected foetuses or with a history of NTD-affected foetuses. Increased S-adenosylhomocysteine has also been noted in plasma of women with a history of NTD, resulting in a decreased S-adenosylmethionine/S-adenosylhomocysteine ratio.[47] Taken together, these results suggest a disturbed remethylation cycle could be involved in the aetiology of NTDs.

A meta-analysis of 32 studies involving 1,890 NTD-affected mothers and 3,995 control mothers, was undertaken to develop an understanding of the relationship between maternal biomarkers related to one-carbon metabolism and NTD. A highly significant increase in homocysteine levels was observed in NTD-affected mothers compared with controls ($p < 0.00001$). The pooled analysis also revealed that NTD-affected mothers had significantly lower levels of folate ($p = 0.002$), vitamin B12 ($p < 0.00001$) and red blood cell folate ($p = 0.01$).[48]

It is established that folic acid supplementation decreases the prevalence of NTDs,[14,49,50] however, several lines of evidence suggest that not only folates but also choline, B12 and methylation metabolites are involved in NTDs.[51] Several gene polymorphisms involved in these pathways have also been implicated in risk of development of NTDs.[52] This suggests that periconceptional supplementation with vitamin B12, choline and other methylation donors in addition to folic acid, may further reduce NTD risk.

Other Conditions

Increased homocysteine levels along with low folate or vitamin B12 status may occur in patients suffering from depression. The MTHFR C677T polymorphism is overrepresented among depressive patients.[53-55]

In type 2 diabetes, treatment with metformin may result in B12 deficiency. Metformin treatment has also been associated with increased homocysteine concentrations, which in turn was found to be independently related to retinopathy.[56]

A number of studies have demonstrated increased levels of circulating homocysteine in patients with rheumatoid arthritis (RA). RA patients also have an increased risk of cardiovascular disease.[57,58] A study of 235 consecutive RA patients found high homocysteine levels were predictive of atherothrombotic events. Patients treated with corticosteroids had significantly higher homocysteine levels ($p < 0.05$) and those taking folic acid supplements had lower levels ($p < 0.01$).[59]

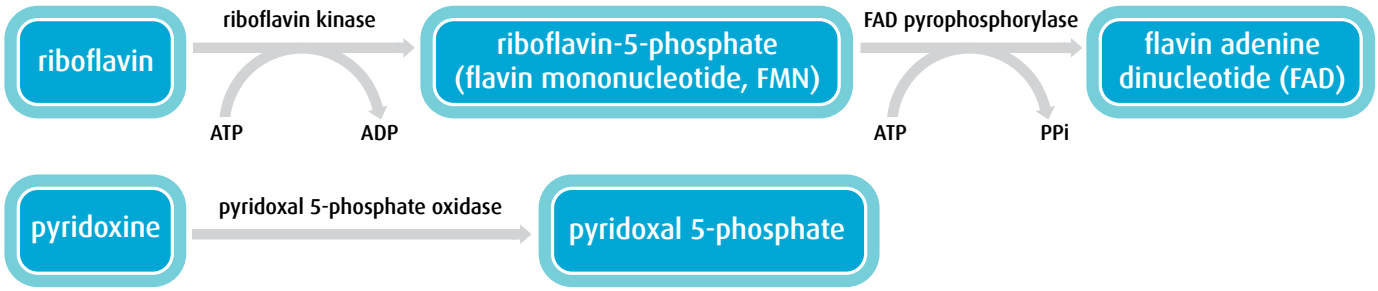
A high quality meta-analysis of 28 studies of homocysteine levels in inflammatory bowel disease (IBD) concluded that levels were significantly higher in IBD patients compared to controls (OR 4.65; 95% CI: 3.04-7.09).[60]

B Vitamins

Activated B vitamins

Recent research highlights the importance of the active metabolites of B vitamins, also called activated B vitamins or vitamers.

Figure 2. Conversion of riboflavin and pyridoxine to active forms.



Activated B vitamins may be important in people with DNA polymorphisms which encode less active forms of enzymes involved in B vitamin metabolism.[61-64]

Riboflavin sodium phosphate

Riboflavin sodium phosphate (flavin mononucleotide or FMN) is an active metabolite of riboflavin (B2). Riboflavin is converted to FMN by riboflavin kinase (RFK) and ATP. FMN is itself a vital coenzyme in metabolism of both pyridoxine (B6) and folate. The conversion of FMN to FAD (flavin adenine dinucleotide, also a coenzyme) is catalysed by FAD pyrophosphorylase and ATP (*see Figure 2*).[65-67] FAD functions as a cofactor for the enzyme MTHFR which is involved in folate metabolism. A polymorphism in the RFK gene has been associated with major depressive disorder due to its influence on both the folate and methionine cycles.[68]

Pyridoxal 5-phosphate

Pyridoxal 5-phosphate (PLP) is an active metabolite of pyridoxine (B6) and is produced in a reaction catalysed by pyridoxal 5-phosphate oxidase (PNPO).[63] In one study of patients with chronic liver disease, only one third of those administered pyridoxine hydrochloride showed an increase in plasma PLP whereas all those patients given PLP showed an increase in plasma PLP. Pyridoxine hydrochloride and PLP were administered by intravenous injection. The study suggested the conversion of pyridoxine to PLP was impaired (so PLP provided better bioavailability) or that elimination of PLP was markedly increased in these patients, possibly as a result of enhanced degradation.[69]

PLP is an important coenzyme in many amino acid related reactions.[70] PLP inhibits purinergic receptors and intracellular influx of calcium ions and has been investigated as an agent to reduce cellular injury during ischaemia.[71] Mutations of PLP have been found to result in several rare inherited diseases, and there is evidence that mutations in PNPO may contribute to overall genetic risk for schizophrenia in the Japanese population.[72] Vitamin B6 was known to improve symptoms in women suffering from morning sickness, but recent research suggests it is PLP that is the antiemetic molecule.[73]

The role of PLP in amino acid metabolism makes it a rate-limiting cofactor in the synthesis of neurotransmitters such as dopamine, serotonin, gamma-aminobutyric acid (GABA), noradrenaline and the hormone melatonin. The role of PLP in amino acid metabolism makes it a rate-limiting cofactor in the synthesis of neurotransmitters such as dopamine, serotonin, gamma-aminobutyric acid (GABA), noradrenaline and the hormone melatonin. As such, even a mild vitamin B6 deficiency will result in a down-regulation of neurotransmitter production, with subsequent effects on sleep, behaviour, cardiovascular function and hormone excretion.[74]

Levomefolate calcium

Levomefolate calcium (also known as 5-methyltetrahydrofolate or 5-MTHF) is the most biologically active form of folate and folic acid from supplements and fortified foods must be converted to 5-MTHF to be used by the body. The mechanism of action of 5-MTHF is through its role as a methyl donor in a range of metabolic and nervous system biochemical processes, as well as being indirectly necessary for DNA synthesis.[75]

After ingestion, the process of conversion of folic acid to the metabolically active 5-MTHF is relatively complex and requires several enzymes, adequate liver and intestinal function, and adequate supplies of vitamins B2, B3, B6, C, zinc and serine.[75] As such, malabsorption, digestive system pathology, liver disease and enzyme defects (as seen in individuals with a genetic defect of the methylenetetrahydrofolate reductase (5-MTHFR) enzyme), can all result in impaired ability to activate folic acid. In these instances, supplementation with 5-MTHF might be preferable to folic acid supplementation as it bypasses all of the enzymatic activation steps in the folic acid pathway (*see Figure 3*).[75-78]

Intake of 5-MTHF may have additional advantages over intake of folic acid. Firstly, the potential for masking the haematological symptoms of vitamin B12 deficiency may be reduced with 5-MTHF. Secondly, 5-MTHF may be associated with a reduced interaction with drugs, such as methotrexate, which inhibits dihydrofolate reductase (DHFR)[79]

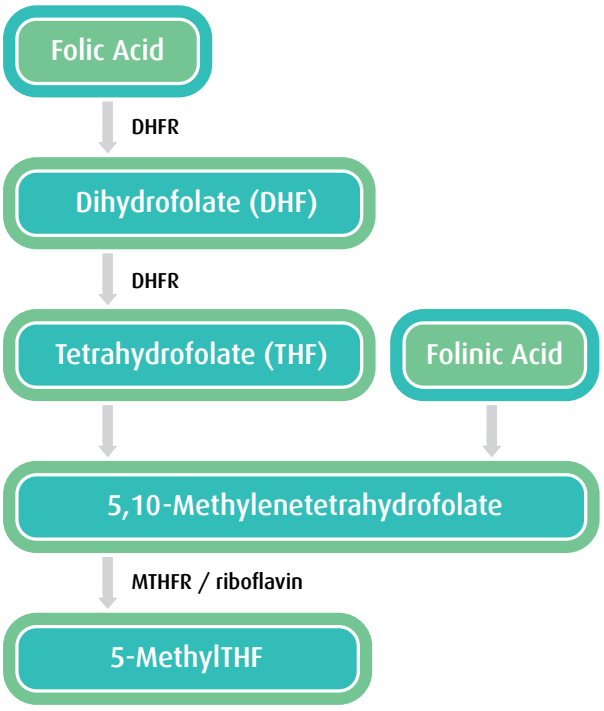
A double-blind, placebo-controlled study compared the relative responsiveness of liver transplant recipients (n = 60) to 5-MTHF (1 mg/day) and folic acid (1 mg/day) over a period of 8 weeks for the treatment of hyperhomocysteinaemia. At the end of the trial period, a significant decrease of total serum homocysteine was observed for the 5-MTHF group ($p < 0.001$), with no significant decrease in plasma homocysteine for either the folic acid or placebo groups.[80] Supplementation with folate-rich foods, folic acid or 5-MTHF significantly reduced homocysteine levels in participants with mild to moderate hyperhomocysteinaemia. Folate-rich food (0.2 mg/day of natural folates), the supplements (0.2 mg/day) or placebo were taken for 13 weeks in addition to their habitual diets.[83]

Methylcobalamin

Methylcobalamin, a biologically-active form of vitamin B12, is particularly important in the folate-dependent methylation of homocysteine to methionine via methionine synthase (MS). As a cofactor for MS, methylcobalamin potentially influences hundreds of S-adenosyl-methionine (SAMe)-methylation reactions including dopamine-stimulated phospholipid methylation, a process which may play an important role in mood, attention and learning.[84]

Further to this, low methylcobalamin levels may result in increased homocysteine and SAMe deficiency, which compromises endogenous glutathione synthesis via the cysteine beta synthase pathway.[85] Abnormal DNA methylation and low levels of glutathione have been implicated in autism and schizophrenia,

Figure 3. Metabolic steps for folic acid and folinic acid.[54,57]



Abbreviations: DHFR - dihydrofolate reductase, MTHFR = methylenetetrahydrofolate reductase.

with brain levels of methylcobalamin found to be 3-fold lower in these populations compared to age-matched controls.[84]

Methylcobalamin is the only form of vitamin B12 that can cross the blood brain barrier without biotransformation. Its methyl group stimulates serotonin synthesis, a neurotransmitter which is responsible for mood enhancement and protects the brain from damage against excitotoxins.[86]

B Vitamin Status

Depending on the particular vitamin, there are a number of potential causes of B-vitamin deficiency including inadequate intake, increased requirements, malabsorption, drug-nutrient interactions and others including genetic disorders and medical conditions (*see Table 1*).

In addition, the ageing process itself can negatively affect the absorption, transport and metabolism of B vitamins and thus older people have increased requirements. A recent systematic review in community-dwelling older adults in developed Western countries (n = 28,000) reported a high prevalence of low-dietary intakes for B vitamins (i.e. below the estimated average requirement).[87]

B vitamins are known to play a significant biochemical role in maintaining cognitive processes within the brain.[88] The elderly are at risk of low folate status. Folate supplementation may prove beneficial however, it should be administered in conjunction with vitamin B12 to reduce the risk of neurological consequences of vitamin B12 deficiency.[89]

In addition to their role as methylating factors, vitamin B6, folate and vitamin B12 are critical to haematopoiesis (blood-building activity) and deficiencies may result in a specific type of anaemia.[90] Pyridoxine plays a key role in haematopoiesis by acting as a coenzyme in the interaction between red blood cells and haemoglobin and enhances the oxygen-binding affinity of the haemoglobin.

Higher plasma concentrations of PLP have been associated with reduced colorectal cancer (CRC) risk.[91] A prospective, case-control study of 613 CRC cases and 1190 matched controls nested within the Northern Sweden Health and Disease Study (n = 114,679) was recently conducted. Participants were followed from 1985 to 2009, and the median follow-up from baseline to CRC diagnosis was 8.2 years. Results of the study indicated that vitamin B6 deficiency as measured by plasma PLP is associated with a clear increase in CRC risk.[91]

In addition to poor diet causing low folate status, some medicines prescribed for treatment of depression can potentially interfere with folate and homocysteine metabolism.[92] However, whether the deficiency is primary or secondary to depression, low level of folate limits the response to antidepressants.[54,93] A wide range of doses of folate or activated forms have been prescribed in clinical trials to drug-medicated patients.[54] In one trial, 0.5 mg/day of folic acid significantly improved response to fluoxetine in female patients, with the women showing significantly decreased plasma homocysteine levels.[94]

Table 1. Causes of B-vitamin deficiency[95]

Inadequate Intake (Occurrence)	Increased Requirement	Malabsorption	Drug-Nutrient Interaction	Other
Folate				
poor cooking techniques(common)	elderly pathological conditions	intestinal diseases coeliac disease Crohn's disease ulcerative colitis	phenytoin phenobarbital primidone trimethoprim methotrexate sulfasalazine metformin	alcohol abuse genetic disorders haemolytic anaemia
B12				
vegan diets (common)	elderly	intestinal diseases coeliac disease Crohn's disease gastric/intestinal resection atrophic gastritis bacterial overgrowth H. pylori infection pancreatic insufficiency pernicious anaemia Zollinger–Ellison syndrome	proton pump inhibitors H2-receptor antagonists metformin nitrous oxide colchicine	alcohol abuse genetic disorders tropical or non-tropical sprue
B6				
chronic dieters (rare)	elderly	HIV	isoniazid anticonvulsants steroids	alcohol abuse genetic disorders liver disease renal dialysis rheumatoid arthritis
B2				
chronic dieters (common)	elderly	diabetes liver disease thyroid and renal insufficiency GI and biliary obstruction	phenothiazines (e.g., chlorpromazine) theophylline	alcohol abuse genetic disorders hypochromic anaemia metals such as zinc, copper and iron

Table 2. Results of cognitive testing in patients with mild cognitive impairment after 2 year's treatment with B vitamins.[81,82]

Overall: All patients
• B vitamins stabilised executive function relative to placebo (p = 0.015). This was the only cognitive domain demonstrating a significant effect.
Subgroup Analysis: Baseline plasma homocysteine
For participants with high baseline homocysteine (≥ 11.3 µmol/L) there was significant benefit of treatment with B vitamins in: • episodic memory: 69% higher likelihood of correct word recall compared to placebo (p = 0.001) • semantic memory: for category fluency, the average number of words was 9.4% greater compared to placebo (p = 0.037) • global cognition: 1.58 times more likely to give a correct answer than placebo (p < 0.001)

Clinical Studies

Cardiovascular Disease

A recent meta-analysis of 30 randomised controlled trials (covering 82,334 participants) assessing folic acid supplementation and the risk of cardiovascular diseases indicated a 10% **lower risk of stroke** and a 4% lower risk of overall CVD with folic acid supplementation.[23]

In patients with stable coronary artery disease, treatment with folic acid (0.8 mg/day) and vitamin B12 (0.4 mg/day) for 24 months was associated with a significant increase in coronary blood flow, which may reflect improved microvascular function.[96]

Peripheral arterial disease affects 8-19% of the population and elevated homocysteine levels have been linked to increased risk and severity. A randomised controlled trial found that plasma homocysteine was significantly reduced in those receiving daily folic acid (0.4 mg) or 5-MTHF (0.4 mg) for a period of 16 weeks compared to placebo-treated patients (p = 0.002 and p = 0.007, respectively). A measure of peripheral artery disease, the ankle brachial index (ABI), improved significantly in both the folic acid and 5-MTHF groups compared to placebo (p < 0.001 and p = 0.009, respectively).[97] In an earlier, and smaller trial (n = 27), oral treatment with B vitamins (10 mg of folic acid, 0.2 mg of vitamin B12 and 20 mg of vitamin B6 per day) for eight weeks had no impact on the ABI despite a significant reduction in plasma homocysteine levels (p < 0.01). These patients had peripheral artery disease, stable intermittent claudication, elevated homocysteine levels and were stabilised on medication.[98]

Cognitive Decline

A meta-analysis reported in 2014 the finding that lowering of homocysteine with B vitamins had no significant effect on cognitive function or cognitive ageing in older adults. Trials that included patients with cognitive impairment or dementia were excluded.[99] This conclusion has however, been robustly critiqued and further qualified.[100-103] A more relevant analysis would be confined to trials that used testing sensitive enough to detect cognitive change and of suitable application (not the MMSE); were of sufficiently long duration; ensured global cognitive measures were obtained; and were stratified for baseline values of B vitamins and homocysteine.[100,102,103] Treatment with B vitamins may assist those with particular characteristics, such as those with the early signs of age-related cognitive decline who have hyperhomocysteinaemia (*see brain atrophy clinical results below*) as the strengths of the associations between high homocysteine and cognitive decline and high homocysteine and incident dementia (including AD), are substantial and consistent.[102]

A randomised trial investigated the effect of taking folic acid (0.8 mg/day), vitamin B6 (20 mg/day) and vitamin B12 (0.5 mg/day) in combination for 2 years on brain atrophy in over 70-year-olds with mild cognitive impairment. In total, 168 participants had MRI scans to measure brain atrophy and the mean rate of atrophy was 0.76% per year in the intervention group compared to 1.08% in the placebo group (p = 0.001). Response to treatment was more apparent in those with high baseline homocysteine levels.[104] Further investigation of the data gathered in this trial demonstrated B-vitamin treatment reduced, by as much as seven-fold, the cerebral atrophy in those grey matter regions specifically vulnerable to the Alzheimer's disease process, including the medial temporal lobe. The beneficial effect of B vitamins was confined to those with high homocysteine levels.[105] Secondary outcomes were also measured in this trial. Supplementation with the B vitamins significantly slowed cognitive impairment over the 2-year period. This was assessed using the global clinical dementia rating score and a questionnaire on cognitive decline. A beneficial effect on some aspects of cognition were also found, particularly in those with high baseline homocysteine levels (*see Table 2*). The authors noted that although the sizes of the effects of treatment with B vitamins were relatively modest, they were highly statistically significant, were found in several areas of cognition and in clinical assessments of cognitive impairment, and as such, were consistent with B vitamins **slowing the progression of cognitive decline.**[106] In summary: the critical thresholds for homocysteine shown in the study were > 11.3 µmol/L (for slowing of brain atrophy and of cognitive decline) and
13 µmol/L (for improvement of clinical measures (of impairment)).[103,107]

A randomised trial investigated the effect of taking folic acid (0.4 mg/day) and vitamin B12 (0.1 mg/day) in combination for 2 years on cognitive decline in 900 community-dwelling older adults (aged 64-70 years) with depressive symptoms. At completion of the trial, supplementation with folic acid and vitamin B12 was shown to significantly improve cognitive function, particularly in immediate and delayed memory performance, as compared to the placebo group. The participants had normal homocysteine levels (on average below 10 µmol/L), folate and vitamin B12 levels at baseline. (Although those who received vitamin treatment had significantly higher concentrations of serum vitamin B12 at baseline than those in the placebo group.) By the end of the study, homocysteine had increased significantly less in the vitamin group than in those taking placebo.[108] A stratified analysis was conducted on baseline homocysteine levels: classifying participants according to low or high plasma homocysteine (the latter defined as > 10.4 µmol/L in women, > 11.4 µmol/L in men). In the group with high homocysteine levels, the significant effect of folic acid and vitamin B12 treatment on cognitive function was confirmed for total score as well as immediate recall, compared to placebo. There were no significant effects found in the low-homocysteine group.[109]

Oxidative damage is a major contributor to many health problems, including cancers.[110] A randomised, double-blind, placebo-controlled trial (n = 450) was conducted in a chronic, low-level, arsenic-exposed population to assess the efficacy of low (0.4 mg/day) and high (0.8 mg/day) folic acid supplementation on **reversing oxidative DNA damage**, which was determined by measuring urine levels of 8-OHdG (8-hydroxy-2'-deoxyguanosine). After adjusting for potential confounding effects, urinary 8-OHdG concentrations decreased significantly and in a dose-dependent manner after intake

of folic acid for 8 weeks, compared to placebo. In addition, the effect of folic acid on reducing urinary 8-OHdG was more pronounced in those individuals with hypercholesterolaemia.[111]

In an open-label trial of 40 children diagnosed with autism, the effect of oral folinic acid (0.4 mg) twice per day plus subcutaneous injection of methylcobalamin (75 µg per kg of body weight) twice per week, for three months, was investigated compared to a group of age-matched unaffected controls. After three months, significant increases in cysteine, cysteinylglycine and glutathione concentrations were observed compared to baseline measures ($p < 0.001$). The results showed the glutathione redox ratio also significantly increased after treatment ($p < 0.008$).[112]

Given the suggested link between elevated homocysteine levels and depressive symptoms, lowering elevated plasma homocysteine levels by supplementing with vitamin B12 and folic acid has been postulated to reduce depressive symptoms and improve health-related quality of life. However, trials investigating these effects are scarce and show inconsistent results. Heterogeneity in study duration, study samples, homocysteine status, and supplement dose may explain the observed differences.

Safety

Avoid high doses exceeding 400 mg daily of vitamin B6. Such high doses may cause sensory peripheral neuropathy.[15] Folate and vitamin B12 should be consumed together, and supplemental intake may be necessary for the elderly and vegetarians.

Green Tea

Green tea contains five main catechins, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and catechin. The amount varies from one tea preparation to another, but generally EGCG and EGC are present at the highest concentrations.[113] Analysis in 2001 of 15 household brands of green tea from the UK and Europe found a typical infusion (less than 1% w/v) contained on average about 600 mg/L of polyphenols.[114]

Epidemiological Data

The results of epidemiologic studies that investigated the association between green tea consumption and cancer risk have not been consistent. Many studies have found no association, while some have found a favourable, although modest, effect. The quality of studies varies, and the 'dosage' (intake) is often quite high and/or occurs over long periods, and it is usually not defined in terms of catechin content (which can vary significantly). The concentration of the tea infusion is also a factor.

In one of the earliest studies reported, the daily consumption of at least 10 Japanese-size cups of green tea resulted in delayed onset of cancer.[115] Surveys of tea-drinking habits at the time and some chemical analysis suggested that this corresponded to between 300 to 540 mg/day of EGCG,[115,116] or about 550 mg/day of EGCG, EGC, ECG and EC.[117] Several more recent epidemiologic studies from China provide possible dosage information: just over 8 g/day of dry leaf is equivalent to 2 cups/day.[118-120] Other analyses estimate one cup of green tea to contain 50-100 mg of catechins.[121]

Meta-analyses of the epidemiological data suggests green tea does not reduce the risk of prostate,[122] bladder[123] or pancreatic cancers.[124] A protective effect was suggested for cancer of the:

- Liver (for those drinking at least 5 cups/day;[125] but may only be significant in women)[126]
- Bowel[127]
- Stomach (for those drinking 6 cups/day, or for 25 years; but increased risk found for drinking very hot tea)[128]
- Lung (especially for those drinking more than 7 cups/day),[129]
- Ovaries[130,131]
- Endometrium[132]
- Breast (for recurrence of cancer – at more than 3 cups/day (Japanese studies)), but not for incidence)[133]

Inverse associations were found between the intake of green tea and the **DNA methylation** of two tumour-related genes CDX2 and BMP-2, in a Japanese study involving patients with stomach cancer. High consumption (7 cups or more per day) showed a significant association with decreased methylation frequencies of CDX2 and BMP-2 after adjustment for other variables.[134]

A meta-analysis assessed the results of 3 case-control studies and 2 prospective cohort studies conducted in Japan and China. The overall results showed a statistically significant 28% reduction in risk of coronary artery disease with the highest green tea consumption. However, the results are not robust. Studies investigating cardiovascular disease including stroke were excluded.[135] It is possible that the association only occurs at high intake, e.g. in two of the trials the highest tea consumption was more than 10 cups/day and greater than about 8 g/day.

In other epidemiological research:

- An increase in consumption of 3 cups/day of green tea was associated with 13% and 24% decreased risks of total stroke and ischaemic stroke, respectively (meta-analysis).[136]
- Green tea consumption was inversely associated with incidence of myocardial infarction (large cross-sectional study),[137] atrial fibrillation (case-control study)[138] and mortality from cardiovascular disease (effect stronger in women; meta-analysis).[139]
- Consumption of 120 to 599 mL/day for one year of green or oolong tea significantly reduced the risk of developing hypertension (cohort study).[140] In a later cross-sectional study, drinking at least 150 mL per week of green tea was associated with a 37% lower risk of having hypertension in those aged at least 40 years, compared to not drinking green tea.[141]
- Green tea consumption was inversely associated with risk of cognitive disorders (meta-analysis).[142]
- Green tea consumption was associated with significantly reduced levels of urinary 1-hydroxypyrene glucuronide (1-OHPG) in Korean shipyard painters. (1-OHPG is indicative of exposure to polycyclic aromatic hydrocarbons.)[143]
- The frequency of sister-chromatid exchange (SCE) in mitogen-stimulated peripheral lymphocytes from smokers who consumed green tea (3 cups/day) was comparable to that of non-smokers, which implies that green tea blocked the cigarette-induced increase in SCE frequency.[144]

Clinical Studies

There are clinical research results available for modest doses of green tea and/or green tea catechins.

Green tea has demonstrated a **genoprotective effect**. Drinking green tea for 4 weeks was associated with a significant decrease in oxidation-induced DNA damage in lymphocytes of healthy volunteers who participated in a placebo-controlled, crossover study.[145] The dose was 2 x 150 mL of 1% w/v dried leaf per day i.e. bags containing 1.5 g of dried leaf were used to make a 150-mL cup of tea. Freshly prepared tea was drunk in the morning and evening. Hot water was used as the control.

A more recent study by the same researchers found that the same dosage and preparation of green tea reduced DNA damage in the lymphocytes of type 2 diabetics. The trial was randomised, placebo-controlled and cross-over in design.[146] Two versions of the comet assay were used to measure aspects of DNA damage: oxidation-induced lesions in DNA and the activity of the repair enzyme hOGG1 (8-oxoguanine glycosylase). Cytoprotective proteins including hOGG1 and heme oxygenase 1 (HMOX-1) are induced by the Nrf2-Keap1 system, for example, in response to oxidative stress. The study also investigated a polymorphism that may affect DNA repair: a microsatellite polymorphism in the HMOX-1 promoter region. This polymorphism was shown to involve the number of guanine-thymidine (GT) repeats ((GT(n)): the S/S genotype has short (GT) n repeats (where n is less than 25) and the L/L genotype has long (GT)n repeats (where n is 25 or greater).

- After administration of green tea, **DNA damage was decreased** by about 13% and the activity of hOGG1 was enhanced ($p < 0.001$ for both).
- Significant increases in Nrf2 and HMOX-1 in lymphocytes were seen after consumption of green tea compared to control (hot water; $p < 0.01$ for both).
- No changes in mRNA expression were observed.
- Baseline HMOX-1 levels and hOGG1 activity were higher in the S/S group ($p < 0.05$), but the responses associated with drinking green tea were similar in both GT(n) groups.
- The daily dose of total catechins in a typical infusion of the green tea prescribed was measured by liquid chromatography at 174 mg.

Green tea infusion reduced oxidative stress in healthy volunteers (1.75 g/day of dried leaf, for 4 weeks),[147] and in workers exposed to benzene (3 g/day of dried leaf, for 6 months).[148] Not all tests for antioxidant activity are robust, or of clinical relevance. These studies measured the more reliable parameters, for example, serum malondialdehyde and plasma glutathione.

Safety

'Antifolate' Activity?

Green tea has been reported as having an 'antifolate' activity, on the basis that EGCG and green tea catechins inhibit DHFR *in vitro*,[149,150] a property also held by antifolate drugs such as methotrexate. This suggests, theoretically at least, that green tea may decrease circulating folate levels. An *in vivo* study (in rats) found only the highest dose of green tea catechins, (0.5 g/kg, over 6 weeks), significantly deceased serum folate (measured as 5-methylTHF). The catechins were included with folic acid in the diet.[150] (Note: Inhibition of DHFR (and the ensuing disruption of DNA synthesis) is the basis of the antitumour action of antifolates. *In vitro* testing found EGCG at physiologically attainable concentrations killed cancer cells through apoptosis, but had little or no effect on normal cells.[149])

In a pilot study with healthy volunteers from the UK, no significant difference in plasma folate concentrations was observed between treatment with aqueous extract of green tea (containing about 670 mg/day of catechins) and placebo. (Folate was not administered in this trial. The study looked at the effect on folate absorbed from the diet. Green tea or placebo capsules were taken for 3 weeks.) The authors noted this dosage of green tea catechins corresponds to about 20 cups of green tea. Participants maintained their normal diet which contained an average of 0.328 mg/day of folate. Mean daily folate consumption did not significantly differ between the groups. The green tea capsules contained less than 0.00005 mg of total folates.[150] Intake of a green tea extract providing 1069 mg/day of total catechins for one week in healthy middle-aged volunteers was associated with a significant decrease in folate levels. Participants were instructed not to make any changes to their diet, alcohol consumption or physical activity.[151]

A pharmacokinetic study investigated the effect of taking green tea catechins on absorption of folate.[152] Clinical significance of the results are unclear however, as it was an acute study (i.e. not ongoing administration), with 50 mg of green tea catechins administered before, during and up to 2 hours after folate (for a total of 250 mg of catechins).

There have been reports from limited epidemiological studies that maternal tea consumption increases the risk of foetal neural tube defects. (The type of tea was usually not defined, and not all studies were conducted in China.)[153-158] In one of these studies folic acid supplementation rates were low, and there were no daily tea drinkers in the supplemented group (they consumed tea less frequently).[155] The early studies have been criticised as being of dubious validity and lacking scientific rigour in design.[157]

Green tea is not likely to produce a clinically-relevant decrease of DHFR. If green tea alone is given to pregnant women, a small addition of folate, or use of activated forms of folate, may be advisable.

Adverse Effect on Liver?

A review conducted by the US Pharmacopoeia and published in 2008 analysed a total of 216 case reports on green tea products from France, Spain, Australia, Canada, UK and USA, including 34 reports concerning liver damage (27 were categorised as possible causality and 7 as probable causality using the Naranjo criteria). In 13 of the liver damage cases, a concentrated aqueous alcoholic extract, standardised to 25% catechins, was used. Four cases involved the use of an aqueous extract containing 40-50% catechins. The Committee recognised that the individual case reports were not strong, but there is a possibility of liver damage caused by products that contain concentrated green tea extracts. It was noted that green tea is used widely as a beverage with a low incidence of a causal relationship to hepatotoxicity. Caution does not pertain to traditional green tea infusions or other beverage preparations.[159]

A safety assessment published in 2017 reviewed the data from epidemiological studies, clinical trials and experimental models. The following summarises the human data regarding the effect of green tea on the liver.[160]

- Green tea infusions have a history of safe use in Asia. There are no reports regarding liver toxicity for consumption of green tea infusions in Japan, despite a mean EGCG intake estimated at 314 mg/day with a maximum of 734 mg/day.
- Twenty clinical studies have evaluated beverages fortified with green tea extracts. The studies had duration of up to one year (median: 12 weeks) and the highest intake corresponded to 498.6 mg/day of EGCG. There were no concerns of liver toxicity, and in 17 studies, liver function was assessed, without adverse effects observed.
- A large number of human studies have evaluated supplementation with dried, concentrated green tea extracts in capsule form for potential therapeutic effects, and many of these studies also monitored liver enzymes or other parameters indicative of liver function impairment. Doses of EGCG ranged from 100 to 4000 mg/day, taken for between 1 day and 2 years (median: 90 days). Effects on liver enzymes were not observed when EGCG dose remained below 600 mg/day. Above this dose, occasional small increases in liver enzymes were observed and were reversible.

Iron Absorption

The evidence for the potential effect of green tea to reduce iron absorption has been found to be conflicting in clinical and epidemiological studies (no effect nearly as often as an effect, particularly at the lower end of the dosage range).[161-168] As a precaution, it may be advisable not to take at the same time with meals or iron supplements in anaemia and cases where iron supplementation is required.

Supportive Formulation

These nutrients and Green Tea would complement each other to support the following actions:

- Maintenance of normal homocysteine levels
- Provision of key nutrients to support methylation and genomic stability
- Antioxidant support
- Maintenance or enhancement of haematopoiesis

Indications

- To reduce the risk of cardiovascular disease, particularly stroke
- May reduce cognitive decline by reducing age-related brain atrophy
- To support genomic stability and to help prevent cancer
- May support patients with depression, particularly those with low folate and/or high homocysteine levels

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