The 4th generation folate

Product overview
Folates are widely distributed in nature and are essential for the maintenance of cellular functions and health. As humans (and other mammals) cannot synthesize folates, they must be obtained via diet.

However, natural folates (the first generation) are susceptible to oxidation, they rapidly lose activity in foods and have a bioavailability range of 25-50%, depending on kind of food. On the contrary, folic acid, the synthetic form of monoglutamyl folate, is almost completely stable for months or even years and can be considered as a “second generation of folate, the stable one”.

The absorption of monoglutamyl folate occurs in the jejunum by a saturable, carrier-mediated, process at physiological (micromolar) concentration of intraluminal folates (1, 2)*. In order to diffuse all cells into body through the circulatory system, the folate monoglutamate must be transformed in the 5-methyltetrahydrofolate form, which passes by diffusion from blood into all body cells. Oral supplementation with folic acid increases the body’s pool of 5-methyltetrahydrofolate in healthy and diseased individuals.

Folate-requiring reactions, collectively referred to as “one-carbon metabolism”, include those involved in
• amino acid metabolism
• purine and pyrimidine synthesis
• formation of the primary methylating agent, S-adenosyl-methionine (SAM).

The principal function of folate coenzymes is to accept or donate one-carbon units in key metabolic pathways. The conversion of tetrahydrofolate (THF) to 5,10-methylene-THF is a crucial first step in the cycle that employs the 3-carbon of serine as a major carbon source.

*References are each numbered and described at the end of the document.

### Total folate content in some common foods

<table>
<thead>
<tr>
<th>VEGETABLES</th>
<th>FRUIT</th>
<th>MEAT</th>
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<tbody>
<tr>
<td>µg/100g</td>
<td>µg/100g</td>
<td>µg/100g</td>
</tr>
<tr>
<td>Spinach</td>
<td>150</td>
<td>Chestnuts</td>
</tr>
<tr>
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<tr>
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<td>Grapes</td>
</tr>
<tr>
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<td>Almond paste</td>
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<tr>
<td>Artichokes</td>
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<td>Grapefruit</td>
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<table>
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<tr>
<th>MILK AND DIETARY PRODUCTS</th>
<th>FISH</th>
<th>OTHER</th>
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</thead>
<tbody>
<tr>
<td>µg/100g</td>
<td>µg/100g</td>
<td>µg/100g</td>
</tr>
<tr>
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<td>102</td>
<td>Tuna</td>
</tr>
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<td>55</td>
<td>Eel</td>
</tr>
<tr>
<td>Gorgonzola</td>
<td>52</td>
<td>Octopus</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>33</td>
<td>Trout (oven)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>7</td>
<td>Crustaceans</td>
</tr>
<tr>
<td>Milk</td>
<td>6</td>
<td>Herrings</td>
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</table>
A portion of the 5,10-methylene-tetrahydrofolate thus produced undergoes irreversible enzymatic reduction to 5-methyltetrahydrofolate by methylene-tetrahydrofolate reductase (MTHFR). The N-5 methyl group of 5-methyltetrahydrofolate is removed and transferred by vitamin B12 coenzyme to homocysteine, thus forming methionine. In addition to protein synthesis, methionine serves as a methyl group donor through conversion to 5-adenosylmethionine (SAM), a key biological methylating agent involved in over 100 methyltransferase reactions with a wide variety of acceptor molecules. The methionine synthase reaction also regenerates THF required for the formation of 5,10-methylene-THF and 10-formyl-THF used directly in thymidylate and purine synthesis, respectively (3).

**Principal Components of the Folate Biochemical Cycle.**

Dietary or genetically determined folate deficiency leads to mild hyperhomocysteinemia, which has been associated with various pathologies. Molecular mechanisms of homocysteine-induced cellular dysfunction include increased inflammatory cytokine expression, altered nitric oxide bioavailability, induction of oxidative stress, activation of apoptosis and defective methylation. Moreover, the involvement of folate and homocysteine metabolism has been documented in ageing-related diseases, in several developmental abnormalities, in pregnancy complications and in male and female subfertility (4).

At repeated doses, Lamers in 2006 demonstrated that the administration of (6S)-5-methyltetrahydrofolate is more effective than folic acid in improving folate status.

Healthy women (n=144) aged 19–33 y received 400 μg folic acid, the equimolar amount of (6S)-5-methyltetrahydrofolate (416 μg), 208 μg (6S)-5-methyltetrahydrofolate, or placebo as a daily supplement for 24 wk. Red blood cell and plasma folate concentrations were measured at baseline and at 4-wk intervals (5).
Quatrefolic®, the fourth generation folate

Until now, 5-methyltetrahydrofolate calcium salt (the third generation) was the only folic acid derivative available on the market, and able to penetrate the body cells without further metabolism. Gnosis R&D objective was to develop an innovative salt form able to overcome the existing limits related to stability and slight solubility.


In 2010, after a deep review of the extensive safety information and the uniqueness of the Quatrefolic® compound, FDA has accepted the New Dietary Ingredient (NDI) notification for the ingredient concluding that a dietary supplement containing the new dietary ingredient is expected to be safe under the conditions of use recommended or suggested on the label.
The new salt advantages

With Quatrefolic® Gnosis has managed to develop an innovative product with significant advantages over previous folate generations. This remarkable step forward is attributed to the development of the glucosamine salt of Quatrefolic®, through two main steps:

- Improving solubility in water of 5-methyltetrahydrofolate, Quatrefolic® is defined as water soluble, a significant opportunity for improving bioavailability, where as the calcium salt version is defined as sparingly soluble.
- Guaranteeing a safety and high-quality stable profile by choosing glucosamine as natural compound naturally present in the body.

The result of this project, Quatrefolic®, claims key relevant benefits as:

- Long lasting stability
- Improved bioavailability
- High water solubility
- Established safety

Stability

Quatrefolic® shows an extraordinary long lasting chemical stability guaranteeing a purity quite unaltered even after several months, and an assay reduction in 1 year less than 1%, allowing easy handling and storage.

The stability of Quatrefolic® powder form was tested according to ICH (international Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines both at room temperature and other conditions keeping samples in airtight containers, protected from light, and measuring purity and assay at different points.

Stability of Quatrefolic® at room temperature

<table>
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<tr>
<th></th>
<th>Baseline</th>
<th>6 Months</th>
<th>12 Months</th>
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<tr>
<td></td>
<td>Purity</td>
<td>Assay</td>
<td>Purity</td>
</tr>
<tr>
<td>Quatrefolic®</td>
<td>99.3%</td>
<td>55.7%</td>
<td>99.0%</td>
</tr>
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</table>
The bioequivalence study of Quatrefolic® and (6S)-5-methyltetrahydrofolate calcium salt in healthy volunteers

Once the animal studies were completed, a human clinical trial was performed on 24 healthy volunteers of both sexes in order to verify the superior bioavailability of Quatrefolic®. It was a single dose, balanced, two sequences, two periods, two treatments randomized crossover study, with a 7 day wash out between two consecutive treatments.

**Results**

The study confirmed the experimental findings in the rat: Quatrefolic® has better bioavailability than (6S)-5-methyltetrahydrofolate calcium salt. Mean $C_{\text{max}}$ for Quatrefolic® was 94.56 nmoles/l vs 85.29 nmoles/l for (6S)-5-methyltetrahydrofolate (+10.9%) and mean $AUC_{0-24}$ was 594.2 nmoles/l (+9.6%) and 501.1 nmoles/l for Quatrefolic® and (6S)-5-methyltetrahydrofolate respectively. Quatrefolic® can be considered 10% more bioavailable than (6S)-5-methyltetrahydrofolate calcium salt.
Safety

In a number of published and unpublished experimental human clinical trials and animal studies, the safety of (6S)-5-methyltetrahydrofolate and Quatrefolic® has been extensively investigated. Based on a critical evaluation of the available data an independent expert panel confirms that Quatrefolic® is “generally recognized as safe” (“GRAS”) for use as a source of folate in conventional and medical foods.

Toxicological studies

Bacterial mutation in *S.typhimurium* and *E.coli*
The bacterial mutation assay was performed in order to assess the compound’s ability to induce gene mutations in *S.typhimurium* and *E.coli*. The reverse mutation assay was run in bacterial strains already mutant at a locus whose phenotypic effects are easily detected, and, since many chemicals can demonstrate mutagenic activity only after metabolism to some reactive forms, the test was performed in presence and in absence of a rat liver metabolic system (S9 microsomal fraction).

The test concluded that Quatrefolic® does not induce reverse mutation in *S.thyphimurium* and *E.coli* at doses up to 5,000 μg/plate.

Mutation in L5178YTK+/− mouse lymphoma cells.
The assay was done in order to confirm the inability of Quatrefolic® to induce mutations in L5178YTK+/− mouse lymphoma cells cultured after in vitro treatment, and in absence or presence of a rat liver microsomal system.
The test concluded that Quatrefolic® does not induce mutations at concentrations up to 5,000 μg/ml.

Chromosome aberrations in Chinese hamster ovary cells (CHO) in vitro.
The assay was made in order to demonstrate the inability of Quatrefolic® to induce any chromosomal aberration in presence or absence of a S9 liver microsomal fraction.
No chromosomal aberrations were observed in CHO after in vitro treatment with concentrations of Quatrefolic® up to 5,000 μg/ml.

Single dose oral toxicity
The acute toxicity of Quatrefolic® was assessed in rats of both sexes, dosing the product by gavage at 500 mg/kg level. After dosing, animals were observed for a 7 day period and finally sacrificed.
No mortality occurred at this dose and during the observation time, and no clinical significant signs were observed in any animal. Changes in body weight were not relevant, and no anomalies were recorded at the autopsy performed after the observation time. The lack of mortality points out that the maximum tolerated dose is greater than 500 mg/kg body weight, a dose some thousand times the one suggested for human use.

Dosage

The intended uses of Quatrefolic® and use levels will be same as that of folic acid, expressed on the basis of the "Recommended Dietary Allowances for Folate for Children and Adults".

**Dosages**

<table>
<thead>
<tr>
<th>AGE (years)</th>
<th>MALES AND FEMALES (μg/d)</th>
<th>PREGNANCY (μg/d)</th>
<th>LACTATION (μg/d)</th>
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<tr>
<td>1-3</td>
<td>150</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4-8</td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-13</td>
<td>350</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14-18</td>
<td>400</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>19+</td>
<td>400</td>
<td>600</td>
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</table>
Folates, folic acid and the link with Vitamin B12

Interactions with Drugs and Vitamin B12

A number of drugs have been shown to affect the normal metabolism of folate and may cause folate deficiency.

Anti-folate drugs - drugs needed for certain conditions that adversely affect folate status
Methotrexate, used to treat neoplastic disease and rheumatoid arthritis, works as a folate antagonist by targeting a key enzyme in folate metabolism, the dihydrofolate reductase. Many of the side effects mimic folate deficiency and include gastrointestinal problems (nausea, diarrhea), stomatitis, headache, vertigo, pneumonitis, leucopenia, thrombopenia, hair loss and infections (6, 7).

Anti-convulsant drugs
Low folate levels have consistently been reported in epileptic patients on phenytoin, phenobarbital or pirimidone, while data on valproate are conflicting (8, 9). On the contrary, it has been demonstrated that supplementation with folic acid can prevent folate deficiency and improve phenytoin pharmacokinetics (10).

Anti-inflammatory drugs
Commonly used non-steroidal anti-inflammatory drugs (NSAIDs) have anti-folate activity via their action as inhibitors of enzymes involved in folate metabolism. Although the dose-response relationships with respect to antagonistic effects on folate metabolism have not been established, folic acid food fortification has not been reported to cause any interference in the treatment actions of these drugs (11).

Oral contraceptives
It is suggested that oral contraceptives impair folate metabolism and tend to slightly, but significantly, reduce the serum and erythrocyte levels of folate and to increase the urinary excretion of formiminoglutaric acid, an intermediary product of histidine that requires THF to be further metabolized. This reduction is probably due to an increase in folate coenzyme utilization (12, 13).

Alcohol
Chronic alcoholism is probably the leading cause of folate deficiency in the Western world, with an incidence of folate deficiency in chronic alcoholic patients as high as 87%, if low serum levels are used as the criterion of folate deficiency, and an incidence of megaloblastic anemia up to a 61% (14, 15). Possible causes of folate deficiency in alcoholic patients include:
- inadequate diet with resultant decreased body storage
- intestinal malabsorption
- altered serum protein binding and tissue affinity
- altered hepatobiliary metabolism.

Vitamin B12
The potential adverse effects of high doses of folic acid supplements in relation to vitamin B12 deficiency relate to intakes above the safe upper intake level of 1,000 μg/day.
Since the metabolism of folate and vitamin B12 are linked, in the case of vitamin B12 deficiency, conversion of 5-methyltetrahydrofolate to THF declines and eventually ceases. The synthesis of 5-methyltetrahydrofolate in cells by the enzyme 5,10 methylene-THF reductase is irreversible. Thus, once formed, 5-methyltetrahydrofolate can only be used by a single enzyme – namely B12-dependent methionine synthase. If vitamin B12 is deficient, the enzyme methionine synthase ceases to function and, as a consequence, the folate present in cells becomes “metabolically trapped” as 5-methyltetrahydrofolate. This situation produces a “pseudo folate deficiency”, because although the cells have adequate levels of folate, it is trapped in the 5-methyltetrahydrofolate form not acting as a co-factor for purine and pyrimidine biosynthesis.

If folic acid is consumed in very high doses (>1,000 μg per day), it can enter cells in “free” form and is converted directly to THF and THF-polyglutamates, through pathways that are not dependent on vitamin B12. In this way, “free” folic acid can restart DNA biosynthesis, and correct anemia without affecting the methylation cycle, which needs intervention of the vitamin B12.

Thus, while the anemia will be treated by folic acid, the neuropathy seen in vitamin B12 deficiency due to interruption of the methylation cycle will not, and some evidence exists to suggest it may become worse. This masking of vitamin B12 anemia by taking folic acid makes the presence of B12 deficiency more difficult to diagnose, allowing the neuropathy associated B12 deficiency to progress. Therefore, the main risk of exposure to large doses of folic acid (>1,000 μg) is the masking of megaloblastic anemia, a diagnostic symptom of vitamin B12 deficiency.

Bioequivalence of 5-methyltetrahydrofolate and folic acid in single dose

The short term bioequivalence of folic acid and (6S)-5-methyltetrahydrofolate has been demonstrated in humans by Pentieva and co-workers in the laboratory setting and by Venn and co-workers in the clinical practice. The implications are that the natural folate derivative could have all the beneficial effects associated with folic acid, but without the potential disadvantage of masking the anemia of vitamin B12 deficiency. Importantly, (6S)-5-methyltetrahydrofolate is a natural folate derivative, a normal constituent of the body, and safety and tolerance of high doses are not issues of concern (16, 17).

At repeated doses, Lamers in 2006 demonstrated that the administration of (6S)-5-methyltetrahydrofolate is more effective than folic acid in improving folate status. Healthy women (n=144) aged 19-33 y received 400 μg folic acid, the equimolar amount of (6S)-5-methyltetrahydrofolate (416 μg), 208 μg (6S)-5-methyltetrahydrofolate, or placebo as a daily supplement for 24 wk. Red blood cell and plasma folate concentrations were measured at baseline and at 4-wk intervals.
The increase observed in red blood cell folate over time was significantly higher in the group receiving 416 μg (6S)-5-methyltetrahydrofolate/day than in the groups receiving 400 μg folic acid/day or 208 μg (6S)-5-methyltetrahydrofolate/day (p<0.001). No plateau was reached in red blood cell folate concentration in the 3 treatment groups during 24 wk of intervention; however, plasma folate plateaued after 12 wk (5).

Willems et al. showed that after administration of (6R,S)-5-methyltetrahydrofolate to patients with established coronary artery disease (CAD) the peak plasma concentration of (6S)-5-methyltetrahydrofolate is more than 7 times higher compared to that after folic acid administration. This difference is independent from the patients’ MTHFR genotype (18).

Pharmacokinetic properties of orally administered (6R,S)-5-methyltetrahydrofolate versus folic acid in patients with coronary artery disease.

Genotype and treatment. (6S)-5-methyltetrahydrofolate plasma concentration (ng/ml) in patients with MTHFR CC genotype or TT genotype following the administration of (6R,S)-5-methyltetrahydrofolate or folic acid, 5 mg each (18).
Gene Variants

Normal MTHFR activity may help maintain the pool of circulating folate and methionine, preventing the build-up of homocysteine. MTHFR catalyzes the conversion of 5,10-methylene-tetrahydrofolate into 5-methyltetrahydrofolate and its gene is located on chromosome 1 at 1p36.3.

It has been shown that two common MTHFR alleles (C677T and A1298C) are associated with congenital anomalies. MTHFR activity among C677T homozygotes is 50-60% lower than in normal subjects. People homozygous for the C677T allele tend to have mildly increased blood homocysteine levels if their folate intake is insufficient, but normal blood levels if their folate intake is adequate.

The activity of the encoded enzyme A1298C allele is decreased, although less than in the case with the C677T allele. People homozygous for the A1298C allele do not appear to have higher serum homocysteine levels than controls. However, people who are heterozygous for the A1298C and C677T alleles (i.e., people with the A1298CIC677T genotype) tend to have a biochemical profile similar to that seen among C677T homozygotes, with increased serum homocysteine levels and decreased serum folate levels.

The population frequency of the C677T allele showed regional and ethnic variations. For example, the allele frequency was high in Italy and among Hispanics living in California and low among U.S. Blacks and in some areas of sub-Saharan Africa, while the population frequency of the A1298C allele is less documented (19).

Population frequency of homozygosity by geographic area and ethnicity

Health Benefits

Pregnancy

Low dietary intake of folic acid increases the risk for delivery of a child with a neural tube defect (NTD). Before conception and in the first part of gestation, folic acid supplementation significantly reduces the occurrence of NTD. Spina bifida and anencephaly are the most common neural tube defects (NTDs) and, in randomized controlled trials, folic acid supplementation before conception and during the first trimester has been shown to reduce the recurrence of NTDs by 72% in women with a previous NTD affected pregnancy.
Data from U.S. birth certificates indicate a 19% decline in the birth prevalence of NTDs and a 23% decline in spina bifida prevalence among births conceived after mandatory folic acid fortification (October 1998 through December 1999) compared with the NTD prevalence before folic acid fortification (October 1995 through December 1996) (20).

**Trend in spina bifida**


Arrows indicate statistically significant increases and decreases by the exponential weighted moving average analyses with parameters of \( p=0.01 \) and weight=0.075 (20).

In Canada, the overall prevalence of neural-tube defects at birth decreased from 1.58 per 1,000 births before fortification mandatory (November 1998) to 0.86 per 1,000 births during the full-fortification period, a 46% reduction (95% confidence interval, 40 to 51). The magnitude of the decrease was higher for spina bifida (53%) than for either anencephaly (38%, \( p=0.02 \)) or encephalocele (31%, \( p=0.03 \)).

A greater reduction in rates was found in regions with a higher baseline prevalence of neural tube defects than in regions with a lower prevalence (21).

**Prevalence of Neural-Tube Defects**

Prevalence of Neural-Tube Defects, According to Diagnostic Category, in Seven Canadian Provinces from 1993 through 2002. NOS denotes not otherwise specified (21).
Plasma folate levels and risk of spontaneous abortion

It has been suggested that the rapidly developing cells in the embryo may suffer by lack of adequate folate. Failure to produce sufficient DNA and to regulate DNA function could lead to spontaneous abortion. In their case-control study, George and co-workers evaluated the relationship between plasma folate levels and the risk of spontaneous abortion. Compared with women with normal plasma folate levels (2.20-3.95 ng/ml), women with low (<2.19 ng/ml) folate levels were at increased risk of spontaneous abortion (adjusted odds ratio 1.47), whereas women with higher folate levels (3.96-6.16 ng/ml) showed no increased risk of spontaneous abortion (22).

Effects on female and male fertility

In 2008, Chavarro and co-workers reported the prospective analysis of incident ovulatory infertility among participants to the Nurses’ Health Study II, a 9 years prospective cohort study designed to investigate the role of diet and other lifestyle factors in common chronic diseases, run in 18,555 married, pre-menopausal women who attempted to become pregnant. Objective of the study was to examine whether the use of multivitamins and intake of specific nutrients in multivitamins was associated with ovulatory infertility.

A first analysis concluded that multivitamin users had approximately one-third lower risk of developing ovulatory infertility when compared with non-users (p<0.001). After a further data adjustment for known and suspected risk factors for infertility, only the intake of folic acid was associated with a reduced risk of ovulatory infertility (23).

Relative risk of ovulatory infertility

Multivariate-adjusted relative risk of ovulatory infertility and intake of folic acid (23).
Effects of different vitamin supplements on plasma concentration of homocysteine

It has been shown that the administration of a daily dose of 15 mg folic acid for 3 months to 65 infertile men with an excessive round cell count in their ejaculate induced statistically significant modifications of all analysed sperm parameters, particularly it was observed an increase in sperm density (from 15 to 22.6x10⁶/ml) and motility (from 17.7% to 27.8%) as well as a decrease in round cell count (from 9.7 to 6.4x10⁶/ml) (4).

**Anemia**

Folic acid has a long history of use in conjunction with vitamin B12 for the treatment of macrocytic anemia. Depending on the clinical status of the patient, the dose of folic acid or 5-methyltetrahydrofolate required to reverse macrocytic anemia varies, but the therapeutic dose is usually 800-1,000 μg daily. Duration of therapy to reverse macrocytic anemia can be as short as 15 days after initiation of supplementation, or it may require prolonged supplementation.

**Hyperhomocysteinemina**

Homocysteine is widely accepted as independent risk factor for coronary, cerebral, and peripheral vascular diseases (24). This has been observed even when presupplementation plasma folate concentrations were well within the range of values currently accepted as reflecting adequate status. In several studies, daily folic acid administration in high (pharmacologic) doses of 0.4 to 10 mg resulted in significant reductions in plasma total homocysteine (25). In a placebo-controlled study on 100 men with hyperhomocysteinemia randomly assigned to 5 groups of daily treatment with placebo, folic acid (0.65 mg), vitamin B12 (0.4 mg), vitamin B6 (10 mg) or a combination of the three vitamins for 6 wk, folic acid supplementation reduced plasma homocysteine concentrations by 41.7% (p<0.001), whereas the daily vitamin B12 supplement lowered homocysteine concentrations by 14.8% (p<0.01). The daily pyridoxine dose did not reduce significantly plasma homocysteine concentrations. The combination of the three vitamins reduced circulating homocysteine concentrations by 49.8%, which was not significantly different (p=0.48) from the reduction achieved by folate supplementation alone (26).

**Effects of different vitamin supplements on plasma concentration of homocysteine**

![Bar chart showing the effects of different vitamin supplements on plasma concentration of homocysteine](image.png)

*Effects of different vitamin supplements on plasma concentration of homocysteine in men with hyperhomocysteinemina (26).*

Reducing elevated plasma homocysteine means lowering not only an independent risk factor for neural tube defects and other birth defect and altered male and female fertility, but also for cardiovascular disease, Alzheimer’s disease, cognitive decline, osteoporosis, rheumatoid arthritis, kidney failure, and cancer.
Cardiovascular Disease (CVD)

Interestingly, a large population-based cohort study of men and women, 40 to 42 and 65 to 67 years old, showed that in the older age group total homocysteine levels are strong predictor for CVD hospitalization in the following 5 years. The relationship observed among the elderly was graded, independent of other measured CVD risk factors, and applied to all of the major categories of CVD. The association was strongest among people with preexisting CVD and/or antihypertensive treatment (27).

Time to first hospitalization with cardiovascular disease (CVD)

In another prospective study on patients with coronary artery disease (CAD) angiographically documented, a strong dose-response relation between the total homocysteine level and overall mortality was observed (28).

Chronic exposure of vascular endothelium to homocysteine compromises the production of adequate amounts of nitric oxide (NO) leading to injury of the endothelial lining and the initiation of atherosclerosis. 5-methyltetrahydrofolate improves NO synthesis by:

- reducing plasma homocysteine levels
- enhancing the availability of key endothelial NO cofactors, such as tetra-hydrobiopterin
- reducing the production of superoxide anions
- substituting for tetrahydrobiopterin as a cofactor in the enzyme nitric oxide synthesis, the net effect of which is improvement of peripheral blood flow.

The effects of 5-methyltetrahydrofolate were documented ex vivo by incubating vessels with 5-methyltetrahydrofolate (1 to 100 μmol/l) and in vivo by intravenous infusion of 5-methyltetrahydrofolate or placebo before vessel harvest. 5-methyltetrahydrofolate improved NO-mediated endothelium-dependent vasomotor responses and reduced vascular superoxide, both ex vivo and in vivo (29).
**Superoxide and peroxynitrite production**

Superoxide and peroxynitrite production were significantly decreased after incubation with increasing concentrations of S-methyltetrahydrofolate for 45 minutes in both SVs (A, n=32, and B, n=6) and IMAs (C, n=23 and D, n=6) compared with control vessels (incubated with buffer) from the same patients. Values are expressed as median (horizontal line), 25th to 75th percentile (box), and range (whiskers). *P<0.01 vs control. RLU/sec/mg indicates relative light units per second per milligram (29).

In a double-blind, placebo-controlled, crossover study on individuals with coronary artery disease, supplementation with high-dose folic acid (30 mg per day) improved blood flow in coronary arteries. Folate was associated with a reduction in mean arterial pressure (100 ± 12 mmHg vs 96 ± 11 mmHg, placebo vs folate, p<0.03) (30).

**Acute effects of folic acid administration on coronary flow in CAD patients**

<table>
<thead>
<tr>
<th></th>
<th>PLACEBO</th>
<th>FOLIC ACID</th>
<th>p VALUE</th>
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</thead>
<tbody>
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<td>96±11</td>
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<tr>
<td>Myocardial Blood Flow</td>
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<tr>
<td>Peak Flow Ratio (Abnormal/Normal)</td>
<td>0.54±0.17</td>
<td>0.75±0.24</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Effect of high-dose folate on coronary dilator reserve**

Folate increased dilator reserve by ~83% in abnormal segments (0.72 ± 0.60 ml/min/g vs 1.31 ± 1.08 ml/min/g, mean ± SD, placebo vs folate, p<0.05), whereas dilator reserve in normal segments remained unchanged (2.00 ± 0.61 ml/min/g vs 2.12 ± 0.69 ml/min/g, placebo vs folate, p=NS). (NS: Not Significant) (30).
References

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Product overview