



 **Quatrefolic[®]**

White Paper



gnosis

advanced biotech

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Introduction

There is a growing amount of data indicating that a series of nutrients, bioactive dietary components and changes in diet habits could modulate the patterns upon a series of organs, systems and vital functions that as a whole determine the health status of people at all the stages of life.

Since we opened our doors in 1989, Gnosis has been committed to improving the quality of life of humans and animals and promoting their well-being, the specific target of worldwide advanced nutrition.

“Science behind health” feeds Gnosis’ challenge to develop the “advanced nutrition concept” with clinically proven bioactive compounds and nutrients involved in one carbon metabolism - the key network of cellular reactions essential to sustain human life - for the whole body health condition.

Quatrefolic®, the fourth generation folate, the biologically active form of the naturally-occurring predominant form of folate, is one of the most impressive results of the capacity of Gnosis to merge excellence in biotech innovation and constant evolution in nutraceutical applications.

Quatrefolic® is also an evidence of how Gnosis is leader and really cares of the world of folate, fostering its knowledge, which is a fundamental and key point for a good health.

With this white paper we are pleased to share with you an informative report on Quatrefolic® and the new research topics of folate application with specific reference to the role of the polymorphism of the enzyme Methyltetrahydrofolate reductase (MTHFR).

The “Advanced Nutrition Concept”

Gnosis Advanced Nutrition redefines the nutrients contained in food to offer customers an extensive range of differentiated solutions improving and boosting human head-to-toe health condition and making up for the contemporary poor dietary quality.



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Overview: Folate

▪ NUTRITIONAL BIOCHEMISTRY OF FOLATE

If the word folate sounds like foliage to you, this is not an accident.

The words share a common root (the Latin word folium, meaning "leaf"), which helps remind us that green plant foods can be among the richest sources of folate. However, as the chart shows, there are outstanding sources of folate in other food groups as well, especially legumes.

Total folate content in some common foods

| Vegetables | Fruit | Meat |
|----------------------|-------------------|-------------------|
| µg/100g | µg/100g | µg/100g |
| Spinach 150 | Chestnuts 62 | Beef liver 330 |
| Brussels sprouts 135 | Pistachio nuts 58 | Pork liver 295 |
| Asparagus (can) 96 | Almonds 48 | Eggs 50 |
| Broccoli 90 | Oranges 31 | Ham 19 |
| Herbs (leaves) 89 | Almond paste 24 | Chicken breast 14 |
| Artichokes 68 | Grapefruits 21 | Sausages 8 |

| Milk and dietary products | Fish | Other |
|---------------------------|-----------------|-------------------|
| µg/100g | µg/100g | µg/100g |
| Camembert 102 | Tuna 20 | Yeast 1,250 |
| Grana cheese 55 | Eel 16 | Adzuki beans 622 |
| Gorgonzola 52 | Octopus 6 | Dried lentils 110 |
| Cheddar cheese 33 | Trout (oven) 15 | Pasta 34 |
| Yogurt 7 | Crustaceans 14 | White bread 29 |
| Milk 6 | Herrings 11 | Rice 20 |

Typical folate intakes are poor in the diets of many individuals for several reasons. Natural folates are susceptible to oxidation, they rapidly lose activity in foods and are largely destroyed by cooking till 90%. Moreover they have a low and incomplete bioavailability.

The most of folate assumption is coming from folic acid man-made version in supplements and added to foods. Folic acid (like food folate) is inactive and needs to be metabolized to 5-methyltetrahydrofolate (5-MTHF) to become metabolically effective. Today we know that the folic acid has a complex metabolism and that people assuming folic acid may still be gravely folate deficient because of the big variations in how efficiently folic acid is converted to the bioactive form in different people ^(1,2).

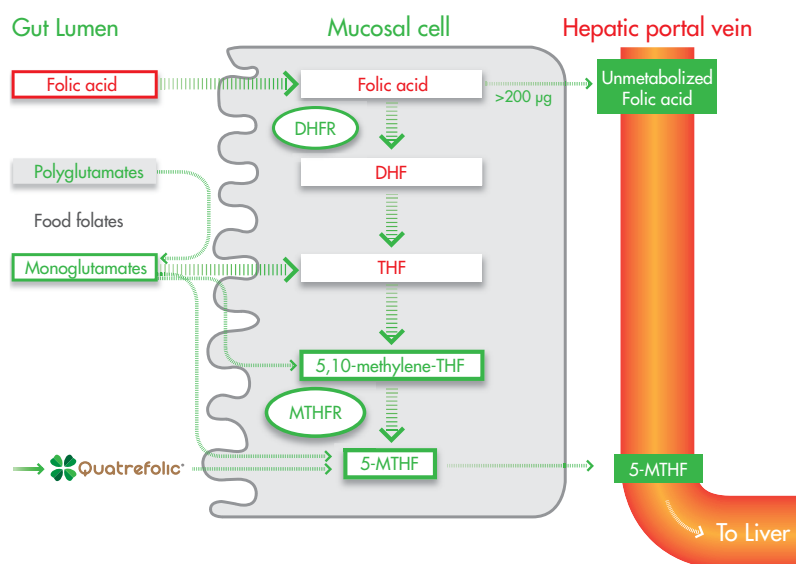
Folic Acid

Folic acid (like food folate) is inactive and needs to be metabolized to 5-methyltetrahydrofolate (5-MTHF) to become metabolically effective.

Overview: Folate

Food folates are hydrolyzed to the monoglutamate form in the gut prior to absorption by active transport across the intestinal mucosa. Therefore, before entering the bloodstream, the monoglutamate form is reduced to tetrahydrofolate (THF) and converted to methyl forms.

On the other hand folic acid is firstly reduced to Dihydrofolate by the enzyme Dehydrofolate reductase (DHFR) and then to tetrahydrofolate. **In humans the gut appears to have a very efficient capacity to convert reduced dietary folates to 5-MTHF but limited ability to reduce folic acid.** As a matter of fact, folic acid reaches the liver in unmetabolized form.



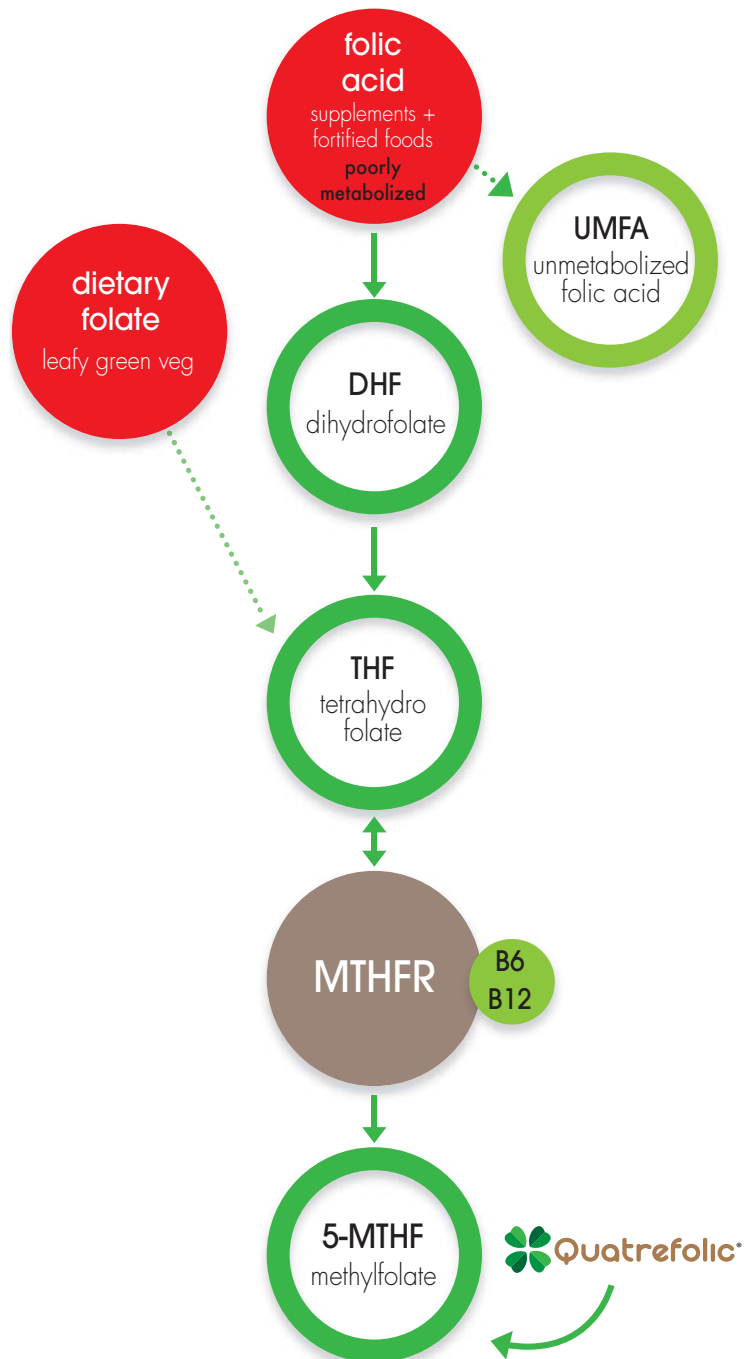
Quatrefolic® passes the gastric barrier and is absorbed mainly in the small intestine by a carrier mediated mechanism. The carrier is not saturated and this enables Quatrefolic® to ensure a higher folate uptake (3,4).

Hepatic biotransformation of folic acid to 5-MTHF is critically regulated by two polymorphic enzymes, the DHFR and the MTHFR. DHFR enzyme appears to have low and highly variable activity. Chronic liver exposure to folic acid may induce saturation, which would possibly explain reports of systemic circulation of unmetabolized folic acid. Additionally, some people have genetic variations that decrease the activity of DHFR. **A new study published in 2014 clearly showed that 86% of folic acid in the hepatic portal vein is unmetabolized, whilst almost all of the natural folate was converted correctly** (3,4).

The active form of folate

Quatrefolic® passes the gastric barrier and is absorbed mainly in the small intestine by a carrier mediated mechanism. **The carrier is not saturated and this enables Quatrefolic® to ensure a higher folate uptake.**

Overview: Folate



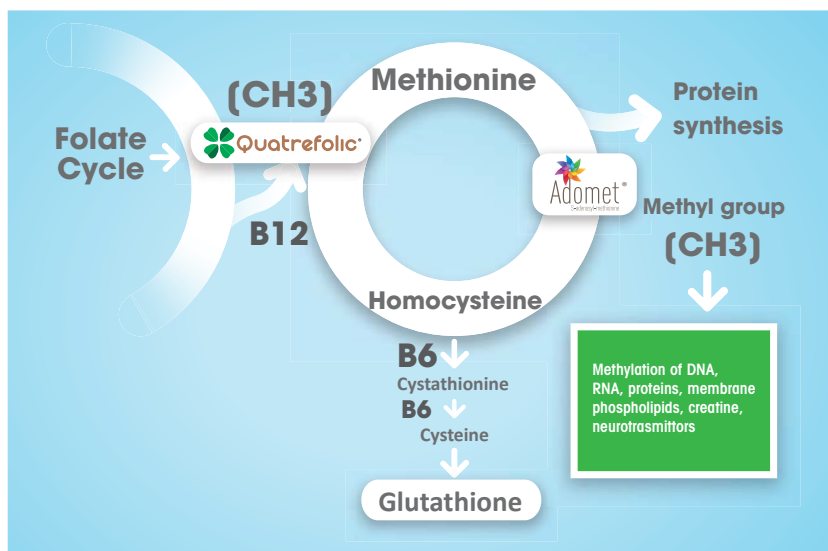
The other enzyme, the Methyltetrahydrofolate reductase (MTHFR) is also a polymorphic enzyme. Genetic variations, such as polymorphisms may impair MTHFR activity and the related metabolism of folic acid in 5-MTHF. **MTHFR polymorphisms are estimated to occur in up to 57% of the population** ^(5,6).

Overview: Folate

▪ FOLATE AND “ONE CARBON METABOLISM”

Folate-dependent one carbon metabolism and the role of nutritional compounds in regulating biochemical pathways in our bodies are the focus of recent and future investigative research.

Often referred to as the Methylation Cycle, the life critical process is a network of interrelated biochemical reactions that involves the transfer of one carbon methyl groups from one compound to another. **Folate and Methionine are the key components of the Methylation Cycle and are required for normal healthy cellular function** (7,8).



One carbon metabolism occurs in all cells of the body and is crucial for a variety of functions, including detoxification energy production, immune function, maintaining and regulating genes, mood balancing and controlling inflammation.

Therefore maintaining the balance and levels of folate, vitamin B12 and S-Adosylmethionine (S-Adomet® - Adomet®) can be critical to support various cellular processes, which influence the development, prevention and treatment of various health issues.

Quatrefolic®, the most bioavailable form of folate, can be the preferred choice for active folate supplementation in synergy with S-Adomet® to support and regulate the methylation cycle.

One carbon metabolism

Gnosis is the pioneer in the development of products involved in supporting the Methylation Cycle and one carbon metabolism network.

Overview: Folate

In recent years clinical and epidemiological evidences have helped to clarify how nutritional supplements can rectify one carbon cycle activity, restoring normal production of key metabolites and the methylation status, supporting the right gene expression.

▪ FOLATE HISTORY

The importance of folate nutritional status in health and wellbeing has been recognized through history for more than 50 years. Today folic acid and folate are often used interchangeably and many health practitioners will not be able to tell the difference, although "folate" is a family of compounds that counts 4 different generations from those naturally present in foods to the innovative Quatrefolic®.

1st generation - Food folate

Refers to the various tetrahydrofolate derivatives naturally present in foods.

2nd generation - Folic acid

Got its name from folium, the Latin word for leaf when it was isolated from spinach in 1941. It is a synthetic oxidized molecule, that does not occur in nature but can be utilized by the human body as a precursor to form natural folates that are biologically active.

Folic acid lacks coenzyme activity and must be reduced to the metabolically active form within the cell, through a series of biochemical steps before it can be used by the body's cells in vital metabolic pathways such as DNA production, cell reproduction and homocysteine metabolism.

3rd generation - (6S)-5-methyltetrahydrofolate calcium salt

In 1989, Bioresearch S.p.A. was the first company to evaluate the real importance of 5-methyltetrahydrofolate and to boost the development and the launch of this innovative natural endogenous compound in the form of stable pharmaceutical composition of calcium 5-methyltetrahydrofolate, being well aware that biological molecules face less risks of adverse effects in humans compared to xenobiotics.

The calcium salt of 5-methyltetrahydrofolate is commercially available and represents the third generation of folate. Until now,

HISTORY OF GNOSIS

- **1989**
Foundation
- **1997**
Starting Large Scale Manufacturing
- **2003**
Opening of R&D site in Desio (MB)
- **2004**
Acquisition of Sant' Antonino (CH) Site from Abbott Knoll Bioresearch (ex Bioresearch S.p.A.)
- **2009**
Acquisition of Pisticci Site from Pfizer
- **2011**
Opening of Gnosis USA Branch Office
- **2012**
Opening of Gnosis China Branch Office
- **2014**
US FDA Approval for both Manufacturing Sites

Overview: Folate

5-methyltetrahydrofolate calcium salt was the only folic acid derivative available on the market and able to penetrate the body cells without needing further metabolism.

The 4th generation: Quatrefolic®

The goal for Gnosis' R&D was to develop an innovative folate salt form able to overcome the existing calcium salt form limitations related to stability and poor solubility. In February 2008, Gnosis patented a new generation of folate derivative, brand named Quatrefolic®, (6S)-5-methyltetrahydrofolate glucosamine salt.

Quatrefolic® represents the fourth generation folate endowed with long lasting stability as well as a peculiarly high water solubility, improved bioavailability and well established safety.

Quatrefolic® has been successfully launched in 2011, after having recognized as a New Dietary Ingredient by FDA and having achieved GRAS status in 2010.

In 2014, the European Commission approved the use of Quatrefolic® in the European Union as a Novel Food Ingredient. In 2015, the Annex II of the Directive 2002/46/EC has been officially amended to include Quatrefolic® which may be used in the manufacture of food supplements sold inside of European Community.

Chronology: The generations of folate

1st generation folate
(food)

2nd generation folate
(synthetic folic acid)

3rd generation folate
(reduced folate)

The 4th generation folate
Quatrefolic®

Open questions: folate & folic acid

■ GENETIC POLYMORPHISM IN FOLATE

In the recent years several evidences of the advantages of reduced folate vs folic acid have been found. **The rational use of reduced folate (particularly reduced and methylated such as Quatrefolic®) is derived from the inability of a part of world population to assimilate and metabolize folic acid from food or supplements** (4,9,10).

Folic acid and also food folate are not biologically active and need to be converted to the metabolically active 5-MTHF through a multi-steps process where the enzyme methylenetetrahydrofolate reductase (MTHFR) owns a key role. Some individuals, due to their unique genetic patterns and expression, have polymorphic forms of this enzyme and do not produce adequate or effective MTHFR.

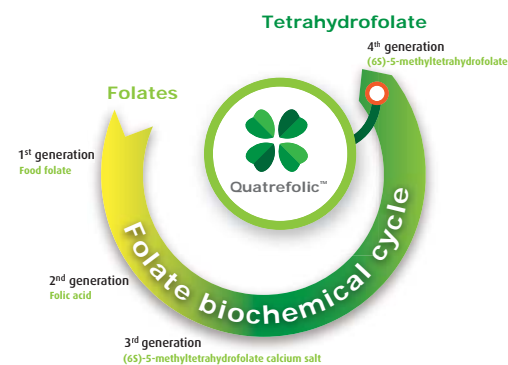
Not the 100% of people

There is a big variation in how efficiently folic acid is converted to the bioactive form in different people



Source: Tsang et al. Am. J. Clin Nutr. 2015

Emerging science of nutrigenomics is shed light how much the MTHFR polymorphism is implicated in chronic disease states and how folate nutrition may contribute to replace adequate methylation and overall health (11).



Open questions: folate & folic acid

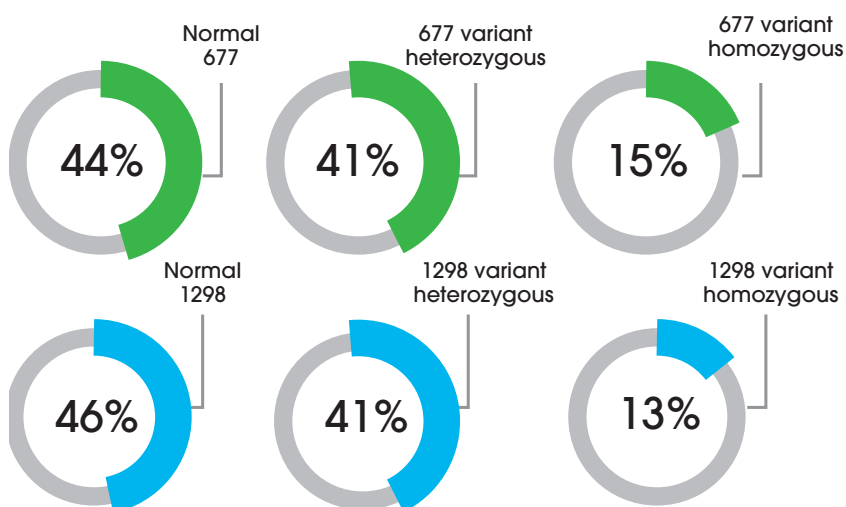
To date, there are more than 50 known MTHFR variants, but the two prime variants are called C677T and A1298C. The numbers refer to their location on the MTHFR gene. The letters refer to the amino acid position on the MTHFR.

The MTHFR is reported as either heterozygous or homozygous (12).

Polymorphic MTHFR enzyme may function approximately 55% to 70% efficiency compared to a normal MTHFR enzyme. Homozygous means two genes are affected and enzyme efficiency decreases to approximately 7% to 10% when compared to normal. They are also more common among those predisposed to diseases such as cancer, heart disease, and autism, where the mutation frequency can exceed 90% of these populations (10).

MTHFR variant genes are common!

Percent of a mixed population containing 677 and 1298 variant genes.



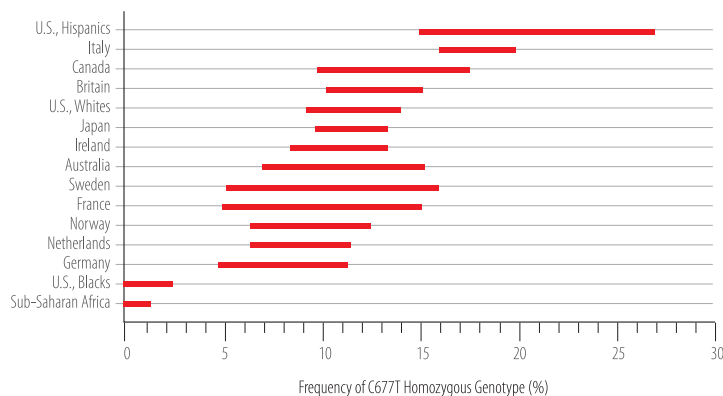
Frequency of the two most common polymorphisms, 677 and 1298 variant genes, found in a mixed population (13).

MTHFR gene mutations affect over 40% of the world population, however sadly this is largely ignored. **Current population data shows gaps especially in some ethnic groups or large geographical areas: about 50% women in Caucasian (higher in the North American Caucasians, Italian and the Hispanics), Mexico (34.8%) and in the North part of China (around 20%)** (14,15).



Open questions: folate & folic acid

Population frequency of homozygosity by geographic area and ethnicity



The inability of a part of world population to assimilate and metabolize folic acid from food or supplements may jeopardize their health and increase the risk of adverse health outcomes. The biological active form 5-Methylfolate such as Quatrefolic®, is kindly recommended because efficiently normalize the folate status of all potential subjects including who owns MTHFR polymorphism (14).

▪ THE “UMFA”, UNMETABOLIZED FOLIC ACID IN SERUM

Several studies have reported an increase in serum of unmetabolized folic acid (UMFA) levels since the implementation of folic acid fortification, with possible concern about its potential ‘overdosing’ and adverse effects.

Variability in the presence or persistence of UMFA in the population suggests that it may be accumulated in the blood as a consequence of different conditions described above, such as the impairment, and/or the slackness of the folic acid reduction pathway to the 5-methyltetrahydrofolate (genetic polymorphism), and the overdosing effect due to uncontrolled folic acid intake.

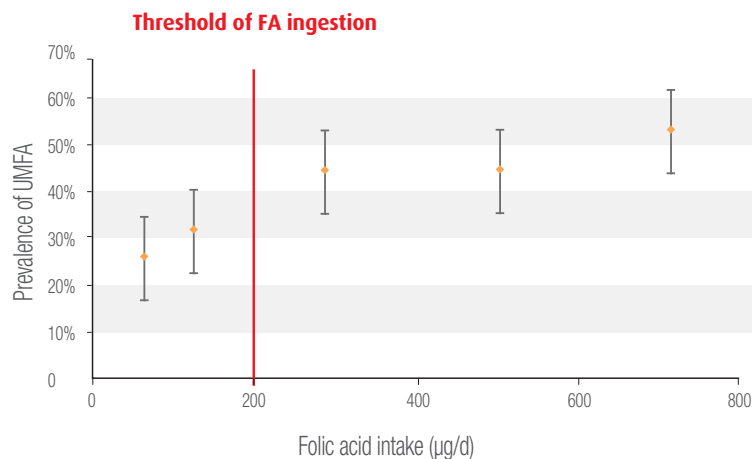
The threshold of ingestion of folic acid that leads to the direct appearance of UMFA in the plasma, results to be highest than 200-300 µg/daily intake. **The consumption of highest dosage of synthetic folic acid results in absorption of unreduced folic acid, which may interfere with folate metabolism for a period of years** (16,1).

UMFA

Several studies have reported an increase in serum and unmetabolized folic acid (UMFA) levels since the implementation of folic acid fortification.

Open questions: folate & folic acid

Prevalence of detectable circulating unmetabolized folic acid by quintile category of estimated folic acid intake (17).



The large amounts of UMFA in the portal vein are probably attributable to an extremely limited capacity of the enzyme dihydrofolate reductase (DHFR) present in the mucosal cell of the intestine, that is responsible for the first step of reduction of synthetic folic acid to 5-MTHF.

Therefore, chronic liver exposure to folic acid in humans may induce saturation, which would possibly explain reports of systemic circulation of UMFA.

Recent studies have confirmed that UMFA is associated with a reduction of natural killer cytotoxicity, which reduces the immune systems capacity to kill off malignant or pre-malignant cells. Moreover it accelerates cognitive decline and anemia in the elderly with low levels of vitamin B12.

Quatrefolic® answers to all consumers' and physicians' concerns relating to potential harmful effects of folic acid administration. As Quatrefolic® provides the metabolic reduced folate form utilized and stored in the human body, the (6S)-5-methyltetrahydrofolate, it does not aid to the potential accumulation of UMFA in the blood, which has no biological function and whose effects are not yet known, also due to the potential uncontrolled assumption of folic acid by diet (10,9).

Quatrefolic® and UMFA

Quatrefolic®, the "active form" of folate does not aid the potential accumulation of UMFA in the blood.

Quatrefolic®: The innovative folate

▪ BIOAVAILABILITY & SOLUBILITY

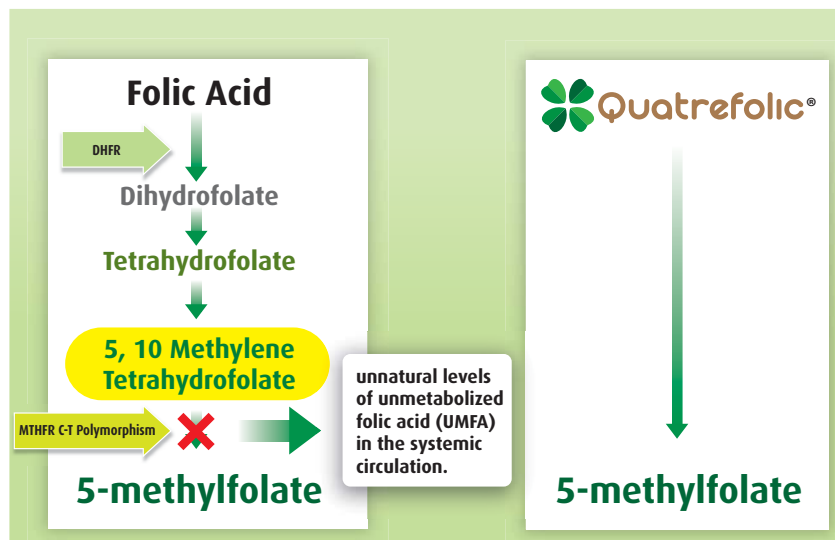
Quatrefolic® is the glucosamine salt of (6S)-5-methyltetrahydrofolate and is structurally analogous to the reduced and active form of folic acid; Quatrefolic® delivers a “finished” folate the body can immediately use without any kind of metabolization.

Choosing Quatrefolic® as a source of folate presents several advantages and solves some problems about folate supplementation:

- 1- The limited ability of human gut to reduce folic acid to 5-MTHF and related risk that the majority of a physiologic oral dose of folic acid passes into the portal venous circulation in an unmodified form (2,4,19).
- 2- The big variations in how efficiently folic acid is converted to the bioactive form in different people due to defect of MTHFR activity (1, 11).
- 3- The low solubility of calcium salt (36).

Quatrefolic® is structurally analogous to the active form of folic acid.

Quatrefolic® delivers a “finished” folate the body can immediately use without any kind of metabolization.

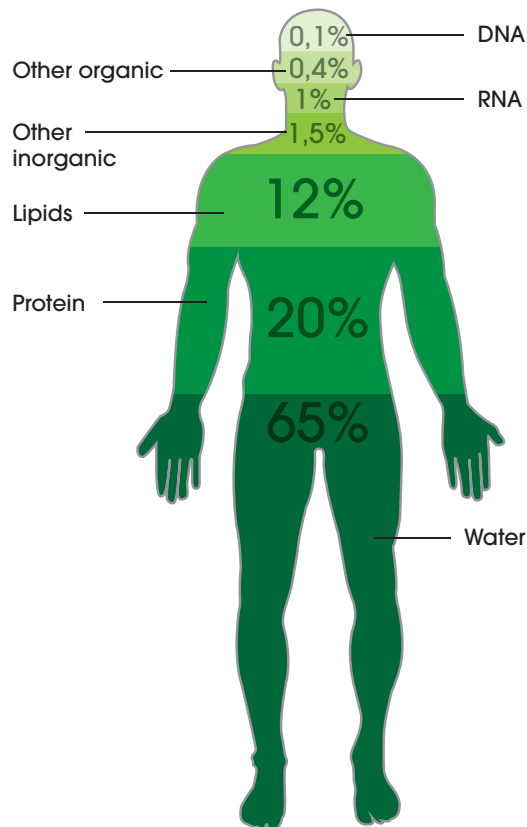


Quatrefolic® supplementation allows 5-methyltetrahydrofolate to be immediately bioavailable for its biological action without being metabolized in the body.

Quatrefolic® demonstrates a high solubility in water – greater than 1g/1ml – compared to the slight solubility of the competitor compound, (6S)-5-methyltetrahydrofolate calcium salt (1g/100ml), 100 times more soluble.

Quatrefolic®: The innovative folate

The oral bioavailability of an active ingredient is highly dependent on its solubility. The absorption of the compound happens after dispersion and solution in gastrointestinal fluid.



The human body is composed of more than 65% of water and any active ingredient to be adsorbed must be present in the form of solution at the site of absorption.

High water solubility means Quatrefolic® may be better absorbed by mucosal cells, which may facilitate access to the blood and circulation with the potential of improving bioavailability.

The extremely higher water solubility of Quatrefolic® is derived from two specific characteristics of the ingredient: the glucosamine salifying agent and the amorphous chemical structure of the product.

The amorphous status provides an intrinsic solubility guaranteeing homogeneous and reproducible solubility process whereas the crystalline alternative cannot.

What does solubility mean?

High water solubility means Quatrefolic® may be better absorbed by mucosal cells which may facilitate access to the blood and circulation.

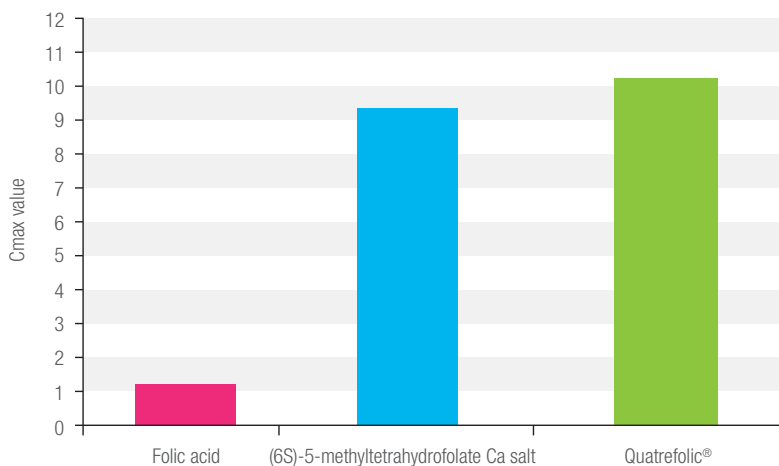
Quatrefolic®: The innovative folate

Glucosamine is a basic, natural, safe and organic salifying agent, chosen after a deep screening operated by Gnosis on more than 100 molecules, as a salt of the biological active form of folate, the (6S)-5-methyltetrahydrofolate, which makes Quatrefolic® 100 times more soluble than the old generation compound, the 5-MTHF calcium salt.



Comparison of solubility in water has been performed evaluating the quantity of solvent needed to solubilize the same amount of each folate derivative, at standard temperature. The improved solubility of Quatrefolic® over the (6S)-5-methyltetrahydrofolate calcium salt represents another advantage achieved by Gnosis in creating a product for applications in the food and beverage industries.

Animal studies and human clinical trial confirm that Quatrefolic® owns a superior bioavailability profile over the (6S)-5-methyltetrahydrofolate calcium salt and folic acid. It was a single dose, balanced, two sequences, two periods, two treatments randomized crossover study.



(6S)-5-methyltetrahydrofolate hematic peak produced by an equivalent dose of the indicated folate source.

Quatrefolic® solubility

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Folic Acid Bioavailability

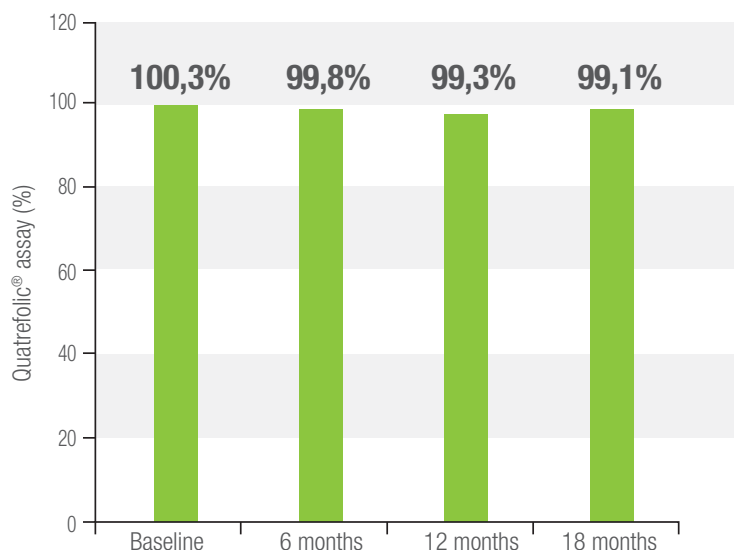
Humans has a limited ability to reduce folic acid to 5-MTHF. The lower bioavailability of folic acid cannot be offset by its increasing intake (2,4,19).

Quatrefolic®: The innovative folate

▪ STABILITY

Quatrefolic® shows an extraordinary long lasting chemical stability guaranteeing a quite unaltered purity even after several months, and an assay reduction in 18 months less than 1%, allowing easy handling and storage. The pH of Quatrefolic®, once dissolved, is neutral. This value also provides greater stability to the molecule, protecting it from hydrolytic degradation.

Stability of Quatrefolic® at room temperature



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.

Gnosis has deliberately chosen to carry out stability tests of Quatrefolic® according to the most strict guidelines, specifically required for pharmaceutical ingredients guaranteeing independent and reliable criteria to claim the long lasting chemical stability to its clients.

The lyophilized ingredient could be handled without specific and restrictive conditions, easily combined with other ingredients and excipients and could be stored at room temperature instead at 2-8°C of the previous folate derivative.

Quatrefolic®: The innovative folate

▪ SAFETY

Quatrefolic®, as glucosamine salt of (6S)-5-methyltetrahydrofolate has been the subject of an extensive and relevant number of biological and toxicological studies in order to prove the safety and tolerability of this revolutionary folate. The safe use of Quatrefolic® in pregnancy and lactation has been deeply supported by Gnosis in the submission of both the New Dietary Ingredient (NDI) Notification at FDA and Novel Food at EFSA (18).



Quatrefolic® is "generally recognized as safe" ("GRAS") for use as a source of folate in conventional and medical foods. The GRAS recognition has been achieved in 2010, by an independent panel of recognized experts - qualified by their scientific and/or medical training and relevant experience - which carried out a critical evaluation of the available data. The FDA has approved Quatrefolic® as New Dietary Ingredient (NDI) in the same year. The European Novel Food approval has been reached in 2015. Kosher and Halal Certification have been reached in 2014.

Toxicological studies

Gnosis has performed several in-vitro and in-vivo studies such as mutagenicity, genotoxicity and acute toxicity on Quatrefolic® and the product has met all the safety requirements.

Toxicological tests carried out proved that Quatrefolic® does not induce mutations and it is not cause of chromosomal aberrations. In vivo single dose oral toxicity has been carried out by Gnosis.

Quatrefolic® is safe

Toxicological tests carried out proved that Quatrefolic® does not induce mutations and it is not cause of chromosomal aberrations.

Quatrefolic®: The innovative folate

Dosage

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

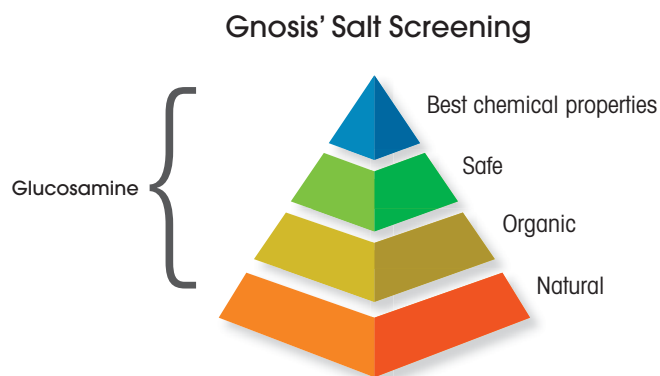
As Quatrefolic® provides the metabolic reduced folate form utilized and stored in the human body, it is totally bioavailable.

| AGE (years) | MALES AND FEMALES (µg/day) | PREGNANCY (µg/day) | LACTATION (µg/day) |
|-------------|----------------------------|---------------------|---------------------|
| | Folate/Quatrefolic® | Folate/Quatrefolic® | Folate/Quatrefolic® |
| 1 - 3 | 150 | - | - |
| 4 - 8 | 200 | - | - |
| 9 - 13 | 300 | - | - |
| 14 - 18 | 400 | 600 | 500 |
| 19 + | 400 | 600 | 500 |

Bibliographic reference: Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. National Academy Press. Washington, DC, 1998.*

Safety of glucosamine

Glucosamine is a naturally occurring, endogenously produced molecule and thus is not an extraneous compound in terms of the human metabolic process.



Glucosamine is made up of glucose and amino acid glutamine and it is a key building block for several structures.

Several studies show that glucosamine is well tolerated by healthy volunteer subjects at very high dosages, when administered intravenously. Relevant scientific data has been provided related to the effect of glucosamine on sensible categories such as pregnant/lactating women, and children.

The intake of Glucosamine from Quatrefolic® is deemed to be insignificant being equal to 552 µg/day where the acceptable daily intake is 184 mg/kg/day.

Quatrefolic®: The innovative folate

Vitamin B12 masking

Quatrefolic® is unlikely to mask the vitamin B12 deficiency and its hematologic symptoms as the pathway where it is involved is vitamin B12 dependent.

Vitamin B12 deficiency is common in old age and may not be easy to recognize. People with vitamin B12 deficiency are at risk for nerve damage, anemia and degeneration of the spinal cord. Even relatively mild deficiency can affect brain functions and the nervous system, and the nerve damage may develop into permanent debilitation if left untreated.

Folate and vitamin B12 deficiency have the same hematologic symptom, megaloblastic anemia, which disappears after supplementation with large amounts of folic acid (that could be reached thanks to mandatory food fortification with folic acid), particularly in the elderly. Folic acid supplementation may activate synthesis of purine and pyrimidine through a specific pathway correcting anemia, although vitamin B12 is still absent; on the contrary nerve and cognitive deterioration related to vitamin B12 deficiency may continue unchecked.



Quatrefolic® supplementation does not activate purine and pyrimidine synthesis and, if vitamin B12 is absent, 5-MTHF remains “metabolically trapped”. This situation produces a “pseudo folate deficiency” because although the cells have adequate levels of folate, it is trapped as 5-MTHF form allowing doctors to diagnostic vitamin B12 deficiency (19,20,21).

People at risk of vitamin B12 deficiency

Vegetarians and vegans, people affected by celiac, autoimmune and Crohn’s disease, gastritis, malabsorption syndrome, HIV infection, multiple sclerosis and under specific drug treatment are also at risk.

Quatrefolic®

Quatrefolic® is unlikely to mask the vitamin B12 deficiency and its hematologic symptoms being the pathway where it is involved vitamin B12 dependent.

Quatrefolic®: Health connections

▪ STATE OF THE ART

The influence of folate nutritional status has been recognized as critical for human health due to its role in one carbon metabolism, the network of cellular interrelated biochemical reactions involving the transfer of one carbon groups from one biological compound to another (methylation). Folate deficiency has far-reaching negative health consequences at all stages of life. In fact, folate-dependent one carbon transfer is required for DNA synthesis and cell division, regulation of gene expression, amino acid metabolism and neurotransmitter synthesis.



Quatrefolic® passes the gastric barrier and is absorbed mainly in the small intestine by a carrier mediated mechanism. The carrier is not saturated and this enables Quatrefolic® to ensure a higher folate uptake.

Humans cannot synthesize folate and because of its water soluble nature, the body stores folate to a limited extent. **Folate deficiency may occur when dietary intake is inadequate or when an increased need is not matched by an increased intake as:**

1. conditions with a high rate of cell turnover such as rapid tissue growth (infants, kids and adolescents) **pregnancy and lactation.**
2. conditions such as **enzyme defects, malabsorption, digestive system pathology, liver disease** but also when metabolism or drug use interferes with the ability of the body to use folate.

The past decade of folate research has taught us much more about the nature of this vitamin and its critical role in supporting our health. **Our goal in these next paragraphs is to provide a framework to simplify key aspects of recent research on folate and health benefits.**

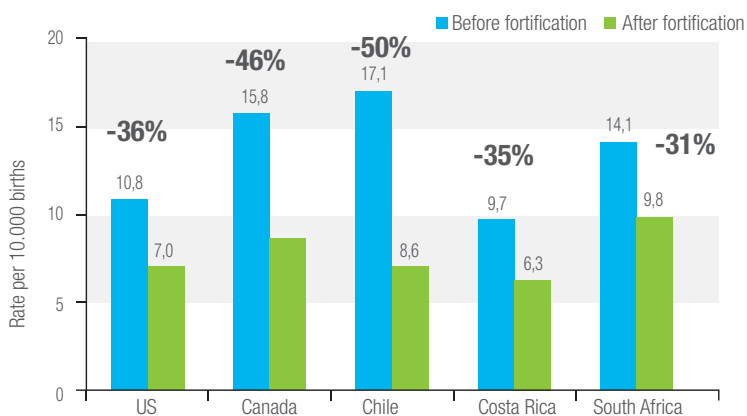
Folate and one carbon metabolism

Folate-dependent one carbon transfer are required for DNA synthesis and cell division, regulation of gene expression, amino acid metabolism and neurotransmitter synthesis.

▪ PREGNANCY AND LACTATION

The demand for folate increases when human cell growth is very active, such as in pregnancy and lactation. Studies have found that low dietary intake of folate increases the risk of delivering a child with several types of birth defects, particularly neural tube defects (NTD) and possibly leading to poor growth in the fetus or placenta (22).

Neural tube defects prevalence changes before and after folic acid fortification



Quatrefolic® as a source of (6S)-5-methyltetrahydrofolate (5-MTHF) might be particularly useful to provide the nutritionally active form of folate during preconception, pregnancy and lactation.

Even today in Europe and United States half of pregnancies are unplanned and expose these women to a serious risk since defects of the brain and spine (Neural Tube Defects) develop in the first 28 days of pregnancy – before many women even know that they are pregnant.

Clinical evidence suggests that supplementation of the natural form, 5-MTHF, is a better alternative to supplementation of folic acid, and that can effectively improve folate biomarkers in young women in early pregnancy in order to prevent NTDs.

Since the association between MTHFR polymorphism and low folate concentration has been assessed, with a major risk of NTD,



Quatrefolic®: Health connections

5-MTHF supplementation may represent a substantial advantage.

Spontaneous Abortion

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 (23).

Down Syndrome

Several studies have investigated maternal enzyme polymorphism in the metabolism of folate as a risk factor for Down Syndrome (24).

Lactation

Breast milk folate concentrations are maintained at the expense of maternal folate reserves.

A lactating woman would require 128 µg/day of additional folate in order to restore her losses.

5-MTHF appeared to be as effective as, and perhaps more effective than, folic acid in preserving RBC folate concentrations during lactation (25,26).

■ WOMEN HEALTH

Contraceptive therapy

New applications and formulations suggest to combine oral contraceptives with folate because women may become pregnant during and after discontinuation of contraceptive drug treatment.

Since birth control pills are the most popular method of reversible contraception in the USA, it would seem quite logical to find a way to supplement them (27).

Infertility

Oxidative stress is one of the factors related to the pathogenesis of fertility disorders such as idiopathic infertility, polycystic ovarian syndrome and endometriosis.

There was an inverse association between frequency of multivitamin use with folate and ovulatory infertility (28).



Quatrefolic®: Health connections

Mood - Post Partum Depression

Folate deficiency can contribute to depressed mood, impairing the production on neurotransmitters through the One carbon cycle. Folate supplementation may be useful for some depressed patients (23).

Post-menopausal

Whether maintained in the long term, 5-MTHF cardiovascular and metabolic effect may contribute to primary cardiovascular wellness of postmenopausal women (30).

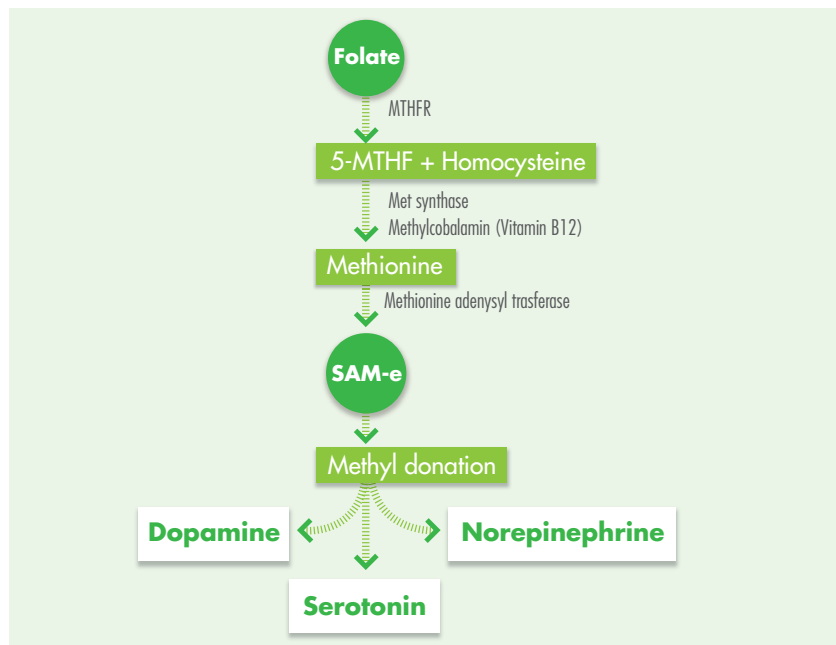


▪ OTHER APPLICATIONS

Mood/Depression

Studies suggest that there might be a direct link between cerebral folate status and depression. Folate deficiency impairs the production on neurotransmitters through the One carbon cycle.

Quatrefolic®: Health connections



A decline in folate levels in the cerebral spinal fluid (CSF) has been recognized in aging and there is evidence causally relating folate levels to mental function, especially depression. Folate deficiency can contribute to depressed mood, and therefore folate supplementation may be useful for some depressed patients (31).



Clinical trials of folate in depression

| Author | Design | Folate Supplement used | Sample characteristics | Outcome |
|------------------------|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Godfrey et al, 1990 | DBC 6 months | D-L-MTHF (15mg) or placebo. Other psychotropic medication allowed. | 24 depressed and 17 schizophrenic subjects with low red cell folate (<200 ng/ml). DSM-III diagnosis. | Significant decrease in mean outcome scores in MTHF group at 3 and 6 months. |
| Passeri et al, 1993 | DBD 8 weeks | 5-MTHF (50 mg/day p.o.) or Trazodone TRZ (100 mg/day p.o.) in addition to standard psychotropic medication. | 96 elderly patients with Hamilton Depression Rating Scale (HDRS) > or = 18. | HDRS score was reduced from 23 +/- 5 to 18 +/- 6 in the 5'-MTHF and from 23 +/- 3 to 19 +/- 5 in the TRZ group. |
| Ginsberg et al, 2011 | A retrospective analysis from patient charts 60 days | 5-MTHF plus SSRI/SNRI at treatment initiation (n=95) and SSRI/SNRI monotherapy (n=147). | Adults 18 to 70 with major depressive episode (single or recurrent). | Major improvement (CGI-S reduced by ≥2 points) for 18.5% of 5-MTHF plus SSRI/SNRI patients compared to 7.04% (CGI-S=4-5) of SSRI/SNRI monotherapy patients. |
| Papakostas et al, 2012 | DBC 30 days | L-MTHF (trial 1 = 7.5mg; trial 2 = 15mg) or placebo, adjunctive therapy to SSRI. | Treatment-resistant depressed patients. Trial 1, n=148. Trial 2, n=75. | 7.5 mg had no significant difference. 15 mg showed significantly greater response rate and Change in HAM-D score. |
| Reynolds et al, 2015 | — 6 weeks | 5-MTHF 50 mgs (25 mgs biologically active) or Amitriptyline 150 mgs. | 31 patients (20 – 69 years) with a Montgomery Asberg Depression Score (MADS) of at least 14. | Of 19 patients randomised (n = 16) or crossed over (n = 3) to treatment with 5-MTHF, 8 responded (42%). Of 20 patients randomised (n = 15) or crossed over (n = 5) to Amitriptyline, 7 responded (35%) responded. |

Quatrefolic®: Health connections

Anemia

Folate has a long history of use in conjunction with vitamin B12 as supplement in macrocytic anemia. Megaloblastic anemia is characterized by red blood cells that are larger than normal. The red blood cells are also deformed and both their rate of production and their lifespan are diminished.

Folate anemia occurs most often in infants, adolescents, pregnant and lactating females, alcoholics, the elderly and in those with malignant or intestinal diseases.

Cardiovascular Disease & Hyperhomocysteinemia

Homocysteinemia is widely accepted as an independent risk factor for coronary, cerebral and peripheral vascular diseases.

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.

Several randomised placebo-controlled trials are currently being conducted to establish whether folic acid supplementation can help to reduce the risk of cardiovascular diseases by lowering homocysteine blood levels. The effects of 5-MTHF are significantly more potent than folic acid itself as reported by Akoglu in 2008 (32,33).

Aging & Older People (Mild Cognitive Impairment)

There are numerous physical and physiological changes which occur during the aging processes.

Aging is associated with changes in gastrointestinal function that could possibly affect the absorption of different folate forms. This deficiency may be important with respect to blood formation, neurologic function and cardiovascular function. Epidemiological studies and case observations have suggested that low concentrations of folate in the blood can be related to poor cognitive function, dementia and Alzheimer's disease. It has been hypothesized that the relationship between folate deficiency and poor cognitive function may be due to the role of folate in reducing homocysteine blood and its effects on the vascular system (34).



Quatrefolic®: Health connections

Men Infertility

Folate levels measured in semen have been associated with sperm count and health. Low folate levels in semen were connected with poor sperm DNA stability (35).

Infants & children

Folate is a critical nutrient when human cells growth is very active and folate deficiency can slow overall growth rate. Infants, children and adolescents represent a critical phase of growth and the proper level of folate is recommended to prevent a variety of medical conditions such as anemia.

Recommended dietary intake of folate for males and females during the age of growth has been defined.



Quatrefolic®: Key points

Quatrefolic® and Folic Acid?

Folic acid

Folic acid itself is not active and must be metabolized through several steps in order to enter the folate cycle.

Unmetabolized folic acid is found in blood at doses >200 µg / day.

Lesser bioavailability.

High doses of folic acid can mask vitamin B12 deficiency and delay its diagnosis by correcting hematological signs.

Folic acid upper tolerable limit is 1 mg /day.

5, 10-Methylenetetrahydrofolate reductase (MTHFR) enzyme polymorphism problem: In carriers of mutated homozygotes **677T->T** genotype the enzyme activity of the MTHFR is about **70% less than normal**, and heterozygotes **677C->T** **30-40% less than normal**. The reduction in MTHFR activity **increases homocysteine levels** and **in turn reduces the availability of the DNA methyl groups**.

Not soluble in water.

Quatrefolic®

Main folate form in blood and cord serum.

It is already the **biologically active form**. It can enter directly the folate cycle.

No unmetabolized folic acid with Quatrefolic®.

Higher bioavailability: Pre-clinical study in vivo with Quatrefolic® showed a plasmatic (6S)-5-MTHF concentration peak about **3 times higher** for Quatrefolic® than folic acid.

As Quatrefolic® is already the biologically active form, it doesn't mask the vitamin B12 deficiency.

No upper tolerable limit of Quatrefolic® in US dietary reference intakes. Safety of (6S)-5-MTHF has been confirmed by several studies.

As Quatrefolic® is already the biologically active form, **the problem of people with 677C->T or 677T->T polymorphisms in folate-related enzymes (especially MTHFR) doesn't exist anymore**.

Quatrefolic® is totally soluble in water.

Quatrefolic® and 5-MTHF Calcium Salt?

(6S)5-MTHF Ca Salt

(6S)5-MTHF Ca Salt is stable only at temperature between 2-8°C.

(6S)5-MTHF Ca Salt is lesser bioavailable.

100 times less soluble in water than Quatrefolic®.

Quatrefolic®

Quatrefolic® is lyophilized and is stable at room temperature 25°C.

Higher bioavailability: Pre-clinical study in vivo with Quatrefolic® showed a plasmatic (6S)-5-MTHF Ca Salt concentration peak about **20% times higher** for Quatrefolic® than Metafolin.

Quatrefolic® is totally soluble in water.

Quatrefolic®: Key points

Main health benefits of Quatrefolic®:

- Women planning pregnancy
- Pregnant women
- Breastfeeding women
- Infants, children and adults with folic acid deficiency
- Macrocytic anaemia
- Hyperhomocysteinemia
- Depression
- Cognitive functions in elderly people
- Sport
- Bone mineralization

Clinical researches emphasize the importance of folate supplementation in:

| | |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| • Neural-tube defect | • Irritable bowel disease |
| • Male and female infertility | • Cognitive deficits in elderly |
| • Spontaneous abortion | • Lifestyle putting people at risk of low folate levels: <ul style="list-style-type: none">• Smoking• Alcohol excess• Eating disorders• Low vegetables intake• Chronic dieting |
| • Coronary heart disease | • Epilepsy |
| • Macrocytic anaemia | • Mood |

PATENTS:

"Folates, compositions and uses thereof." (U.S. Patent No. 7,947,662 – European Patent No. EP 2245032)
"Process for the diastereoisomeric resolution of 5-methyltetrahydrofolic acid." (EP 2254890 - PCT/EP2008/52034)

TRADEMARK

Quatrefolic® trademark and four-leaf clover logo is a proprietary trademark of Gnosis S.p.A.
Registered in EU, USA, CA, CN, AR, IN, MX, TW.

LEGISLATION AND COMPLIANCE

- NDIN (New Dietary Ingredient) Notification
- GRAS (Generally Recognized as Safe) Self-Affirmation
- EFSA Novel Food
- Manufacturing Operations FDA Inspected
- HACCP
- 21CFR Part 111

CERTIFICATIONS

- UNI EN ISO 22000:2500
- KOF-K Certificate
- Halal Certificate

Links & Contacts

▪ WEBSITES

www.gnosis-bio.com

www.advancednutrition-bio.com

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▪ SOCIAL PAGES

www.facebook.com/quatrefolic

www.linkedin.com/company/gnosis-bioresearch

▪ CONTACTS

HEADQUARTERS & INNOVATION CENTRE

Gnosis S.p.A.

Via Laboratori Autobianchi, 1
20832 Desio (MB), Italy

Tel +39.0362.48.84.1
Fax +39.0362.48.84.40
info@gnosis-bio.com

OPERATIONS

Gnosis Bioresearch S.A.

Via Lischedi, 4
6592 Sant'Antonino
Switzerland

Tel +41.(0).91.851.91.91
Fax +41.(0).91.851.91.92
info@gnosis-ch.com

Gnosis Bioresearch S.r.l.

Via Pomarico
75010 Pisticci Scalo
Pisticci (MT) - Italy

Tel +39.0835.46.18.01
Fax +39.0835.46.18.160
infobio@gnosis-bio.com

BRANCH OFFICES

Gnosis USA Inc.

4259 West Swamp Road,
3rd Floor Suite 305
Doylestown, Pennsylvania 18902 - USA

Tel +1.215.340.7960
Fax +1.215.340.1351
info@gnosis-us.com

Gnosis China

Room 1001, Hong Kong Plaza (South Tower), 283 Huaihai Middle Rd.,
LuWan District, 200021
Shanghai, China

Tel +86.021.530.875.12
Fax +86.021.530.875.13
info@gnosis-china.com



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