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Is there a multidisciplinary role for 5-methyltetrahydrofolate? The obstetric evidence in perspective

M. RUBINI[1], G. DI MINNO[2], E. FERRAZZI[3]

[1]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy
[2]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli "Federico II", Clinica Medica, Naples, Italy
[3]Department of Woman, Unit of Obstetrics, Child and Neonate, Mangiagalli Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

ABSTRACT. – 5-methyltetrahydrofolate (5-MTHF), or its synthetic precursor, folic acid, is traditionally used as a supplement for improving fertility and for the prevention of embryonal neural tube defects. However, in the last decade, starting from the effectiveness of this preventive treatment in the gynecological setting, the use of 5-MTHF was extended to other medical and pathological areas. Thus, there might be a rationale for the use of 5-MTHF for purposes other than the protection of the growing embryo linked to the possible effect of MTHFR variants in different pathological conditions. A narrative review was conducted to provide an overview of the available evidence on the use of 5-MTHF in the obstetric field and to critically discuss the available data regarding the use of 5-MTHF across other different therapeutic areas. Results showed that the use of 5-MTHF in pregnancy presents some advantages if compared with folic acid, such as immediate action, the non-necessity of metabolic activation, and the immediate bioavailability of the mother and fetus. Otherwise, the role of 5-MTHF in the management of cardiovascular risk is still debated due to the multiple confounding factors that characterize this patient setting. A link between folate deficiency in pregnancy and postpartum depression has been proposed, as well as between folate levels and the onset of depression. In conclusion, evidence from the literature supports the additional role of 5-MTHF as a pleiotropic drug with a transversal effect in different therapeutic contexts. With regard to the prevention of cardiovascular disorders, available evidence is not conclusive.

Key Words:
5-methyltetrahydrofolate, 5-MTHF, Folate, Folic acid, Neural tube defects, Obstetrics, Gastroenterology, Rheumatology, Cardiology, Neurology.

Introduction

Folates play a crucial role in the metabolic regulation of amino acids and nucleic acids and one-carbon metabolism, providing the production of methyl groups[1]. In particular, folates are essential in the conversion of homocysteine into methionine, a limiting amino acid in the synthesis of many proteins[2]. This process, known as methionine synthesis, requires the one-carbon units provided by folate. The metabolism of methionine affects several biochemical pathways involving the production of nutrients, which are essential for the optimal functioning of the cardiovascular, skeletal and nervous systems.

Homocysteine is an intermediate product of methionine metabolism. It is itself metabolized by two pathways: the remethylation pathway, which regenerates methionine, and the trans-sulfuration pathway, which degrades homocysteine into cysteine and then taurine (Figure 1)[3,4]. Folate deficiency can lead to disruptions in these pathways, leading to several health issues. Among them, hyperhomocysteinemia has been associated with an increased risk of cardiovascular disease, osteoporosis, renal failure, diabetic microangiopathy and neuropsychiatric disorders, as well as spontaneous abortion, hypertensive disorders of pregnancy, and placental abruption[3-7].

To prevent folate deficiency, it is important to consume a diet rich in folate-containing foods, such as leafy greens and legumes. Healthcare professionals may recommend the assumption of folate supplements to individuals with low folate status or to people with increased folate needs, such as women during pregnancy or lactation,

Corresponding Author: Michele Rubini, MD; e-mail: rub@unife.it

or individuals bearing genotypes that affect the one-carbon metabolism, such as variants in the *MTHFR* gene encoding the 5-10-methylenetetrahydrofolate reductase enzyme. Homozygote carriers of a common variant in the *MTHFR* gene, the c.665C>T (historically known as c.677C>T or C677T), may present reduced serum and red blood cell folate levels and increased plasma homocysteine concentrations. This single nucleotide polymorphism (SNP) determines a p.Ala222Val substitution in the MTHFR enzyme, causing reduced catalytic activity, which, in turn, affects the remethylation of homocysteine to methionine, ultimately determining reduced synthesis of methionine and the accumulation of homocysteine[8]. This SNP is highly prevalent as heterozygotes, but c.665TT homozygous can easily reach 25% in certain populations (Iran, China, Turkey, Spain, southern Italy)[8,9].

Among different formulations, 5-methyltetrahydrofolate (5-MTHF) has been evaluated as a better alternative to folic acid supplementation[10-14]. Indeed, folic acid is a strong oxidative molecule and a product of cheap chemical synthesis that is not present in edible natural products. It requires five deoxidation steps in the liver cells to be transformed into its active form, 5-MTHF (vitamin B9)[8]. On the contrary, 5-MTHF is a naturally occurring product and, as a product of chemical synthesis, which does not require biotransformation processes, it is readily absorbed by the digestive tract and distributed to all tissues, including the central nervous system (CNS), with a greater rapidity of action compared with other formulations[8,13,15-17].

Traditionally, 5-MTHF, or its synthetic precursor, folic acid, is widely used as a supplement for improving fertility and for the prevention of embryonal neural tube defects (NTDs)[18-20]. However, in the last decade, starting from the effectiveness of this preventive treatment in the gynecological setting, the use of 5-MTHF was extended to other medical and pathological areas.

Based on this evidence, there might be a rationale for the use of 5-MTHF for purposes other than the protection of the growing embryo, linked to the possible effect of *MTHFR* variants in different pathological conditions. This narrative review provides an overview of the available evidence on the use of 5-MTHF in the obstetric field. It critically discusses the available data regarding the use of 5-MTHF across other different therapeutic areas. To this aim, a literature search for clinical data supporting the use of 5-MTHF in different therapeutic contexts was conducted by using articles published in the English language in the last 10 years (up to October 2023) to provide only updated evidence. The research about the biological rationale for the use of 5-MTHF was conducted without restriction of time. All types of study designs were included to expand the scope of this narrative review. Search terms used on PubMed/MEDLINE included "5-MTHF AND pregnancy," "5-MTHF AND vascular disorders," "5-MTHF AND rheumatology," and "5-MTHF AND neurological disorders". References of the articles were screened by title and abstracts to identify relevant information on these topics. Further full-text screening was done for previously published review articles to identify gaps in the selected literature and elaborate on the importance of this topic comprehensively.

The Role of 5-MTHF During Pregnancy

Folate deficiency in pregnancy is traditionally related to the increased risk of development of neural tube and abdominal wall (omphalocele, gastroschisis) defects and adverse pregnancy outcomes[5,21-23]. Folate deficiency is also responsible for the development of hyperhomocysteinemia. This condition can lead to endothelial dysfunction with an increased risk of vascular disorders, which can result in preeclampsia[6,24,25]. Pregnancy complications related to hyperhomocysteinemia and polymorphisms of homocysteine genes also include recurrent pregnancy loss, preterm delivery, placental abruption, fetal growth restriction, and gestational diabetes[25]. Of note, mean homocysteine levels in pregnancy in large cohort studies[25] ranged around 6.5 μmol/L.

The normal homocysteine cycle maintained by adequate 5-MTHF concentration is a key factor for a number of metabolic pathways, all of which are of critical importance in a rapidly growing and differentiating tissue (the trophoblast, the embryo, and the fetus) that require an efficient and performing maternal cardiovascular system. Figure 2 summarizes these key metabolic pathways in pregnancy.

Moreover, a link between folate deficiency in pregnancy and postpartum depression has been proposed[26-28].

Prenatal care often includes recommendations for 5-MTHF supplementation before conception and during the early weeks of pregnancy to reduce the risk related to folate deficiency and hyperhomocysteinemia, as suggested by reported evidence in Table I.

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Table I. Literature evidence supporting the use of 5-MTHF during pregnancy (the last 10 years were considered).

Aim of the study	Study design	5-MTHF administration modality	Main results and conclusions	Ref.
To correlate 5-MTHF concentrations during pregnancy with birth outcomes	An observational study including unselected consecutively admitted healthy mothers and their term and preterm newborns. Umbilical cord plasma and maternal serum were analyzed	NA	The concentration of 5-MTHF in umbilical cord blood is significantly associated with gestational age at birth, newborn weight, length and head circumference; the concentration of 5-MTHF in umbilical cord blood erythrocytes was significantly lower in preterm than in term newborns	Weber et al[29], 2013
To assess the risk of recurrence of preeclampsia in relation to 5-MTHF supplementation	Retrospective cohort study	Acetylsalicylic acid (100 mg) and 5-MTHF (15 mg per day for the duration of pregnancy, starting in the first trimester) or only acetylsalicylic acid	A significant reduction in the incidence of preeclampsia was observed in the group treated with 5-MTHF	Saccone et al[30], 2016
To evaluate the impact of 5-MTHF on Assisted Reproductive Technology (ART) outcomes	Retrospective study	Daily supplementation with vitamin B complex (5-MTHF, vitamin B12) or with only folic acid	Women supplemented with 5-MTHF and vitamin B12 have a higher chance of conceiving after ART, clinical pregnancy and live birth in comparison to those supplemented with only folic acid	Cirillo et al[31], 2021
To assess the effect of folate supplementation on blood folate status during pregnancy	Systematic revision and meta-analysis of randomized clinical trials	0.6 mg/die folic acid or 5-MTHF for 16 weeks	Supplementation with 5-MTHF proved to be effective in reducing unmetabolized folic acid in maternal plasma	Cochrane et al[32], 2023
To monitor the blood level of folate in women with an uneventful pregnancy	Longitudinal observational study with two sampling points during the pregnancy, one sample at delivery and one from the umbilical cord	400 µg of folate daily during pregnancy	5-MTHF was found to be the main form of folate in the umbilical cord blood, supporting the conclusion that active placental transport only affects 5-MTHF and no other forms of folate	Kubo et al[33], 2020

5-methyltetrahydrofolate (5-MTHF).

Elevated 5-MTHF concentrations during pregnancy have been associated with improved birth outcomes. One important research[29] showed that the concentration of 5-MTHF in umbilical cord blood is significantly associated with gestational age at birth, newborn weight, and its length and head circumference. The study[29] also showed that the concentration of 5-MTHF in umbilical cord blood erythrocytes was significantly lower in preterm than in term newborns.

In a retrospective cohort study[30], the risk of recurrence of preeclampsia in relation to 5-MTHF supplementation was assessed in a population of women who suffered from preeclampsia in a previous pregnancy. Recruited patients were retrospectively divided into two groups based on the intake of acetylsalicylic acid (100 mg) and 5-MTHF (15 mg per day for the duration of pregnancy, starting in the first trimester) or only acetylsalicylic acid. A significant reduction in the incidence of preeclampsia was observed in the group treated with 5-MTHF (21.7% *vs.* 39.7%; OR 0.57, 95% CI 0.25-0.69). Gestational age at delivery and newborn weight were increased according to the lower incidence and severity of preeclampsia in the treated group[30].

The impact of 5-MTHF was underlined[31] in a group of 269 women of south European Caucasian macro-ethnicity referred for assisted reproductive technology (ART). These women were daily supplemented with vitamin B complex (5-methyltetrahydrofolate, vitamin B12) or with only folic acid. Women supplemented with 5-MTHF and vitamin B12 had a higher chance of conceiv-

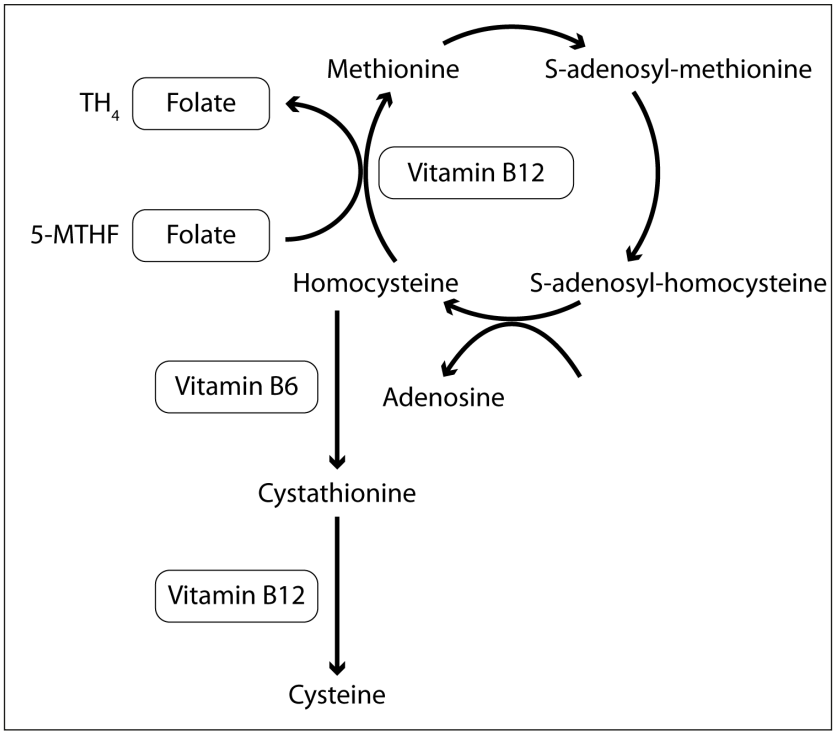

Figure 1. Homocysteine metabolism in the body. Folate and methionine cycle: folate is essential for the conversion of homocysteine to methionine. In the methionine cycle, 5-MTHF donates a methyl group to homocysteine, converting it to methionine. This reaction is catalyzed by methionine synthase. Vitamin B12 is a cofactor for methionine synthase, and its deficiency can lead to impaired methionine synthesis, resulting in elevated homocysteine levels. Transmethylation pathway: homocysteine is formed by transmethylation of methionine *via* S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) by methionine adenosyltransferase (MAT). Remethylation pathway: homocysteine can be remethylated to form methionine. This process requires a methyl group donor, which is often provided by either 5-MTHF or betaine. Vitamin B12 is essential for this reaction, as it helps convert homocysteine to methionine in conjunction with methyl groups from the aforementioned donors. Trans-sulfuration pathway: homocysteine can also be converted into cysteine through the trans-sulfuration pathway. This pathway involves several steps and requires vitamin B6 (pyridoxine).

ing after ART, clinical pregnancy and live birth (p=0.01; p=0.02, respectively) in comparison to those supplemented with only folic acid.

In a recent randomized trial[32] including pregnant women in Canada, supplementation with 5-MTHF, while it appeared as effective as folic acid in maintaining maternal folate status, proved to be effective in reducing unmetabolized folic acid in maternal plasma. However, the biological relevance of this observation is still unclear.

Lastly, In a Japanese longitudinal study[33], women with uneventful pregnancies were monitored

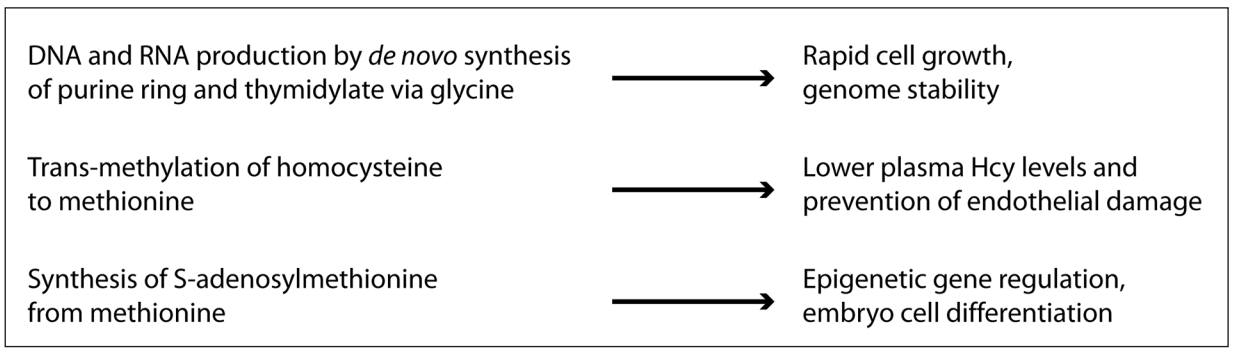

Figure 2. Key metabolic pathways involving homocysteine in pregnancy.

for the blood level of folate, with two sampling points during the pregnancy, one sample at delivery and one from the umbilical cord. All women took 400 μg of folate daily during pregnancy. The results showed a progressive reduction in blood levels of 5-MTHF in maternal blood during pregnancy and a parallel increase in homocysteine levels, with an inverse correlation between the levels of the two molecules[33]. 5-MTHF was found[31] to be the main form of folate in the umbilical cord blood, supporting the conclusion that active placental transport only affects 5-MTHF and no other forms of folate. This interesting relationship of 5-MTHF between maternal plasma and fetal plasma at birth, where the latter has a higher concentration, suggests an active uptake of 5-MTHF, but not of folic acid, by the placenta, as observed by Obeid et al[34].

5-MTHF for the Management of Cardiovascular Disorders

Hyperhomocysteinemia has been linked to an increased risk of endothelial damage, particularly in the presence of homocysteine concentrations >15 μmol/L. Indeed, hyperhomocysteinemia causes the dimerization of the molecule, resulting in oxidation of the endothelium, hypercoagulation, vasoconstriction, and platelet aggregation[35]. Nitric oxide, which generally neutralizes homocysteine in conditions of excess of the latter, is no longer able to perform its function, accelerating cardiovascular events (Figure 3)[36]. In this context, 5-MTHF can stimulate its production and thus favor the elimination of homocysteine excess, favoring its remethylation to methionine (Figure 1)[37]. Thus, the correction of hyperhomocysteinemia through the administration of 5-MTHF can be suggested to reduce the risk of endothelial damage and cardiovascular events[38]. In particular, it has been suggested[39-41] that the impact of folate administration in patients with cardiovascular disease does not end with the reduction of homocysteine but involves wider effects that lead to an improvement in endothelial function and oxidative stress, thanks to a direct antioxidant property of 5-MTHF and an interaction with the endothelial nitric oxide synthase (eNOS) enzyme. In this regard, the hy-

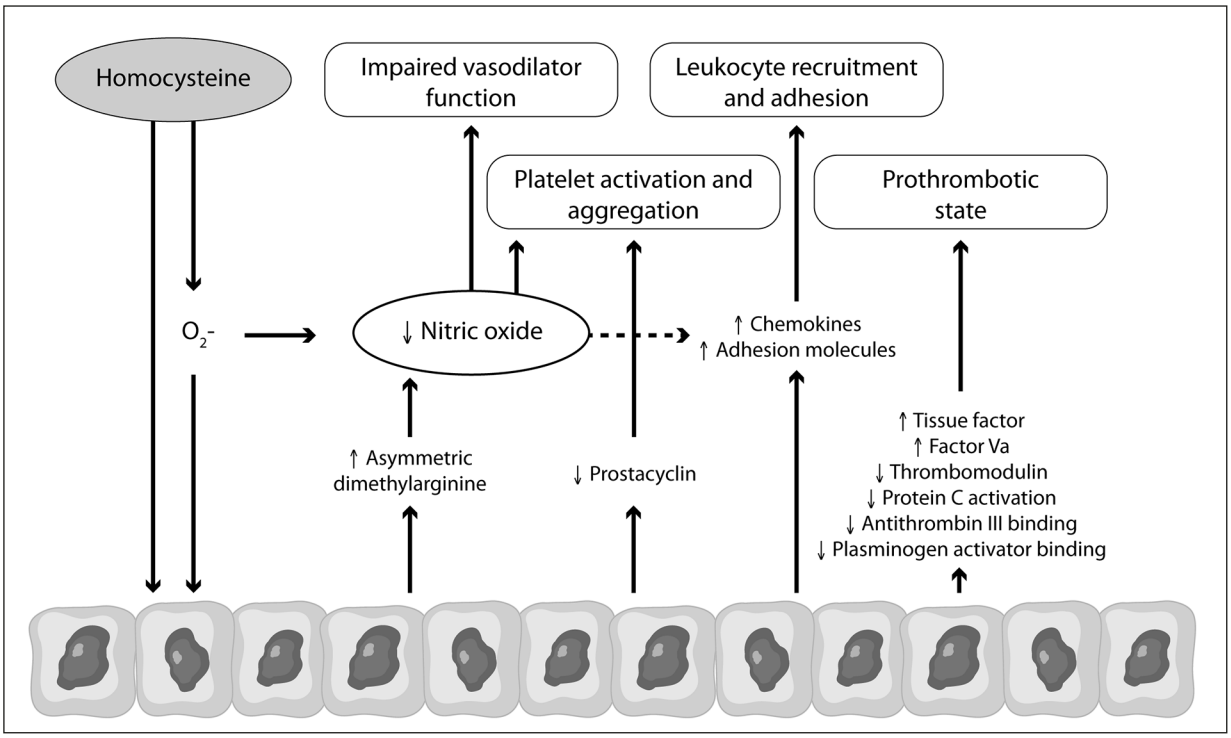

Figure 3. Hyperhomocysteinemia and endothelial dysfunction. Exposure of endothelial cells to homocysteine leads to reduced bioavailability of nitric oxide, resulting in impaired endothelium-dependent vasodilator function. Decreased bioavailable nitric oxide, together with a decrease in the endothelial synthesis of prostacyclin, promotes platelet activation and aggregation. Elevated homocysteine levels induce the expression of several chemokines and adhesion molecules by endothelial cells, which leads to increased recruitment and adhesion of circulating inflammatory cells. Homocysteine may further promote thrombosis by activating endothelium-dependent prothrombotic mechanisms.

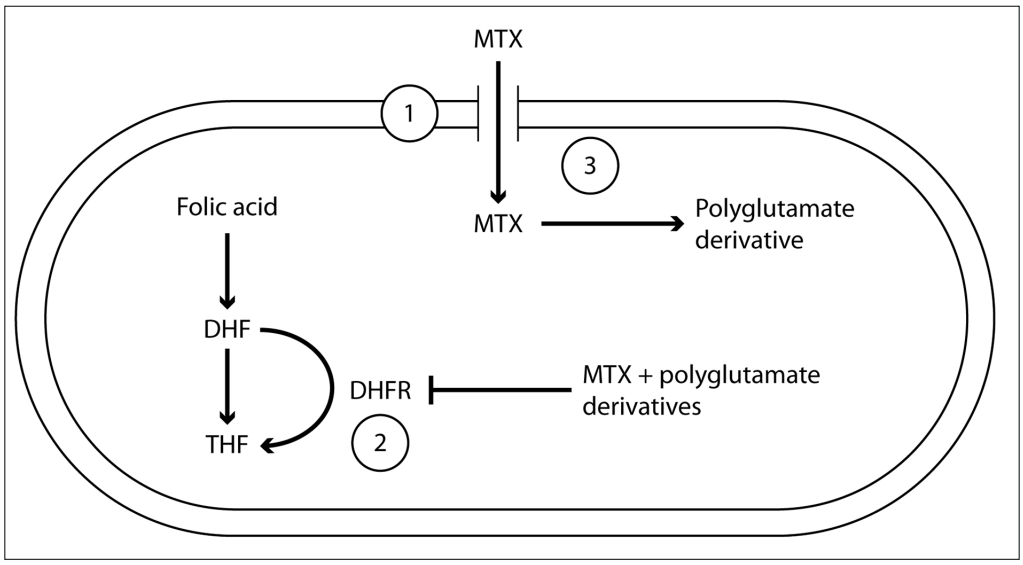

Figure 4. Methotrexate (MTX) blocks the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by inhibiting the dihydrofolate reductase (DHFR) enzyme.

pothesis that a cyclical supplementation (1 month of therapy followed by 2 months of withdrawal) with 5-MTHF could reduce total plasma levels of fasting homocysteine was assessed in a prospective study[42]. Therefore, patients with mild or moderate hyperhomocysteinemia were treated with a cyclical supplementation of 5-MTHF and, after one 5-MTHF treatment cycle, both a reduction of 54.4% of total homocysteine levels ($p<0.001$ *vs.* baseline) and a parameter normalization in over 50% of patients (51.3%) were observed. The best outcomes were recorded after eight cycles of supplementation (2 years).

A population-based study[43] of 608 elderly people explored to what extent 5-MTHF and other folates are associated with endothelium-dependent, flow-mediated vasodilation and whether these associations were stronger in individuals with diabetes or other cardiovascular risk factors. 5-MTHF was associated with endothelial and smooth muscle cell function; the effect of homocysteine on endothelial function was relatively small compared with 5-MTHF. No interactions were observed for diabetes or cardiovascular risk factors.

The acute effects of intravenous 5-MTHF on endothelial function and asymmetric dimethylarginine (ADMA) were assessed in patients with chronic heart failure (CHF) in a randomized, parallel-group, placebo-control study[44]. Even if 5-MTHF did not affect endothelial function in this study, it significantly reduced serum ADMA concentrations both in CHF patients and controls, suggesting a direct effect of 5-MTHF on ADMA metabolism[44].

It is worth mentioning that meta-analysis[45] results of >50,000 patients from high-quality randomized clinical trials document that lowering homocysteine levels by folic acid, vitamin B12 and vitamin B6 little affected the cardiovascular risk. These data appear to be in contrast with the literature evidence[46] deriving from clinical trials and supporting the role of homocysteine-lowering treatments in reducing cardiovascular risk. However, several explanations can be provided, such as a shorter duration than appropriate for modulating an established progressive vascular disease, or several confounders might have affected the results of the observational studies[47]. Moreover, individuals with pre-existing atherosclerosis have higher homocysteine levels than those without; some drugs may increase homocysteine levels, and the effect of some drugs on homocysteine is reversible following treatment withdrawal. Lastly, most randomized trials[47] have been performed in populations with high folate concentrations. Thus, the controversy over homocysteine and cardiovascular risk called attention and denotes that this correlation is still very controversial[47,48].

The Role of 5-MTHF in the Rheumatological Field

Folate deficiency is common among patients with autoimmune diseases, including rheumatoid arthritis (RA) and other rheumatological condi-

tions. Folate supplements are generally recommended to patients with RA to counteract reduced folate levels. First-line therapy with methotrexate or, in the second line, with monoclonal antibodies is significantly influenced by common *MTHFR* gene polymorphisms[49,50].

The use of 5-MTHF in rheumatology mainly refers to administration associated with methotrexate therapy. Methotrexate (4-amino-10-methylfolic acid hydrate, MTX) has been used in the treatment of RA since the 1980s and, up to date, is often the first-line medication for RA treatment[51]. MTX leads to competitive inhibition of the enzyme dihydrofolate reductase (DHFR) and, therefore, results in systemic depletion of active forms of folates and consequent impairment of crucial molecular processes, including DNA synthesis and methylation.

In approximately one-third of RA patients, the administration of MTX causes a wide range of side effects, including stomach, intestine, hematologic system, liver, lung, and CNS[52]. Moreover, inadvertent exposure to MTX treatment during early pregnancy has been associated with spontaneous abortion and birth defects, even at low doses[53].

The rescue from MTX toxicity is generally performed by supplementation with folic acid or folinic acid (5-FTHF), with uncertain results. However, overall, a reduced incidence of hepatotoxicity and gastrointestinal side effects has been observed[54,55]. As MTX has a much higher affinity to DHFR compared to folic acid[56,57], according to pharmacokinetics, folic acid would not be effectively turned into active folate in the presence of MTX, and this would preclude its rescuing effect to MTX-induced adverse effects (Figure 4). For this reason, in clinical practice, folic acid is administered 1-3 days after MTX application, although no clinical study has shown that co-administration of FA with MTX reduced the treatment efficacy[58].

An *in vitro* study[59] on the rescuing effects of folic acid compared to active folates on MTX-treated human trophoblasts has provided evidence of the inefficacy of folic acid, while 5-MTHF and 5-FTHF appeared effective in protecting trophoblast cells from MTX-induced toxicity.

Overall, this evidence suggests that to avoid adverse effects resulting from the administration of MTX, it is preferable to use natural folates, such as 5-MTHF or 5-FTHF, which are already active and do not require the action of the DHFR enzyme. Folic acid, instead, to become active, needs to be reduced by the DHFR enzyme, whose activity is inhibited by MTX.

5-MTHF and Neurological Disorders

It has been proposed[60-63] that there is a correlation between folate levels and the onset of depression. Moreover, folate deficiency has been linked[64] to an elevated risk of more severe depressive symptoms, longer episodes of depression, and an increased likelihood of depressive symptom relapse.

An RCT[65] showed that monotherapy with 5-MTHF showed comparable effectiveness to a standard antidepressant in the treatment of mild-to-moderate depression. Response rates were 42% for 5-MTHF and 35% for amitriptyline[65]. No side effects were reported with 5-MTHF, while three patients had to withdraw from the study due to side effects from amitriptyline[65].

A real-world study[66] assessed changes in severity and medication satisfaction in patients with a major depressive disorder prescribed with 5-MTFH (7.5 or 15 mg per day). Patients reported a significant reduction in depression severity scores, with 67.9% of participants responding to treatment and 45.7% achieving remission after an average of 95 days of therapy[66]. Patients also experienced improvements in work/home/social life and expressed higher satisfaction with 5-MTHF compared to their prior medication[65].

Among patients with major depressive disorders, at least 15% remain refractory to treatment. Two multicenter, sequential, parallel comparison design trials[67] investigated the effect of 5-MTHF augmentation in the treatment of major depressive disorder in patients who had a partial response or no response to selective serotonin reuptake inhibitors (SSRIs). In these resistant patients, the combination of SSRIs with 15 mg of 5-MTHF led to an increased response to SSRIs[67]. In a subsequent post-hoc analysis, it was observed that the patients who responded best to the combined treatment were those with a predisposition to folate deficiency[68]. The correlation between treatment-refractory depression and low cerebrospinal fluid levels of 5-MTHF was also observed in a case-control study in a small group of adolescent and young adult patients with well-characterized histories of treatment-refractory depression[69]. Based on these data, future research on folate supplementation in depression is warranted, and clinicians may wish to consider folate supplementation for patients with depression[70].

A narrative review[71] also proposed that psycho-organic syndrome (defined as cognitive dysfunction) in the elderly may be caused by folate deficiency. This hypothesis is further supported

by the results of a cross-sectional observational study[72] based on the data from the National Health and Nutrition Examination Survey, including 2,524 adults aged 60 years and older. In this study, an inverse correlation was found between the dietary intake of folic acid and cognitive impairment[72]. Similarly, a correlation has been proposed between increased plasma homocysteine levels and the development of dementia and Alzheimer's disease[73,74], as well as between the *MTHFR* c.665C>T polymorphism and Alzheimer's disease[75,76].

5-MTHF and Anemia

Literature evidence reports[77] that anemia is caused by folate, iron or vitamin B12 deficiency in about one-third of the elderly population. In particular, macrocytic or megaloblastic anemia [Mean Corpuscular Volume (MCV) ≥100 fL] is determined by any factor that can alter DNA synthesis, such as vitamin B12 and/or folate deficiency[13]. Documented anemia diagnosis, MCV >100 fL and high levels of homocysteine are significant indicators for the use of folates in the elderly[78].

Folate deficiency or vitamin B12 deficiency-related anemia is also reported[79] during pregnancy, further supporting the use of folate supplementation in this setting. Of note, pregnant women at risk of vitamin B12 deficiency should always be prescribed folate supplementation along with vitamin B12. Indeed, megaloblastic anemia can be hidden by folic acid supplementation in case of vitamin B12 deficiency because a large part of folate remains trapped in the 5-MTHF form and does not participate in DNA synthesis, with consequent insufficient production of DNA and the onset of anemia. Otherwise, 5-MTHF presents a lower probability of concealing hematological symptoms due to vitamin B12 deficiency; thus, its administration should be preferred in this condition[13].

Conclusions

Folate represents a methyl donor in numerous metabolic reactions, including the biosynthesis of glycine from serine, the conversion of homocysteine into methionine, and the DNA and RNA precursors biosynthesis. Therefore, its appropriate intake by nutritional sources and/or prudent nutraceutical supplementation is fundamental for rapid cellular growth and for key epigenetic modulation phenomena during embryonic and fetal life[13]. Moreover, folate deficiency has been related to hyperhomocysteinemia, which can lead to cardiovascular complications in pregnancy associated with endothelial dysfunction[25].

The use of 5-MTHF in pregnancy presents some advantages if compared with folic acid, such as immediate action, the non-necessity of metabolic activation, and the immediate bioavailability of the mother and fetus. In addition to these positive aspects, the eventual *MTHFR* gene variants that vary among populations have no impact on 5-MTHF. Indeed, this condition dampens the liver's de-oxidative steps to transform this acid molecule into a body-friendly vitamin for the growing tissues in pregnancy[13]. This is of major importance for the south European Caucasian population and other macro-ethnicities where the prevalence of functional variants in the gene coding for the MTHFR enzyme is generally higher than in northern European populations and can be as frequent as one in four women in Iran, China, Turkey, Spain, and southern Italy[13]. This is, therefore, an area to be studied further to optimize indications regarding folate supplementation.

Moreover, a link between folate deficiency in pregnancy and postpartum depression has been proposed, as well as between folate levels and the onset of depression and neurological conditions, highlighting the importance of folate supplementation also in these settings[26-28,60,61].

On the other hand, the role of 5-MTHF in the context of the management of cardiovascular risk is still debated due to the multiple confounding factors that characterize this patient setting[48]. Thus, pregnancy can be considered a privileged niche for studying the effects of 5-MTHF on the cardiovascular system as well. Indeed, during pregnancy, the concomitant use of additional drugs is very rare, as is the presence of important diseases. This allows for a better understanding of the protective mechanisms of 5-MTHF to reduce the risk of endothelial damage and vascular disorders, considering that both high vascular impedance as it occurs in severe early disorders of pregnancy or high-volume dominant hypertensive disorders of late pregnancy are associated with endothelial damage and vasocontraction of resistance arteries. In this clinical context, a stable, active homocysteine cycle promoted by appropriate levels of 5-MTHF was shown[38] to prevent homocysteine-mediated endothelial damage.

In conclusion, evidence from the literature supports the additional role of 5-MTHF as a pleiotropic drug with a transversal effect in therapeutic contexts other than the obstetric field. With regard to the prevention of cardiovascular disorders,

available evidence is not conclusive, limiting the role of 5-MTHF in this therapeutic context. In the future, it is desirable that RCTs involving pregnant patients treated with acetylsalicylic acid in addition to 5-MTHF will be conducted. This setting is, in fact, the ideal model to elucidate the protective mechanisms of 5-MTHF in the absence of confounding factors.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Availability of Data and Materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent
Not required due to the nature of the study.

Authors' Contributions
All authors contributed to the definition and contextualization of the paper's contents, critically edited the manuscript, and approved its final version for submission.

ORCID ID
Michele Rubini: 0000-0003-1448-9516
Enrico Ferrazzi: 0000-0001-5243-0537
Giovanni Di Minno: 0000-0003-4235-7166

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