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Abstract

The importance of folate in normal fetal development and wellbeing has been recognized only during the past three decades and knowledge concerned is still far from complete. In man, folate acts as a substrate in the transfer of one-carbon moieties and thereby, plays an essential role in the synthesis of several amino acids such as methionine and nucleic acids. Consequently, folate requirements are related to the amount of tissue growth. Epidemiological, clinical and teratological research showed that this B-vitamin is particularly involved in the prevention and pathogenesis of neural tube defects. Therefore, in this review the metabolism of folate has been outlined. Furthermore, the characteristics of the various genetically determined folate 'deficiencies' as well as a possible biochemical explanation of the relationship between folate and neural tube defects are being discussed. Finally, the new recommendations launched in November 1993 by the Dutch Health Council as well as the Food and Nutrition Council with regard to folate supplementation in the prevention of neural tube defects are presented.

Keywords: Folate; Neural tube defects; Prevention; Biochemistry; Recommendations

1. Introduction

Maternal folate status is important in the prevention and pathogenesis of neural tube defects (NTDs) [1]. It is, however, unclear which precise mechanisms are involved. Pregnant women are prone to develop a folate deficiency, probably due to their increased folate demands for the growing fetal and maternal tissues. Other factors provoking folate deficiency include a poor diet, the physiological haemodilution of pregnancy, increased plasma clearance and hormonal influences [2]. Our hypothesis is that a subset of NTDs might be due to a disbalance in the maternal and/or embryonic homocysteine metabolism, partly genetically determined, in which folate plays a particularly important role [3,4].

Therefore, in the first part of this review, attention is given to the biochemistry and daily requirements of folate, the diagnosis of folate deficiency and the genetic disorders in folate metabolism. In the second part, a possible underlying mechanism will be described which may explain (in part) the beneficial effect of folate supplementation in the prevention of NTDs. Finally, new recommendations regarding folate supplementation are presented.

2. Folate

2.1. Structure

In 1930, folic acid was first recognized as a factor present in the yeast preparation marmite, which was able to cure a megaloblastic anaemia occurring among Hindu women in India, particularly during pregnancy [5]. The term 'folic acid' (Latin folium, 'leaf') for pteroyl-glutamic acid was introduced in 1941, and the substance was successfully synthesized in the form of pteroylmonoglutamate a couple of years later. Folic acid and folate are the preferred synonyms for pteroylglutamic acid (Fig. 1).

Folic acid comprises a pteridine, p-aminobenzoic acid and glutamic acid. The natural-occurring folates consist of a large group of derivatives derived from the reduction and addition to the basic structure. Folates are available in part as small molecules with one to three
sidechains, i.e. mono and oligoglutamates, and in part as larger molecules, i.e. polyglutamates. Folates are present in all body tissues. Folate is stored to the greatest extent in the polyglutamate form in liver, pancreas, kidneys and brain. Mono and oligoglutamates (free folate) and polyglutamates together represent 'total folate'.

2.2. Biochemistry of folate

Transport mechanism. The absorption process of dietary folate (mainly as 5-methylpteroylpolyglutamates) is divided in two steps (Fig. 2). In the first step, polyglutamates are converted to monoglutamates by a conjugase present in the jejunum. Only monoglutamates or, at most oligoglutamate derivatives are taken up by the intestinal cell. The second step includes absorption by active as well as passive transport of monoglutamates into the intestinal cell. In the intestinal cell, the absorbed monoglutamates are fully reduced to tetrahydrofolate (THF) by dihydrofolate reductase. Depending upon folate supply they are transported directly into the portal circulation, converted to 5-methyltetrahydrofolate (5-methyl-THF), and transported to the portal circulation, or converted directly into THF-polyglutamate stores.

The stored THF-polyglutamates can be degraded to THF-monoglutamates by intracellular conjugase and released into the circulation. 5-methyl-THF-monoglutamate is the predominant form of folate in serum and in many tissues. In the liver, polyglutamate derivatives of 5-methyl-THF are the major stored folates.

About two-thirds of 5-methyl-THF in serum is loosely bound to a non-specific low-affinity folate binder such as albumin. A second folate binder has also been described. Although this binder has a high affinity for folate, its capacity is low. This protein may represent a storage form [6]. Blood of the umbilical cord contains both low and high-affinity folate binders [7]. Folate binding proteins are involved in the transfer of folate against a concentration gradient from the mother to the fetus, which suggests active placental transport by which the fetus is able to accumulate folate. Strelling [8] determined higher folate levels in fetal blood as compared with maternal blood. The placenta seems able to supply the fetus with adequate folate. However, little is known
about folate provision for the growth of the conceptus before the placenta has developed.

Metabolism. The prime function of folate is to provide one-carbon moieties for the synthesis of three of the four bases of DNA, i.e. guanine, adenine and thymine, as well as for synthesis of other compounds. These one-carbon moieties are formate required for the synthesis of the purines 5-formamidoimidazole-4-carboxamide ribonucleotide (FAICAR) and formylglycinamide ribonucleotide (FGAR), methylene for deoxymethylidine synthesis (dTMP), and the methyl group for methionine synthesis [9,10]. The one-carbon moiety is derived from the conversion of serine into glycine, formiminoglutamic acid (FiGlu) into glutamic acid (Glu), or the cleavage of glycine. The folate metabolism in man is depicted in Fig. 3.

The vitamin B12-dependent enzyme 5-methyl-THF-homocysteine methyl-transf erase or methionine synthase (EC 2.1.1.13) catalyses the conversion of 5-methyl-THF and homocysteine into THF and methionine. Methionine reacts with adenosine to form S-adenosylmethionine. S-Adenosylmethionine is the most important methyl donor and regulates its own synthesis by inhibition of 5,10-methylenetetrahydrofolate reductase (EC 1.1.1.68) and stimulation of cystathionine synthase (EC 4.2.1.22) [11].

Methionine synthase requires reduced vitamin B12, i.e. methylcobalamin, as a cofactor. Cobalamin accepts its methyl group from 5-methyl-THF [12]. Both folate and vitamin B12 deficiency will decrease methionine formation which may result in decreased levels of S-adenosylmethionine. In methylenobalamin deficiency, folate is trapped as 5-methyl-THF because methionine synthase is the only reaction able to convert 5-methyl-THF into THF (methyl-folate trap) [13]. In such cases, a functional folate deficiency exists, although normal to increased folate levels will be present in blood.

2.3. Requirements

Humans are totally dependent on dietary sources of folate, and major contributors are green leafy vegetables, liver and kidney. Requirements of folate can be met by a variety of folate derivatives, as long as the essential structure of pteroylglutamic acid remains intact [14]. The inactivation of folate occurs after oxidation and exposure to ultraviolet light and heat. Thus, naturally occurring folates are lost in storage and cooking. Most naturally occurring folates are present as polyglutamates. This is in contrast to pharmaceutically available folate, which contains only monoglutamates. In the gut, monoglutamates are absorbed much faster than polyglutamates.

The demand of folate is increased in infancy, adolescence and pregnancy. The recommended dietary intakes for folate are presented in Table 1.

2.4. Diagnosis of folate deficiency

Folate deficiency leads to clinical symptoms such as diarrhoea, cheilosis and glossitis [15]. The haematological manifestations of folate deficiency are macrocytosis, low reticulocyte, leucocyte and platelet count, anisocytosis, poikilocytosis, macroovalocytes and hypersegmentation of the nucleus of the neutrophils. Pathological circumstances, resulting in an increased folate requirement are: malabsorption due to sprue, restoration of blood cells after loss or haemolysis, alcohol abuse, treatment with folate antagonists (methotrexate) and antiepileptic drugs (phenytoin, phenobarbital, primidone).

Different methods are available for the measurement of folates. However, they are difficult to compare. Serum folate concentrations vary rather widely with respect to nutrition, sex, and the use of drugs. Folate is incorporated into red cells during erythropoiesis. Therefore, red cell polyglutamates reflect a more accurate and less variable index of folate status than serum folate levels [16]. Morphologic blood examination showing hypersegmentation of the polymorphonuclear leucocyte nucleus, used to be the best way to diagnose a folate deficiency [14]. Function tests to determine folate status include the deoxyuridine suppression test and the FiGlu function test. Both methods are used to study folate levels indirectly. The deoxyuridine suppression test is based on the observation that, in case of folate deficiency, the uptake of tritiated thymidine by bone marrow cells is suppressed because of decreased folate-dependent conversion of DUMP into DTMP. The FiGlu function test is based on the increased urinary excretion of FiGlu after histidine loading, because folate deficiency leads to a decreased conversion of FiGlu into glutamic acid (Glu) (Fig. 3) [17]. In addition, folate deficiency will result in a mild to moderate hyperhomocysteinaemia. Therefore, the determination of the concentration of total homocysteine in blood seems to be a valid indirect method, as well to diagnose a metabolic folate deficiency [18,19]. Microbiologic assays of folate depend upon the metabolic requirements of folate in certain microorganisms. Lactobacillus casei is generally accepted as the standard assay organism, because it responds to the greatest variety of different folate
Table 1
Recommended dietary intake of folic acid (RDI) (modified from Health Council/Food and Nutrition Council [62])

<table>
<thead>
<tr>
<th></th>
<th>USA(RDA) 1990 µg/day</th>
<th>UK 1991 µg/day</th>
<th>FAO/WHO 1992 µg/day</th>
<th>Netherlands 1992 µg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male age &gt; 19 years</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200–300</td>
</tr>
<tr>
<td>Female age &gt; 19 years</td>
<td>180</td>
<td>200</td>
<td>170</td>
<td>200–300</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>400</td>
<td>300</td>
<td>&gt;350</td>
<td>400–600</td>
</tr>
<tr>
<td>Lactation</td>
<td>280</td>
<td>250</td>
<td>270</td>
<td>400–600</td>
</tr>
</tbody>
</table>

*aFAO: Food and Agriculture Organization
bWHO: World Health Organization

derivatives [20]. A more sophisticated technique is the radioassay based on competitive binding of labelled and unlabelled folates to a limited number of binding sites on a folate-binding protein. The results are comparable to microbiologic assays. High performance liquid chromatography (HPLC) separation of folates is favourable when specific folate derivatives need to be measured [20].

2.5. Genetic disorders of folate metabolism

Up to now, five inherited disorders of folate metabolism have been described. These are: (a) 5,10-methylene-THF reductase deficiency; (b) functional 5-methyl-THF-homocysteine methyltransferase (methionine synthase) deficiency; (c) glutamate formimino transferase deficiency; (d) hereditary folate malabsorption; and (e) dihydrofolate reductase deficiency. Their clinical and biochemical characteristics are summarized in Table 2.

5,10-methylene-THF reductase deficiency. Mudd et al. [21] first described 5,10-methylene-THF reductase deficiency. About 30 patients have been described (Fig. 3, conversion 4 and Table 2). The severity of the enzyme deficiency and the corresponding level of 5-methyl-THF parallels the clinical and biochemical symptoms. Clinical symptoms are: generalized seizures, delayed psychomotor development and peripheral neuropathy. Severely affected patients often die at infancy or early childhood [22]. Patients with milder symptoms tend to survive into adult life [21]. Their typical laboratory fea-

Table 2
Inherited defects in the metabolism of folate (modified from Rosenblatt [9]; Surtees and Leonard [57])

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MTHFR</th>
<th>Methionine synthase deficiency</th>
<th>Glutamic formimino-transferase deficiency</th>
<th>Hereditary folate malabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td>Cbl E,G</td>
<td>Cbl A,B</td>
<td>Cbl C,D,F</td>
</tr>
<tr>
<td>Megaloblastic anaemia</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>+</td>
<td>+</td>
<td>-a</td>
<td>-</td>
</tr>
<tr>
<td>Speech abnormality</td>
<td>-</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Apnoea</td>
<td>+</td>
<td>-</td>
<td>-a</td>
<td>-</td>
</tr>
<tr>
<td>Osteo abnormality</td>
<td>+</td>
<td>-a</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Biochemical data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Homocysteinaemia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hypomethioninaemia</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Low serum folate</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Low red cell folate</td>
<td>+a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low serum B12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Increased MMA in urine</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Increased FIGLU in urine</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme deficiency detectable in</td>
<td>F,L,e,LI,LY</td>
<td>F</td>
<td></td>
<td>K, Li</td>
</tr>
<tr>
<td>Treatment</td>
<td>Betaine folic acid</td>
<td>Methionine</td>
<td>Hydroxocobalamin, Folinic acid, Betaine Folic acid</td>
<td>Folic acid</td>
</tr>
</tbody>
</table>

+a/- Clinical symptom or biochemical abnormality present/absent.

* Exceptions described in some cases.

Cbl, cobalamin; FIGLU, formiminoglutamic acid; MMA, methylmalonic acid; MTHFR, 5,10-methylene-THF reductase deficiency; F, fibroblasts; K, kidney Le, leucocytes; Li, liver; Ly, lymphocytes.
tures are given in Table 2. The enzyme defect is inherited in an autosomal recessive trait and can be assessed in liver, fibroblasts, lymphocytes and leukocytes. Kang et al. [23] recently discovered a 'new variant' of 5,10-methylene-THF reductase deficiency, which is characterized by the absence of neurological abnormalities, an enzyme activity of about 50% of the normal value, and distinctive thermolability of the enzyme in lymphocytes. Reportedly, the incidence of thermolabile 5,10-methylene-THF reductase was 5% in controls and 17% in patients with coronary artery disease. This suggests a high mutated gene frequency among the general population. The mild hyperhomocysteinemia assessed in some subjects with thermolabile 5,10-methylene-THF reductase was effectively treated by oral folic acid therapy. Because severe 5,10-methylene-THF reductase deficiency is resistant to folate treatment, betaine is often prescribed.

**Functional methionine synthase deficiency.** Vitamin B$_{12}$ as methylcobalamin is an essential cofactor for methionine synthase, and as adenosylcobalamin for the enzyme methylmalonyl-CoA mutase (EC 5.4.99.2). Therefore, an enzymatic disorder in the synthesis of these cobalamins will lead to a functional defect in methionine synthase or methylmalonyl-CoA mutase activity (Fig. 3, conversion 5 and Table 2). The reported enzymatic defects may affect either methylcobalamin synthesis (cobalamin E and G mutation), the synthesis of adenosylcobalamin (cobalamin A and B mutation), or both (cobalamin C, D and F mutation). The biochemical characteristics are shown in Table 2.

**Glutamate formiminotransferase deficiency.** The enzyme glutamate formiminotransferase (EC 2.1.2.5) is involved in the catabolism of histidine (Fig. 3, conversion 9 and Table 2). This pathway may be present only in liver and kidneys, and represents, quantitatively, a minor single-carbon-pool. Glutamate formiminotransferase deficiency syndrome, an autosomal recessively inherited disease, can be divided into two different phenotypes. The severe type is probably caused by a primary block in the cyclodeaminase enzyme (Fig. 3, conversion 10). These patients suffer from mental and physical retardation and cortical atrophy. The milder type of the disease may be due to a defect in the glutamate formiminotransferase enzyme (Fig. 3, conversion 9 and Table 2) without symptoms of mental retardation [9]. Folate, vitamin-B$_{12}$ or serine treatment is hardly influencing biochemical symptoms such as FiGlu excretion and formiminoglutamic acidemia. At present, the determination of enzyme activity is only possible in hepatocytes.

**Defective folate absorption.** An autosomal recessive inherited disorder in the absorption of folate was first described by Lubhy et al. (Figs. 2 and 3, conversion 1 and Table 2) [24]. About a dozen patients have been reported, who fail to absorb oral folic acid monoglutamates or reduced folates (food) [9,14]. These folate malabsorption disorders support the existence of a specific carrier mechanism in the transport of folate across the intestinal wall, and into the cerebrospinal fluid. The common clinical presentation in hereditary folate malabsorption is megaloblastic anaemia, diarrhoea, mouth ulcers, severe physical and mental retardation, and seizures in the first few months of life. Some patients respond to treatment with very large doses of folic acid monoglutamate (up to 40 mg/day). In others, the vitamin was successfully given intramuscularly. Some showed very low folate levels in the cerebrospinal fluid possibly due to a transport defect from the serum into the cerebrospinal fluid. Intramuscular treatment with reduced folates such as 5-formyl-THF (folic acid (5′-Leucovorin)) can produce neurologic improvement [9].

**Dihydrofolate reductase deficiency.** A rare inherited disorder, dihydrofolate reductase deficiency, has been reported in three cases (Fig. 3, conversion 2). Depending on the degree of enzyme deficiency, this disease presents in infancy and may result in stillbirths and spontaneous abortions during reproductive life [25,26]. Clinical symptoms of surviving infants are: failure to thrive, severe megaloblastic anaemia, glossitis, mental retardation and splenic enlargement. The diagnosis is based primarily on low hepatic levels of dihydrofolate reductase activity. Treatment with 5′-Leucovorin bypasses the enzyme block and corrects the defect.

3. **Neural-tube defects**

In 1952, Thiersch first suggested an association between NTDs and a maternal lack of folate, due to the prenatal use of folate antagonists [27]. Hibbard [17] reported a higher prevalence of birth defects in the offspring of folate-deficient mothers (3%), judged by an increased urinary excretion of FiGlu after histidine loading, compared to controls (1.6%). However, the difference was not statistically significant and the malformations did not include NTDs. In 1965, Hibbard and Smithells reported a possible link between folate deficiency and the development of human fetal malformations, in particular central nervous system malformations, including NTDs [28]. Their results suggested the presence of an underlying defect of folate metabolism, such as a disturbance in folate absorption or turnover, possibly precipitated by the increased demands of folate during pregnancy. This has been supported by Yates et al. [29] who demonstrated an association between maternal folate levels and NTD births, which could not be entirely explained by a lower intake of folate. Because the red cell folate levels in particular were lowest in mothers who had three or four previous NTD pregnancies, they hypothesized that a maternal genetic disorder of folate metabolism was responsible for the development of NTDs. The data reported by Kirke et al. [30] are in line with this. They found that maternal plasma folate and vitamin B$_{12}$ lev-
els, determined at the first antenatal visit, were independent risk factors for having a child with a NTD.

The NTD prevalence in the products of spontaneous abortion is 10-fold increased as compared to the prevalence at birth [31]. Regarding this, it is of interest, that already in 1964, a possible relationship was demonstrated by Hibbard [17] between the occurrence of spontaneous abortion and folate deficiency.

As it can be concluded that a maternal shortage of folate during the periconceptional period may play a role in the development of NTDs, maternal folate supplementation in early pregnancy might therefore be a preventive measure against the development of NTDs. Several observational studies have been carried out, most of which showed the protective effect of an increased maternal folate intake by dietary measures or tablets [32–36]. These studies have been extensively reviewed previously [1]. Finally, the intervention studies, summarized and presented in Table 3, definitely proved the beneficial effect of an increased maternal folate intake in the periconceptional period on the first occurrence and recurrence risk of NTDs [37–41].

Women taking antiepileptic drugs for the treatment of epilepsy have an increased risk of having an infant with a congenital malformation, particularly NTD [42]. Meadow [43] speculated that the teratogenicity of antiepileptic drugs may reside in the induction of folate deficiency. This is supported by the relationship between the use of antiepileptic drugs, folate levels and adverse pregnancy outcome, including spontaneous abortions and congenital malformations [44]. Antiepileptic drugs such as phenobarbital, phenytoin and primidone can reduce folate levels by inhibiting intestinal absorption, or by increasing folate turn-over due to induction of the cytochrome P-450 glucuronyl transferase enzymes [45,46]. The maternal use of valproic acid and carbamazepine is considered to increase the risk of spina bifida in particular [47,48]. For both antiepileptic drugs, no strong evidence is available for the presence of abnormally low serum folate levels, but several studies report some form of interference with folate metabolism [49,50]. Recently, in mice, it has been demonstrated that valproic acid interferes with the conversion of THF into 5-formyl-THF, which is possibly due to inhibition of the enzyme glutamate formyltransferase, through which the teratogenicity of valproic acid may be explained by an altered folate metabolism [49].

In case a lack of maternal folate in early pregnancy predisposes to the development of NTDs, these malformations might be prevented by folic acid supplementation. Biale and Lewenthal [51] prescribed folic acid tablets to epileptic women using antiepileptic drugs and found a reduced risk of congenital malformations. However, there have been some criticisms regarding this study, i.e. historical controls were used, no tests of compliance were incorporated, and several women participated more than once.

Although folate is generally accepted as non-toxic in man, high-dose folic acid supplementation may have several disadvantages. A high dose of folic acid (>1.0 mg folic acid per day) may mask the haematologic effects of vitamin B₁₂ deficiency, while its neurologic manifestations progress [52]. Possible risks of a high level of circulating folate are noted by Scott and Weir [13]. Folic acid is a neurotoxin and causes convulsions in laboratory animals. Although the blood/brain barrier normally restricts

### Table 3: Summary of intervention studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Design, Country</th>
<th>Supplement per day</th>
<th>Results</th>
<th>Risk reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smithells et al. [32]</td>
<td>Multicenter, Non-randomized</td>
<td>Multivitamins + 0.36 mg folate</td>
<td>3 NTD/ 545 supplemented</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>UK</td>
<td></td>
<td>24 NTD/ 519 non-supplemented</td>
<td></td>
</tr>
<tr>
<td>Vergel et al. [33]</td>
<td>Non-randomized, Recurrence</td>
<td>5.0 mg folate</td>
<td>0 NTD/ 114 partially supplemented</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Cuba</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laurence et al. [34]</td>
<td>Randomized, Recurrence</td>
<td>4.0 mg folate/placebo</td>
<td>0 NTD/ 81 supplemented</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wales</td>
<td></td>
<td>0 NTD/ 20 partially supplemented</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 NTD/ 114 non-supplemented</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 NTD/ 60 supplemented</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 NTD/ 51 placebo</td>
<td></td>
</tr>
<tr>
<td>MRC Vitamin Study</td>
<td>Multicenter, Randomized</td>
<td>4.0 mg folate/placebo</td>
<td>6 NTD/ 593 supplemented</td>
<td>72%</td>
</tr>
<tr>
<td>Research Group [35]</td>
<td>Recurrence, 7 countries</td>
<td></td>
<td>21 NTD/ 602 placebo</td>
<td></td>
</tr>
<tr>
<td>Czeizel and Dudás [36]</td>
<td>Randomized, Occurrence</td>
<td>0.8 mg folate/placebo</td>
<td>0 NTD/ 2104 supplemented</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Hungary</td>
<td></td>
<td>6 NTD/ 2052 placebo</td>
<td></td>
</tr>
</tbody>
</table>

*a started at least < 1 month before conception and continued through the 1st trimester
access of folic acid in such a way that very high doses are required to produce these effects, similar toxicity occurs at much lower doses if the blood/brain barrier is damaged. In epileptic women, therefore, supplementation of folic acid with doses similar to that used in the MRC trial [40], might result in increased seizure frequency [53]. As the developed brain may be protected from the neurotoxic effects of synthetic folic acid by the blood/brain barrier, it is unknown whether neural tissue during very early embryonic development is protected.

A low dose of folic acid may also be favourable, as it is unclear whether a high-dose folate interferes with the transfer and metabolism of zinc in man, especially in cases with a marginal zinc status [54]. This may be important as dietary zinc deficiency and a relative shortage of maternal zinc has been associated with NTDs in human [55]. Furthermore, the findings of Quinn et al. [56] suggest that in the presence of a zinc deficiency, the administration of high-dose folate increases the teratogenicity of such a deficiency. The enzyme, γ-glutamyl hydrolase, converts polyglutamates to monoglutamates, which is an important step in the absorption of folate. Its activity is zinc-dependent, which may be another regulatory mechanism in the availability of folate [57].

4. A possible biochemical explanation

As folate is an important substrate in the metabolism of homocysteine, it was suggested in 1991 that a subset of NTD might be due to a maternal disturbance in this metabolism, partly genetically determined, in which folate plays a particularly important role [3].

The metabolism of homocysteine is shown in Fig. 4. Methionine is an essential amino acid, present in eggs, meat and especially liver, which is converted to homocysteine. Homocysteine lies at a metabolic branch point, from which it may be transsulphurated to cystathionine or remethylated to reform methionine. The transsulphuration of homocysteine to form cystathionine is catalyzed by the enzyme, cystathionine synthase, which is present in liver, skin fibroblasts, the central nervous system and lymphocytes. This enzyme requires pyridoxine. The remethylation of homocysteine back to methionine is enabled by two alternative reactions, of which one is most important. It is the remethylation reaction by which the essential conversion takes place of folate as 5-methyltetrahydrofolate to tetrahydrofolate and, whereby vitamin B₁₂ acts as methylcobalamin acts as a cofactor. Apart from the enzymes concerned in folate conversion such as 5,10-methylenetetrahydrofolate reductase, another important catalyzer in this reaction is the enzyme methionine synthase.

In case of a deficiency of folate as 5-methyl-THF, the remethylation of homocysteine is blocked, resulting in elevated homocysteine levels, which might be embryotoxic [58]. Such deficiency also leads to decreased methionine concentrations. Because methionine is an important donor of methyl-groups, which are essential in the methylation of DNA and tRNA, decreased methionine levels, next to elevated homocysteine concentrations, might possibly disturb the development of the neural tube [59,60].

The circumstances causing a derangement in homocysteine metabolism, leading to increased blood levels of homocysteine and other metabolites, which might interfere with the closure of the neural tube are: deficiencies of cystathionine synthase, 5,10-methylene-THF reductase or 5-methyl-THF-homocysteine-methyl transferase, which is another name for the enzyme, methionine synthase; a lack of 5-methyl-THF, methyl-cobalamin, or pyridoxal phosphate is supposed to result in increased homocysteine levels as high as found in carriers for the metabolic disease, homocystinuria. Therefore, apart from a direct influence of mild to severe vitamin deficiencies on maternal, fetal and trophoblastic tissues, the indirect effect might be a moderate maternal hyperhomocysteinaemia. However, the possible effects of such a disorder on the course and outcome of pregnancy are, as yet, unknown.

Our studies, handling a possible relationship between a derangement of maternal homocysteine metabolism and the occurrence of NTD, were performed in non-pregnant women, who had given birth to an infant with a spina bifida or anencephalus, and a control group consisting of healthy non-pregnant women [3,4]. None of the women had been using vitamin preparations or oral contraceptives for a period of at least 3 months before Fig. 4. Homocysteine metabolism in man [4]. (Source: Steegers-Theunissen et al. [4]. Reprinted by kind permission of Metabolism).
the study was performed. The metabolism of homocysteine was studied by performing a standardized oral methionine loading test [4]. In addition, vitamin levels, as well as biochemical parameters, were measured. The study was performed on the 21st day of the ovulatory cycle, because one of our previous studies showed changes in the metabolism of homocysteine during the normal ovulatory cycle [61]. The fasting homocysteine concentrations revealed to be lowest during the midluteal period, and therefore screening on a disorder in the metabolism of homocysteine is most sensitive during this period of the cycle. Therefore, all studies were performed at the midluteal phase of the cycle.

The results revealed that in 22% of the women, who had given birth to an infant with a NTD, methionine intolerance was present after excluding folate and other vitamin deficiencies, and liver or renal disturbances. Therefore, a maternal disorder in the metabolism of homocysteine strongly suggests being a risk factor for having an infant with anencephalus or spina bifida.

Because folate treatment without the existence of a folate deficiency increases the conversion of homocysteine into methionine, a certain percentage of the prevention of NTDs, through folate supplementation, might be mediated by this beneficial effect upon homocysteine metabolism. Finally, to gain more information about the association of maternal hyperhomocysteinaemia with the occurrence of NTDs, we determined the homocysteine and relevant vitamin concentrations in amniotic fluid and blood of mothers carrying a child with a NTD, and compared those with a control group. Increased homocysteine levels in amniotic fluid were found in women carrying a child with a NTD (Steegers-Theunissen et al, in press), which confirms the data of our previous study.

In future, further investigation is needed to study the possible toxic effects of increased homocysteine and decreased methionine levels on the formation of the neural tube. Apart from that, the remethylation enzymes, especially those involved in folate and vitamin B₁₂ metabolism, have to be investigated in methionine-intolerant women. Then, we might be able to further prevent the occurrence of these congenital malformations in the future.

5. Recommendations

At the beginning of this decade, two intervention studies definitely proved the beneficial effect of maternal folate supplementation to prevent the first occurrence and recurrence risk of a NTD-child [40,41]. Therefore, the Dutch Health Council as well as the Food and Nutrition Council were necessitated to launch new recommendations with regard to the improvement of periconceptional maternal folate status [62]. The Dutch Council distinguishes between women who had had a child with a NTD and therefore have a recurrence risk, and those who did not previously have a NTD-affected child and therefore have an occurrence risk. Women with a NTD family history, those who suffer from diabetes, or using drugs interfering with folate metabolism and those who have a normal population risk are belonging to the latter category.

In order to significantly reduce the recurrence risk of a NTD-affected child, women are being advised to take a daily folate supplements of 4–5 mg from at least 4 weeks before conception until at least 8 weeks after conception. This should be done under medical supervision. It is recommended that women with an occurrence risk should take care of a daily folate intake of 400 µg from at least 4 weeks before conception until at least 8 weeks after conception. The consumption of foods which are rich in folate is encouraged in order to reach this level. Introduction as soon as possible, of foods which have been fortified or restored with folate, is also recommended. The choice of foods to which folate has been enriched and the amount added should be such that there is as little chance as possible of an intake in excess of 1 mg/day. If the introduction of such food-products cannot be achieved within a reasonable period of time, women are advised to take one 400 µg folate tablet daily.

The proposed measures to increase folate intake will have to be evaluated after a certain period, in order to determine their effect on public health.

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