The importance of folic acid in the primary prevention of congenital malformations

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Abstract

Folic acid is a vitamin essential to the normal functioning of all human cells. Tetrahydrofolate (THF) is the biologically active form of folic acid. Its main function is to transfer one-carbon units between compounds participating in many important biochemical processes such as purines and thymidylate biosynthesis, and S-adenosylmethionine synthesis. A deficiency of folates in a daily diet or disturbances in their metabolism may be the cause of congenital malformations. Findings of many epidemiological studies showed that periconceptional use of folic acid, alone or in multivitamin supplements, can significantly decrease the risk of neural tube defects (NTDs), as well as some other congenital malformations, including some defects of the heart, limb, urinary tract, digestive system and orofacial clefts. According to the recommendations regarding the primary prevention of NTDs, to prevent of these congenital malformations in offspring, all women in childbearing age who could become pregnant should take 0.4 mg of folic acid daily. Women who have already had a NTD-affected child should take 4 mg of folic acid daily, and they should have an opportunity to get a genetic counselling before the next pregnancy.

Key words: congenital malformations; folic acid, multivitamins, supplementation, prevention

Introduction

Folic acid (pteroylomonoglutamic acid, pteroyloglutamic acid – PGA) belongs to water-soluble B vitamin. For the first time folic acid was isolated from leaves of spinach in 1941, and synthesized five years later.

Pteroylomonoglutamic acid and its derivatives belong to a group of compounds classified as folates (folacin, B_9 vitamin). In this group folic acid is the most oxidized and stable compound and its molecule contains derivative of pteridine (2-amino-4-hydroxy-6-metylopteridine), p-aminobenzoic acid (PABA), and glutamic acid. Derivatives of folic acid differ between themselves with regards to oxidation pteridine ring, type of one-carbon unit (methyl, methylene, methenyl, formyl, or formimino) and number of glutamic acid residue.

In the human organism folates transform into biologically activated form – tetrahydrofolate (THF). Two-stage reaction of reduction of folic acid, first to dihydrofolate (DHF), and then tetrahydrofolate, catalyzes enzyme dihydrofolate reductase (DHFR). Polyglutamate derivatives of tetrahydrofolate function as coenzymes in many biochemical reactions occuring in an organism. Their main function is to transfer one-carbon units between compounds participating in cellular metabolism, including purines and thymidylate biosynthesis, and amino acid metabolism (e.g. serine, histidine, homocysteine).

B_9 vitamin plays fundamental role in biosynthesis and methylation of nucleic acids (DNA and RNA) essential for cell division and differentiation, and regulation of gen expression. Therefore it is indispensable for normal growth and functioning of all cells of the human organism.

Folate deficiency in everyday diet leads to megaloblastic anaemia; furthermore it can increase the risk of cardiovascular diseases (e.g. ischaemic heart disease, cerebral stroke, thrombotic-embolic disease), degenerative diseases of the nervous system (e.g. Alzheimer's disease, parkinsonism), depressive disorder, and some type of cancers (e.g. neoplasm of the large intestine, breast, cervix, lung, pancreas).

Folates are necessary for normal embryogenesis. As a result of folate deficiency in the organism of a pregnant woman, the occurence of congenital malformations in offspring is possible, as well as reduced birth weight of an infant, hypoplasia of placenta, higher incidence of spontaneous abortions and other complications of pregnancy.

Research demonstrates that folic acid intake by women before and during the first weeks of pregnancy can reduce the risk of some congenital malformations in offspring. Well documented is the impact of folic acid supplementation on decreased risk of neural tube defects (NTDs). Findings of many studies suggested, that maternal use of folic acid during periconceptional period can be effective for prevention of other congenital malformations, including defects of the heart, limb, urinary system and orofacial clefts.

The preventive programs of NTDs are conducted in many countries all over the world, which lead to popularization of diet rich in folates and folic acid supplementation among women in childbearing age, and the food fortification with folic acid.

The National Primary Prevention Program of Neural Tube Defects has been implemented in Poland since 1997 for the purpose of popularization of folic acid in the everyday diet of women in childbearing age and education about the prevention of NTDs among all people.

Dietary sources of folates

Folates naturally occur both in vegetable and animal food, mainly as reduced polyglutamate derivatives of folic acid, tetra-

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hydrofolate or dihydrofolate. Polyglutamate forms, generally methyl and formyl, constitute about 80% of all food folates [53].

The folate-rich dietary source is yeast, liver, pulses, dark-green leafy vegetables, cereal products (whole grains) and chicken eggs (Table 1).

Small amount of folates is synthesized by saprophytic intestinal flora of the human organism.

Modicum of folates is synthesised by the saprophytic intestinal flora of human organism.

Pteroylomonoglutamic acid, which is contained in food in small quantities, can be obtained in synthetic form as folic acid tablets, multivitamins and fortified foods.

Table 1. The content of folates in food products [for 22, 23, 28]

Content of folates (μg/100 g)	Foodstuffs*
≤ 5.0	jams, pork loin, butter, plums, cream, peaches, pollock, herring, turkey fillet, milk, apricots
5.1-50.0	apples, cornflakes, chicken, hake, mackerel, cod, turkey, strawberries, potatoes, ripening cheeses, barley groats, bananas, fresh cheeses, wheat-rye bread, white rice, oranges, buckwheat, carrots, kiwi, tomatoes
50.1-150.0	brown rice, wheat flour type 500, cauliflower, avocado, green peas, chicken eggs, walnuts, string-beans, hazelnuts, lettuce, shantung cabbage,savoy cabbage, rolled oats, pig's liver, broccoli, brussels sprouts, broad beans, asparagus
150.1-250.0	peas (dried), soya bean sprouts, cornflakes fortified, parsley (leaves), white beans (dried), spinach, calf liver
≥ 250.1	wheat bran, soya beans (dried), ox's liver, wheat germs, chicken liver, baker's yeast

* Products are ordered at the given compartment in ascending order of folate content

Folate absorption and transport

Folate absorption in the digestive tract is a complex process. It depends on pH value, activity of enzymes, concentration and normal function of carrier proteins. It takes place mainly in the proximal part of the small intestine. The major pathway of folate absorption is active transport, passive diffusion only in small degree.

In the small intestine polyglutamates (naturally present in food) are hydrolyzed to monoglutamyl forms by a conjugase and methylated to 5-methyltetrahydrofolate (5-methylTHF) or 10-formyltetrahydrofolate (10-formylTHF). In these forms, mainly as 5-methylTHF, they are transported with blood to the liver and other tissues. Synthetic folic acid is reduced in the intestine to tetrahydrofolate (THF), and transported unmodified by the enterohepatic circulation or methylated to 5-methylTHF [53].

The active intracellular folate transport occurs by the reduced folate carrier (RFC) or folate receptors (FRs). Inside cells monoglutamyl folates are resynthesized again to polyglutamate derivatives and accumulated and metabolised in these forms.

Folate assimilation

Folate absorption, usage and storage in the human organism depend on many external and internal factors. There are for example: dietary folate forms, diet composition (type and way of food consumption), food processing, stimulant usage, physiological factors (e.g. state of human nutrition), diseases of the digestive system and some medicines.

The degree of folate assimilation from foods is significantly diverse. It depends on the content of mono- and polyglutamate derivatives and it fluctuates between 30% to 80% [53]. The natural inhibitors of specific intestine enzymes (conjugases), which occur in some vegetable products, such as cabbage, oranges, tomatoes and beans, decrease folate absorption. It is assumed that assimilation of natural folates is half less than synthetic folic acid, which is absorbed almost completely.

The most dietary folates are very sensitive to exposure to high temperature, light (especially UV), concentration of oxygen, cupric and ferric ions and alkalinity. Folates oxidise to less digestible forms during storage. Even 50% to 80% of dietary folates can be destroyed in cooking and processing [53].

Other factors which decrease folate absorption are stimulants. Alcoholic abuse inhibits folate absorption in the small intestine, transport to cells and storage in the liver. Smoking decreases folate concentration in blood serum.

A deficiency of some vitamins and minerals, especially iron, copper, ascorbic acid and B_{12} vitamin disturbs folate assimilation in the human body.

The folate absorption may be impaired by structural and functional disorders of the digestive system (e.g. coeliac disease, Crohn's disease) and enteritis, furthermore by some medicines including folic acid antagonists (e.g. aminopterin, methotrexate, trimethoprim), antiepileptic drugs (e.g. carbamazepine, phenobarbital, phenytoin), anti-inflammatory drugs (e.g. aspirin, ibuprofen), cholestyramine, antacid drugs (e.g. aluminium and magnesium formula), tuberculostatic drugs and oral contraceptive pills.

Folate function and metabolism

In the human organism folic acid and its derivatives are used as coenzymes in metabolism of amino acids and nucleic acids. They are of great importance in tissues where intensive cell divisions take place, particularly in the embryo, bone marrow and digestive system epithelium.

Folates play a fundamental role in biosynthesis of purines and thymidylate, which are elements of nucleic acids (DNA and RNA) and in methionine synthesis, which is subsequently transformed in its metabolic active form – S-adenosylmethionine (SAM). SAM is essential in many methylation reactions, for example methylation of DNA and RNA, proteins, phospholipids and neurotransmitters.

Numerous enzymes (e.g. dihydrofolate reductase (DHFR), thymidylate synthase (TS), methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS)) and cofactors (e.g. vitamin B_2, B_6 and B_{12}) play a significant role in folate metabolism. During the folate metabolic pathways particular coenzymes (THF; 5-formylTHF; 10-formylTHF; 5-formiminoTHF; 5,10-methenylTHF; 5,10-methyleneTHF and 5-methylTHF) which act as one-carbon units acceptors or donors, can be converted into each other. Figure 1 presents folate metabolic pathways.

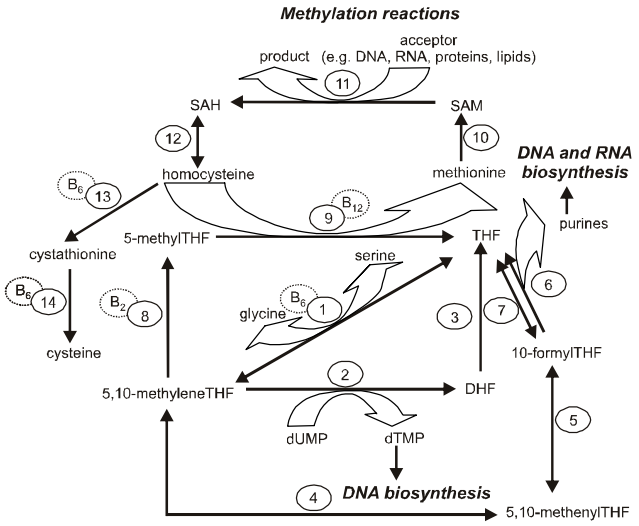

Figure 1. Metabolism of folates [for 44]

THF – tetrahydrofolate; 5,10-methyleneTHF – 5,10-methylenetetrahydrofolate; DHF – dihydrofolate; 5,10-methenylTHF – 5,10-methenyltetrahydrofolate; 10-formylTHF – 10-formyltetrahydrofolate; 5-methylTHF – 5-methyltetrahydrofolate; dUMP – deoxyuridylic acid; dTMP – thymidylate; SAM – S-adenosylmethionine; SAH – S-adenosylhomocysteine; Numers represent enzymes: 1) Serine hydroxymethyltransferase (SHMT); 2) Thymidylate synthase (TS); 3) Dihydrofolate reductase (DHFR); 4) Methylenetetrahydrofolate dehydrogenase (MTHFD); 5) Methenyltetrahydrofolate cyclohydrolase; 6) Glycinamide ribonucleotide formyl transferase (GARFT) and aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFT); 7) Formyltetrahydrofolate synthetase and formyltetrahydrofolate dehydrogenase; 8) Methylenetetrahydrofolate reductase (MTHFR); 9) Methionine synthase (MS); 10) Methionine adenosyltransferase (MAT); 11) Various methyltransferases; 12) S-adenosylhomocysteine hydrolase (SAHH); 13) Cystathionine β-synthase (CBS); 14) Cystathionase

Importance of periconceptional folic acid supplementation in the prevention of congenital malformations

A folate deficiency or a metabolic disturbance in the folate pathways may be the cause of congenital malformations due to an impaired biosynthesis and/or methylation of DNA, essential processes in cells division and differentiation during organogenesis.

The most important causes of folate deficiency are an inadequate dietary intake, malassimilation or an increased requirement of folates (e.g. gestation and lactation).

Folate matabolism may be disturbed due to genetic polymorphism of some enzymes, particularly methylenetetrahydrofolate reductase (MTHFR). The MTHFR gene is located in the short arm chromosome 1. The most common mutation of the MTHFR gene is the transition of cytosine (C) to thymine (T) at postition 677 (C677→T), which induces the conversion of alanine to valine. The C677T mutation is associated with decreased activity of MTHFR from about 30% to 50% [13]. Its consequence is an impaired production of 5-methylTHF, which participates in metabolic pathways of homocysteine. A result of disturbed homocysteine metabolism may be the lack of methionine and/or hyperhomocysteinemia, which is a teratogenic factor. The frequency of C677T MTHFR gene polymorphism depends on race and varies in different ethnic groups. In Caucasian, it concerns from 10% to 13% T/T homozygotes and 50% C/T heterozygotes [8]. In women with T/T genotype a folate deficiency and reduced activity of MTHFR could be a risk factor for NTDs in their offspring.

Findings of many epidemiological studies sugested that folic acid supplementation during periconceptional period and early pregnancy may reduce the risk of some congenital malformations in offspring. The mechanism of folic acid protective effect for prevention of these malformations is being investigated.

Table 2 presents findings of some epidemiological studies, which confirm the significant impact of folic acid or multivitamin supplementation on reduction of the incidence of congenital malformations.

Many epidemiological studies conducted in the recent three decades showed that folic acid or multivitamin supplementation plays an important role in prevention both the first occurrence [1, 9, 10, 12, 14, 15, 30, 33, 42, 50] and recurrence of NTDs [24, 32, 35, 43]. Among others the eight-year-long international multicenter study, which was based on randomization and double blind trial, published in 1991 stands out. This study estimated an impact of periconceptional folic acid supplementation on the recurrence risk of NTDs. Its findings demonstrated that women who have already had a NTD-affected child, and took 4 mg of folic acid daily, had the risk of recurrence of NTDs reduced by 72% [32]. Another very important study was the Hungarian randomized controlled double blind trial. This study showed that the intake of a multivitamin containing 0.8 mg of folic acid before and during early pregnancy is effective for the prevention of first occurrence of NTDs [10, 12, 14]. The Chinese population-based study confirmed the efficacy of periconceptional intake of 0.4 mg of folic acid daily for the primary prevention of NTDs, both in provinces with high (5 to 6 per 1000 births), and with low rates of NTDs (approximately 1 per 1000 births) [1].

Some epidemiological studies showed that folic acid or multivitamin supplementation during periconceptional period and the first weeks of pregnancy reduced the risk of congenital malformations of cardiovascular system [3, 9, 12, 14, 15], particularly outflow tract defects [3, 12] and ventricular septal defects [3, 9, 12, 14]. Among outflow tract defects the most

significant protective effect of periconceptional usage of multivitamins was evident for transposition of the great arteries [3] and tetralogy of Fallot [41]. Other studies did not confirm the efficacy of this metod of primary prevention for both outflow tract defects [37,49] and ventricular septal defects [49].

This is assumed that the risk of orofacial clefts may be reduced by periconceptional folic acid supplementation, although results of studies are inconsistent. Some studies showed the significant efficacy of folic acid or multivitamin supplementation for reduction of the risk of cleft lip (with or without cleft palate) [21, 26, 48, 51] and cleft palate alone [15, 26, 49]. However, other studies did not confirm that periconceptional intake of multivitamin supplements containing folic acid decreased the occurrence of both types of orofacial cleft [9, 12, 14, 15, 17, 39]. The Czech nonrandomized recurrence prevention trial demonstrated that use of multivitamins and high dose of folic acid (10 mg daily) significantly reduced the recurrence risk of cleft lip (with or without cleft palate) [45].

An intake of multivitamin supplements containing folic acid, before and during early pregnancy, significantly reduced the risk of congenital malformations of the urinary system, particularly obstructive defects [12, 14, 15, 25, 49]. The Hungarian cohort study showed the insignificant difference between occurrence of congenital malformations of the urinary tract in babies born by women who were taking a multivitamin with 0.8 mg of folic acid and the offspring of women who were not. However, it was found that the occurrence of obstructive defects of the urinary tract in supplemented cohort, particularly pelvicureteric junction was significantly lower [9].

Some epidemiological studies sugested that multivitamin supplementation, starting before conception and continued during the first weeks of pregnancy, could prevent limb reduction congenital malformations [49, 52]. The US case-control study conducted in California showed lower occurrence of these congenital malformations among the offspring of women who were taking multivitamin supplements too, but this connection was less significant [41].

The Chinese cohort study demonstrated that the risk of anal atresia was reduced by half after the periconceptional supplementation of 0.4 mg of folic acid daily [34]. The Hungarian case-control study showed a similar impact on the occurrence of rectal/anal atresia or stenosis if women used high dose of folic acid (3 to 9 mg daily, mainly 6 mg) in the second month of gestation [15].

The pooled findings of two Hungarian intervention studies, the randomized controlled trial and the two-cohort controlled trial, indicated the effectiveness of periconceptional intake of a multivitamin containing 0.8 mg of folic acid for congenital pyloric stenosis [15].

The US case-control study conducted in Atlanta suggested that multivitamin supplementation before and during the first trimester of pregnancy could reduce the risk of omphalocele in offspring [4].

In the Hungarian case-control study it was observed that the intake of high dose of folic acid (3 to 9 mg daily) during the first weeks of pregnancy may influence risk reduction of hypospadias, polydactyly or syndactyly and multiple congenital malformations [15]. However, the Hungarian intervention trials (randomized and cohort) did not show a decrease in occurrence of these congenital malformations after using a multivitamin containing 0.8 mg of folic acid in periconceptional period [9, 11, 14, 15].

No significant relationship between maternal periconceptional multivitamin supplemenatation and the risk of the following congenital malformations was also detected: congenital hydrocephaly [9, 49], anophthalmia and microphthalmia [38], Down syndrom [5, 9, 11] and other common autosomal trisomies (Edwards and Patau syndroms) [5].

In some studies, for example the Hungarian intervention trials, the low number of cases of some congenital malformations (e.g limb reduction defects, orofacial clefts and congenital hydrocephaly) might limit statistical power, which confirmed or excluded the impact of multivitamin supplementation on their occurrence.

The meta-analysis of 27 case-control studies, 4 randomized controlled trials, and 10 cohort studies was published in 2006. In this research the efficacy of periconceptional multivitamin supplementation for prevention of some congenital malformations was evaluated. The meta-analysis confirmed that the maternal multivitamin supplementation before and in the first trimester of pregnancy was associated with a decreased risk of NTDs, cardiovascular and limb defects in offspring. For congenital hydrocephaly, cleft lip (with or without cleft palate), cleft palate alone and congenital malformations of the urinary system, the protective effect of supplementation was shown too, but only in case-control studies. In contrast, multivitamin supplementation was not associated with a protective effect for Down syndrome, congenital pyloric stenosis, undescended testis, and hypospadias [16].

Recently there has been a debate regarding the primary prevention of congenital malformations whether the use of folic acid alone or multivitamin supplements containning folic acid is better and whether use of pharmacological dose of folic acid (e.g. 5 mg daily) might be better than use of prophylactic dose of folic acid (< 1 mg daily).

It seemes that for the primary prevention of NTDs and some other congenital malformations, including heart defects, urinary tract defects and limb reduction defects, the use of multivitamins containing usually 0.4 to 0.8 mg of folic acid, may be more effective than a use of high dose of folic acid alone. The additive and/or synergestic effect of B vitamins such as folic acid, vitamins B_2, B_6 and B_{12}, can improve the preventive effectiveness of multivitamins [15].

Recommendations regarding the primary prevention of NTDs by periconceptional folic acid supplementation

The epidemiological studies, which demonstrated that intake of folic acid by women before and during first weeks of pregnancy, significantly decreased the risk of NTDs, initiated

Table 2. The impact of folic acid or multivitamin supplements on reduce the risk of some congenital malformations – review of selected studies

Author	Country	Time of study	Type of study	Type of supplementation	Time of supplementation	Risk reduction
Neural tube defects						
Berry et al. (1999) [1]	China	1993-1995	cohort	FA (0.4 mg)	(–1 → +3)	79%[#], 41%[§]
Czeizel (1998) [12]	Hungary	1984-1991	RCT	MV (0.8 mg FA)	(–1 → +3)	94%
Czeizel et al. (2004) [9]	Hungary	1993-1996	CCT	MV (0.8 mg FA)	(–1 → +3)	89%
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	92% 32%; ns
Milunsky et al. (1989) [30]	USA (Massachusetts)	1984-1987	cohort	MV (0.1-1.0 mg FA)	(+1 → +2)	73%
MRC (1991) [32]	Australia, Canada, France, Hungary, Israel, United Kingdom, and the USSR	1983-1991	RCT	FA (4 mg)	(–1 → +3)	72%[*]
Mulinare et al. (1988) [33]	USA (Atlanta)	1968-1980	case-control	MV	(–3 → +3)	60%
Nevin and Seller (1990) [35]	United Kingdom (Northern Ireland)	1976-1989	cohort	MV (0.36 mg FA)	(–1 → +2)	84%[*]
Shaw et al. (1995) [42]	USA (California)	1989-1991	case-control	MV	(–3) (+1 → +3)	35% 40%
Smithells et al. (1989) [43]	United Kingdom (Yorkshire)	1977-1987	cohort	MV (0.36 mg FA)	(–1 → +2)	88%[*]
Werler et al. (1993) [50]	USA (Boston, Philadelphia) and Canada (Toronto)	1988-1991	case-control	MV	(–1 → +1)	60%
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	33% 48%
Cardiovascular congenital malformations						
Botto et al. (2000) [3]	USA (Atlanta)	1968-1980	case-control	MV	(–3 → +3)	T: 24% (54%[1], 39%[2])
Czeizel (1998) [12]	Hungary	1984-1991	RCT	MV (0.8 mg FA)	(–1 → +3)	T: 58% (71%[1, 2])
Czeizel et al. (2004) [9]	Hungary	1993-1996	CCT	MV (0.8 mg FA)	(–1 → +3)	T: 40% (74%[2])
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	43% 19%; 25%
Shaw et al. (1995) [41]	USA (California)	1987-1988	case-control	MV	(–1 → +2)	47%[1]
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	22% 39%
Cleft lip with or without cleft palate						
Itikala et al. (2001) [21]	USA (Atlanta)	1968-1980	case-control	MV	(–3 → +3) / (+1→ +3)	48%
Loffredo et al. (2001) [26]	Brazil (Sao Paulo)	1991-1992	case-control	MV	(+1 → +4)	42%
Shaw et al. (1995) [40]	USA (California)	1987-1989	case-control	MV	(–1 → +2)	50%
Tolarova and Harris (1995) [45]	Czech Republic	1976-1980	cohort	MV + FA (10 mg)	(–2 → +3)	65%[*]
van Rooij et al. (2004) [48]	Netherlands	1998-2000	case-control	FA or MV	(–1 → +2)	47%
Wilcox et al. (2007) [51]	Norway	1996-2001	case-control	FA (≥ 0,4 mg)	(–1 → +2)	39%
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	37% ns
Cleft palate						
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	ns 50%; ns
Loffredo et al. (2001) [26]	Brazil (Sao Paulo)	1991-1992	case-control	MV	(+1 → +4)	40%
Werler et al. (1999) [49]	USA (Boston, Philadelphia) and Canada (Toronto)	1993-1996	case-control	MV	(–1 → +4)	60%
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	24% ns

cont. of tab. 2

Author	Country	Time of study	Type of study	Type of supplementation	Time of supplementation	Risk reduction
Congenital malformations of the urinary system						
Czeizel (1998) [12]	Hungary	1984-1991	RCT	MV (0.8 mg FA)	(–1 → +3)	T: 79% (88%[3, 4])
Czeizel et al. (2004) [9]	Hungary	1993-1996	CCT	MV (0.8 mg FA)	(–1 → +3)	81%[5]
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0,8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	81%[3] ns[3]; ns[3]
Li et al. (1995) [25]	USA (Western Washington)	1990-1991	case-control	MV	(– n → +n)	T: 83% (88%[6])
Werler et al. (1999) [49]	USA (Boston, Philadelphia) and Canada (Toronto)	1993-1996	case-control	MV	(–1 → +4)	40%
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	52% ns
Limb reduction congenital malformations						
Shaw et al. (1995) [41]	USA (California)	1987-1988	case-control	MV	(–1 → +2)	30%
Werler et al. (1999) [49]	USA (Boston, Philadelphia) and Canada (Toronto)	1993-1996	case-control	MV	(–1 → +3)	70%
Yang et al. (1997) [52]	USA (Atlanta)	1968-1980	case-control	MV	(–3 →+3)	53%
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	52% 43%
Anal atresia						
Myers et al. (2001) [34]	China	1993-1995	cohort	FA (0.4 mg)	(–1 → +3)	50%
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	ns ns; 61%
Congenital pyloric stenosis						
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	80% ns; ns
Omphalocele						
Botto et al. (2002) [4]	USA (Atlanta)	1968-1980	case-control	MV	(–3 → +3)	60%
Congenital hydrocephalus						
Goh et al. (2006) [16]	meta-analysis		case-control cohort and RCT	MV	(– n → +3)	63% ns
Hypospadias						
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	ns 32%; 22%
Poly/syndactyly						
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	ns 38%; ns
Multiple congenital malformations						
Czeizel (2004) [15]	Hungary	1984-1991 + 1993-1996 1980-1996	RCT + TCT (combined) case-control	MV (0.8 mg FA) FA (3-9 mg)	(–1 → +3) (+1); (+2)	ns 36%; 25%

RCT – randomized controlled trial; CCT – cohort-controlled trial; TCT – two-cohort controlled trial; MV – multivitamin supplement; FA – folic acid supplement; T – total
[1] outflow tract defects; [2] ventricular septal defects; [3] obstructive defects; [4] renal agenesis; [5] pelvicureteric junction; [6] hydronephrosis; [*] recurrence; [#] one northern province of China with high rates of NTDs (5 to 6 per 1000 births); [§] two southern provinces of China with low rates of NTDs (approximately 1 per 1000 births)
(–1 → +1) – one month before pregnancy → first month of pregnancy
(–1 → +2) – one month before pregnancy → first two months of pregnancy
(–1 → +3) – one month before pregnancy → first three months of pregnancy
(–1 → +4) – one month before pregnancy → first four months of pregnancy
(–2 → +3) – two months before pregnancy → first three months of pregnancy
(–3 → +3) – three months before pregnancy → first three months of pregnancy
(+1 → +2) – one month before pregnancy → first two months of pregnancy
(+1 → +3) – first three months of pregnancy
(+1 → +4) – first four months of pregnancy
(–n → +3) – before pregnancy → first three months of pregnancy
(–n → +n) – before and during pregnancy
(–3) – three months before pregnancy
(+1) – first month of pregnancy
(+2) – second month of pregnancy
ns – statistically non-significant

an introduce the primary prevention programs of NTDs in many countries, also in Poland. These programs were based on the nutrition education, folic acid supplementation and food fortification (mandatory or facultative).

In Poland according to the recommendations of Experts Group brought by the Department of Health and Welfare in 1997, to prevent of NTDs in offspring, all women in childbearing age who could become pregnant should take 0.4 mg of folic acid daily.

Women who have already had a NTD-affected child have increased recurrence risk of NTDs in the next pregnancy. The risk is 4% and increases to 10% if there were two NTD-affected children in the family [36]. Before the next pregnancy they should take higher dose of folic acid – 4 mg daily, and they should have an opportunity to get a genetic counselling.

Recommendations regarding the primary prevention of NTDs in offspring by folic acid supplementation

- **All women in childbearing age**, who could become pregnant, should take **0.4 mg of folic acid daily,** do not exceed 1 mg of folic acid daily, because it makes difficult diagnosis of B_{12} vitamin deficiency anaemia.
- **Women who have already had a NTD-affected child** should take **4 mg of folic acid daily,** and they should have an opportunity to get **a genetic counselling before the next pregnancy**

The folic acid supplementation should be started at least four weeks before planned conception and should be continued during the first trimester of pregnancy.

Folic acid may be taken as folic acid tablets or multivitamins. Table 3 presents vitamin and mineral preparations containing folic acid in the recommended doses 0.4 to 1 mg and 4 mg for pregnant women or women planning a pregnancy.

Folic acid is not toxic for the human organism. Allergic reactions such as rash, flushing, bronchospasm or fever are very rarely observed after folic acid intake. However, the intake of a high daily dose of folic acid may reduce the activity of antiepileptic drugs or it may make it difficult to diagnose B_{12} vitamin deficiency anaemia.

High dose of folic acid may lead to reduction of effectiveness of some anticonvulsants, because of metabolic acceleration of these drugs in the liver and their elimination from the organism, and consequently to an increased intensity of symptoms of epilepsy. Moreover, high dose of folic acid is masking heamatologic symptoms of pernicious anaemia, may slow down its diagnosis and treatment, and consequently to increase the risk of permanent neurological complications.

Because of the above reasons, the total consumption of folic acid should not exceed 1 mg daily, with the exception of control treatment by a doctor.

The evaluation of the primary prevention programs of NTDs by folic acid supplementation carried out in countries associated in EUROCAT showed that the reduction in frequency of NTDs is not significantly different from the reduction seen in countries without folic acid supplementation policy [2, 7]. It is connected to a high percentage of unplanned pregnancies and the delayed intake of folic acid by women. In countries where mandatory fortification of staple foods with folic acid is implemented (e.g. the USA and Canada), a lowering of NTDs rates was observed [2].

In Poland, in 2007 folic acid supplementation during periconceptional period was administered by 35% of pregnant women aged 20-34 years old. 31% of them started using folic acid at least four weeks before conception. However, the percentage of women from that age group, who where taking vitamins containing folic acid in the recommended dose at least 0.4 mg daily was very low (12%) [29].

Table 3. Vitamin and mineral preparations containing folic acid for pregnant women or women planning a pregnancy [18, 19]

Medicine name	Folic acid dose	Category	Producer
Acifolik	0.4 mg	OTC	Hasco-Lek
Bio-Kwas Foliowy	0.4 mg	S	Pharma Nord
Calibrum BabyPlan	0.4 mg	S	Zentiva
Centrum Materna	0.4 mg	S	Wyeth
Elevit Pronatal	0.8 mg	OTC	Bayer
Falvit M	0.6 mg	OTC	Jelfa
Feminatal Metafolin	0.4 mg	S	Merck
Feminital N	0.8 mg	S	Merck
Folacid	0.4 mg	OTC	Synteza
Folifem	0.4 mg	OTC	Biofarm
Folik	0.4 mg	OTC	Polfa Grodzisk
Folimin	0.4 mg 4.0 mg	OTC Rp	Polfa Łódź
Folovit	0.4 mg	OTC	Polfarmex
Kwas foliowy	0.4 mg	S	Puritan's Pride
Prenatal Classic	0.8 mg	S	Puritan's Pride
Vitrum Folicum	0.4 mg	S	Unipharm
Vitrum Prenatal	0.8 mg	OTC	Unipharm
Vitrum Prevital	0.4 mg	S	Unipharm
Vitrum Prevital Forte	0.8 mg	OTC	Unipharm

Rp – prescription drug,
OTC – over the counter drug,
S – supplement

Conclusions

Folic acid is a nutrient essential to the correct functioning of all human cells. It fulfills a very significant role in tissues, where cell divisions take place.

A deficiency of folates in a daily diet and disturbances in their metabolism may be a danger to normal intrauterine development.

Folic acid supplementation before conception and in the first trimester of pregnancy can significantly decrease the risk of NTDs, as well as some congenital malformations of the heart, limb, urinary tract, digestive system and orofacial clefts.

It is essential to conduct more studies to explain the mechanism of folic acid protective effect and to define its effectiveness for the pimary prevention of congenital malformations.

It is important to popularise knowledge about the role of folic acid in the primary prevention of congenital malformations and to encourage all women in childbearing age to adequate nutrition and use of 0.4 mg of folic acid every day.

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