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THE QUESTION IS WHETHER INTAKE OF FOLIC ACID FROM DIET ALONE DURING PREGNANCY IS SUFFICIENT

PITANJE JE DA LI JE UNOS FOLNE KISELINE SAMO ISHRANOM U TRUDNOĆI DOVOLJAN

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Summary

Pregnancy and Folic Acid. Pregnancy is the most important period in life of every woman, partially for the number of physiological adaptations she is going through, partially for the expectance of new life. In addition, pregnancy is the "critical window" for development later in childhood, as a period of foetal programming during which nutrition plays one of crucial roles. Despite the general belief that nutrition through pregnancy is adequate and characterized by better nutritional habits, a number of studies do not corroborate this belief. **Role of Folic Acid.** An adequate folate blood level is necessary for normal cell growth, synthesis of several compounds including deoxyribonucleic acid and ribonucleic acid, proper brain and neurologic functions; it is included in the regulation of homocysteine level, and closely related to the vitamin B_{12} metabolism. Folate deficiency in pregnancy is related to neural tube defects, other neurological disorders, preterm delivery and low birth weight. **Food sources.** A correlation between folate and the prevention of broad spectrum of chronic diseases has been confirmed. Emerging evidence from the epigenetic studies is now bringing even more light on the level of significance of folic acid. A wide range of plant and animal foods are the natural sources of folate; liver, yeast, mushrooms, and green leafy vegetables being the most significant. Different ways of food preparation influence the folate stability and its bioavailability varies from 25 to 50% from foods, 85% from enriched foods or 100% from supplements. **Conclusion**. A great amount of scientific results has led to official recommendations for folic acid supplementation in pregnant women as well as in a number of obligatory or voluntary fortification programmes in order to prevent the folate deficiency on the level of different population groups. Nevertheless, there must be a certain level of precaution for elderly because folate can mask the vitamin B_{12} deficiency with possible fatal outcomes.

Key words: Folic Acid; Pregnancy; Folic Acid Deficiency; Dietary Supplements; Food, Fortified; Food; Biological Availability

Sažetak

Trudnoća i folna kiselina. Trudnoća je najvažniji period u životu svake žene, što zbog fiziloških prilagođavanja kroz koje žena prolazi, što zbog iščekivanja novog života. Ujedno, trudnoća predstavlja „kritičan prozor" u kasnijem razvoju deteta, period fetalnog programiranja u kojem prehrana igra jednu od vodećih uloga. Uprkos prihvaćenom mišljenju kako je ishrana u trudnoći adekvatna i boljeg kvaliteta, sa boljim prehrambenim navikama, rezultati brojnih istraživanja to ne ukazuju. **Uloga folne kiseline.** Adekvatan nivo folne kiseline je neophodan za normalnu ćelijsku deobu, regulaciju sinteze različitih molekula, uključujući dezoksiribonukleinsku i ribonukleinsku kiselinu, za pravilno funkcionisanje mozga i neurološke funkcije, uključena je u regulaciju nivoa homocisteina, te je u uskoj vezi s metabolizmom vitamina B_{12}. Deficit folne kiseline u trudnoći je odgovoran za defekte neuralne cevi fetusa, druge neurološke poremećaje, prevremeni porođaj i malu porođajnu masu. **Izvori folne kiseline.** Potvrđena je i povezanost unosa folata sa prevencijom niza hroničnih bolesti. Rastući broj dokaza iz epigenetičkih istraživanja baca novo svetlo na stvarnu važnost folne kiseline. Folati su prisutni u različitoj biljnoj i hrani životinjskog porekla, a najznačajniji izvori su jetra, kvasac, gljive i zeleno lisnato povrće. Različiti načini pripreme hrane utiču na stabilnost folata, a njihova bioraspoloživost varira 25–50% u hrani, 85% u obogaćenoj hrani ili 100% iz suplemenata. **Zaključak**. Količina nepobitnih naučnih dokaza je rezultirala službenim preporukama suplementacije folnom kiselinom kod trudnica, kao i nizom mandatornih ili dobrovoljnih programa obogaćivanja hrane folnom kiselinom kako bi se prevenirao deficit na nivou različitih populacijskih grupa. Ipak, određena doza opreza je prisutna kada je u pitanju starija populacija jer folna kiselina može maskirati deficit vitamina B_{12} koji u ovoj populaciji može imati fatalan ishod.

Ključne reči: Folna kiselina; Trudnoća; Deficit folne kiseline; Suplementi; Obogaćena hrana; Hrana; Bioraspoloživost

Pregnancy and Folic Acid

Pregnancy is a truly very special condition for every woman. Throughout its duration of 280 days on average (or 40 weeks from the first day of the last menstrual cycle), women experience a number of deep adaptations of cardiovascular system, change in volume of body fluids, respiration, energy metab-

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Abbreviations

DNA – deoxyribonucleic acid
RNA – ribonucleic acid
NTD – neural tube defects
FH4 – tetrahydrofolate
C1 – carbon atom
eNOS – endothelial nitric oxide synthase
DFE – dietary folate equivalent
USA – United States of America

olism and nutrition [1, 2]. Different hormones induce these changes including the growth of uterus as the site of foetal growth and development [1].

For foetal metabolism, glucose is a main source of energy. Vitamin B complex, especially vitamin B_{12} and folic acid are necessary for proper synthesis and maturation of erythrocytes, development of nervous system and growth in general. Vitamin C is important for proper formation of tissues, especially bone matrix and connective tissues; vitamin D is essential for normal bone growth; whereas vitamin E is necessary for normal course of early stage of foetal development, and vitamin K is used by the foetal liver to synthesize the factors II, VII, IX, and X [2]. Due to growing needs of foetus during gestation, the need for nutritional intake of these nutrients increases. As pregnancy advances through trimesters, the daily need for energy increases [3]. From the aspect of vitamins and minerals, main deficiency usually develops for folic acid and iron. Suboptimal intake of both of these micronutrients has been the main focus of interest for pregnancy outcomes, developing foetus and foetal programming [4]. Problems related to iron intake during pregnancy have been discussed thoroughly earlier [5].

The increased need in pregnancy for folate partially reflects the need for blood cells production, growth in uterus tissue and placenta, growth of the foetus itself and increase in blood volume [1, 5, 6]. The need for folate is 5 to 10 times higher in pregnant women, which makes them susceptible to folate deficiency [6]. This results in a lower blood concentration of folate and even megaloblastic anaemia may develop in extreme cases [1, 5]. Maximal amount of folate is needed in the last trimester for the extensive growth of foetus and because foetus is stocking up folate stores [6]. In addition, haemodilution in the later stages of gestation and the way it modulates blood folate should be taken into consideration [1, 2, 7]. Fekete et al. [6] have confirmed that the increased intake of folate, even after the first trimester of pregnancy, is related to higher birth mass and lower risk for delivering low birth weight infant. Birth weight is one of the most important factors in infant death within the first 12 months of age. On the other hand, birth weight is a crude outcome measure of complex processes and is influenced not only by the nutrient status of the mother but also by a number of other factors, such as mother's anthropometry, the length of gestation, sex of the baby etc. [7].

Prolonged folate deficiency in pregnancy causes neural tube defects (NTDs) in a developing foetus. NTDs are congenital malformations leading either to death in case of anencephaly or disability for life in case of spina bifida [1, 5, 8]. Neural tube is a foetal structure from which the brain and spinal cord with nerves and their membranes develop [2]. The neural tube closes on the 24th day from conception or around that time [2], that being long before the woman even realizes she is pregnant. This is the reason why prophylaxis is recommended to all women of reproductive age, i.e. pregnancy planning should be the definitive aim.

Folic Acid

Folic acid or vitamin B_9 is one of eight vitamins of B complex. It is a water-soluble vitamin, isolated from spinach for the first time in 1940s. Its name originates from the Latin word *folium* meaning leaf, because of its high abundance in green leafy vegetables [9].

Folate or folacin is a generic name for folic acid. Folic acid (pteroylmonoglutamic acid) is the highest oxidized form and the most stable form used in supplements and for food fortification. The majority of folate to be found in nature are termed dietary folic acid, i.e. dietary folate, and they represent different derivates of glutamic acid (pteroylpolyglutamates), which have one joint name "folate" [8–10]. A subgroup of folate, 5,6,7,8-tetrahydrofolate (FH_4) has the highest biological importance. It acts as a coenzyme and an intermediate transporter of groups with one carbon atom (C1), e.g. methenyl and methylene groups [10].

Role of Folate in Organism

The function of folate in the organism is closely related to the function of vitamin B_{12}, other B complex vitamins (B_2 and B_6), and some minerals, iron being the most interesting of them from the aspect of pregnancy, because of their interrelation in the physiology of pregnancy [1, 4, 5, 11, 12]. In addition to the above-mentioned role in the neural tube closure and coenzyme activity in C1 metabolism, folic acid is a growth factor and it is also necessary for the synthesis of other compounds. Among these are deoxyibonucleic acid (DNA) and ribonucleic acid (RNA) synthesis and maintenance, cell division, and the regulation of homocystein levels [8, 9, 11]. The magnitude of the interrelation between folic acid and other vitamins of B complex, its importance in C1 metabolism and DNA methylation have been studied extensively in epigenetic studies (see the text below) [7, 13, 14]. **Table 1** shows some of the neurological and psychiatric disorders associated with folate deficiency [11].

From the aspect of public health significance, the involvement of folic acid in homocysteine levels is the most interesting one. An increased homo-

Table 1. Some of non-haematological manifestations of folic acid deficiency [11]
Tabela 1. *Neke od ne-hematoloških manifestacija nedostatka folne kiseline [11]*

Deficit consequences *Posledice deficita*	Deficit manifestations *Manifestacije deficita*
Neurological *Neurološke*	Depression, psychosis, peripheral neuropathy, subacute combined spinal cord degeneration, neural tube defects/*Depresija, psihoza, periferna neuropatija, subakutna kombinovana degeneracija kičmene moždine, defekti nervne cevi*
Metabolic *Metaboličke*	Increase of homocysteine and other metabolites levels *Povećanje nivoa homocisteina i drugih metabolita*
Cardiovascular *Kardiovaskularne*	Cardiovascular, peripheral vascular and cerebrovascular diseases with atherosclerosis and thrombosis/*Kardiovaskularne, periferne vaskularne i cerebrovaskularne bolesti sa aterosklerozom i trombozom*
Neoplastic *Neoplastične*	Preneoplastic alterations of cervix and uterus epithelium and gastrointestinal and pulmonary epithelium/*Pre-neoplatstične alteracije epitela cerviksa i uterusa i gastrointestinalnog i pulmonalnog epitela*
Developmental *Razvojne*	Congenital abnormality (of neural tube and others) *Kongenitalna anomalija (neuralne cevi i druge)*
Epithelial/*Epitelne*	Megaloblastic alterations/*Megaloblastne alteracije*

cysteine level resulting from vitamin B_{12} and folate deficiency is a significant risk factor for cardiovascular diseases [11]. Cardiovascular diseases are the number one death cause around the globe; Croatia, the Republic of Serbia and Montenegro are no exception [15]. Besides regulating homocysteine level, folic acid acts as a cofactor for endothelial nitric oxide synthase (eNOS) leading to improved vasodilatation and end-organ perfusion [14]. Additionally, the increased level of homocysteine in plasma can be embriotoxic. Homocysteine level shows a correlation with bone density; disturbances in the folate-homocysteine-methionine axis could be the basis of pathologies related to the loss of normal organization and strength of skeletal tissue [14], thus presenting a risk factor for osteoporotic bone fractures. Heppe et al. [16] have shown that an increased concentration of homocysteine in the mother in the first trimester of pregnancy shows a correlation to the lower mineral content and bone density in her child.

A significant number of studies have suggested that folic acid can prevent other congenital anomalies and low birth weight among infants, together with some chronic diseases such as cardiovascular diseases, stroke, different types of cancer, depression, dementia and osteoporosis [7–9, 14, 17, 18]. On the other hand, recent studies have found no effect of folic acid on the prevention of some of important chronic diseases, such as cardiovascular diseases or cancer [19, 20].

From the aspect of pregnancy, the main role of folic acid is observed through NTDs. The main risk factors are previous pregnancy with a neural tube defect, inadequate folate intake by the mother, diabetes, some drugs (which interfere with folate metabolism), obesity and inadequate intake of vitamin B_{12}. Several studies have confirmed an undoubted correlation between the consumption of folic acid supplements before conception and a lower risk for the development of neural tube defects [9, 21]. Intake of folic acid at the time of conception of 4 mg per day prevents the development of neural tube defects in women with the previous history of a defect by 72% [5, 11]. These results have led to the official recommendations for women of reproductive age: intake of folic acid in dose of 400 μg per day during pregnancy planning, 600 to 800 μg per day during pregnancy, and 4 mg per day for the prevention of recurrent defect [5, 8, 21–23]. The World Health Organization recommends supplementation with folic acid before conception until the 12th week of pregnancy [24]. A randomized prospective longitudinal observational study on pregnant women from Eastern Croatia has shown that women rarely use folic acid before pregnancy; however, as many as 81.1% of them take folic acid supplements from the time when the pregnancy was confirmed until the 12th week of gestation. Out of them, 59.9% of women continue to take supplements containing folic acid throughout gestation [12]. These data are in accordance with the research conducted by Vandevijvere et al. showing that less than one pregnant woman out of two take folic acid before pregnancy. The authors have shown that 39% of pregnant women from Belgium do not have adequate level of folate in the first trimester to prevent neural tube defects [25]. Vitale et al. conducted research on a group of parturient women from Zadar County and showed that although 75.2% of women consumed folic acid or multivitamin supplement containing folic acid, only 16.5% of these women took folic acid supplement before conception [22].

Folic Acid from the Aspect of Epigenetics

In order to be able to comprehend the overall complex role of folic acid in our organism we need to think „on top of genetics“. For the last 20 years or so, compiling evidence has been supporting the thesis on nutrition altering our epigenome. The un-

derlying changes on epigenome include a group of modifications to chromatin structure that do not alter the DNA sequence, but confer the transcriptional regulation over a range of timescales. The major epigenetic processes include DNA methylation, histone modification and non-coding RNAs. So far, most studies on the interaction between nutrition and epigenetic regulation of genes have been focused on DNA methylation although there are emerging findings about other epigenetic marks [26]. Even though the majority of existing evidence goes towards the correlation between maternal diet and offspring, paternal influence should not be neglected, especially when it comes to environmental factors such as smoking [27].

Maternal diet deficient in macronutrients during pregnancy affects metabolism of a growing foetus. This has been extensively reported both in animal models and humans, especially for protein restricted diets [13, 17, 26] and high-fat diets [26]. Probably one of the most famous studies showing the correlation between dietary restriction, the duration of such restriction and the impact on offspring's development is the Dutch famine cohort [28]. It should be noted that macronutrient and energy deficient diet during pregnancy is accompanied by the micronutrient deficiency [4, 12, 29]. As earlier mentioned, iron and folic acid (together with other B complex vitamins) have been put forward as the most important nutrients in the foetal programming. The role of iron is extensively covered elsewhere [27, 30, 31]. Attention is mainly oriented towards the DNA methylation reactions [26], bringing more attention on folic acid from the aspect of epigenetics.

Speaking of folic acid deficiency, low dietary intake of folate or genetic polymorphism alters the folate metabolism leading to the impaired purine and pyrimidine metabolism, DNA synthesis and/or cell proliferation; and affect foetal growth and result in teratogenesis and congenital malformations [7, 27]. Studies on rodents have shown that a low-protein diet supplemented with folic acid improves offspring's outcomes [13, 17]. In other words, marginally low protein intake and micronutrient insufficiency induce perturbations in C1 metabolism, which is the primary contributor to impaired foetal growth and associated long term consequences in the offspring [7, 27]. Nevertheless, findings are inconsistent. Several studies showed a positive correlation between the red blood cell or plasma folate levels and infant birth weight, while others did not find any relation in a well-nourished population of pregnant women between dietary folate, and plasma folate and the size of an infant at birth [7].

Elevated levels of homocysteine during pregnancy are associated with pregnancy-related disorders such as preeclampsia, early pregnancy losses etc. and the correlation has been found with the homocysteine effect on the vascular endothelial cell function and increased pro-oxidant activity [32]. Interesting epigenetic findings were reported by the Pune Maternal Nutrition study, the cohort study from India [33]. They found that a high level of circulating homocysteine was a predictor of intrauterine growth restriction, and showed a correlation with a low vitamin B_{12} status (folate deficiency in the studied population was rare). At 6 years of age, children born of mothers with high folate status in pregnancy were of higher adiposity and had insulin resistance. The combination of vitamin B_{12} deficiency and high folate status resulted in the most insulin resistant children [33].

Despite a limited number of available studies, transgenerational inheritance of epigenetic modifications induced by the exposure to macro- and micronutrients is getting more attention [27, 34], especially the gender-specific inheritance (Vanhees et al. 2014, Hussain 2012). For example, a study by Dunn and Bale [35] showed that the *in utero* exposure to a high-fat diet resulted in increased body size in third-generation (F3) female offspring, and the interesting part was that the inheritance seemed to be passed by the paternal line.

Cancer has recently gained a lot of attention due to a high increase in the incidence and mortality throughout the world [36], and the fact that the field of epigenetics has been studied largely in the context of tumorogenesis may be even more important [23]. This is especially interesting in cancers with a strikingly high correlation with diet, such as colorectal cancer [36]. There are clear DNA methylation pattern changes in tumors [23, 26, 37]. Folic acid has been suggested as both suppressor for some and promoter for other neoplasias [23]. It has been hypothesized that the early exposure to folic acid might prevent tumors through the provision of enough methyl groups to maintain proper methylation patterns and repair of DNA. In contrast, after the development of tumors, higher intake of folic acid might promote growth of existing tumors [23, 36]. According to some studies on rodents the tumor-suppressor gene *p53* seems to be less methylated in the intestines of adult mice born from mothers fed a diet low in folate during pregnancy. Importantly, this low folate intake by the mother during pregnancy resulted in global hypomethylation of the adult offspring, which is associated with a higher risk of developing cancer [27]. Still, folic acid is more likely to be taken in excess due to wide supplementation and fortification programmes. It is reasonable to mention dangers of the excess *in utero* exposure. Studies have shown that timing is crucial for epigenetic modulation by folic acid. In other words, a high-dose folic acid supplementation during early pregnancy is associated with increased neurodevelopment, resulting in enhanced vocabulary development, communicational skills and verbal comprehension at 18 months of age [18, 27]. However, the excessive exposure to folic acid during late pregnancy seems to have an association with childhood asthma and atopic dermatitis [27, 38]. Vitamin B_{12} in relation to folic acid shows the

potential in modulating epigenetic marks related to cancer, as well as for NTDs, impaired neurodevelopment, and insulin resistance [7, 33, 37].

Needs and Recommendations for Folate

Folate intake from foods, its metabolism, some drugs, certain life conditions and health status, as well as inter-individual genetic variations and gender differences all influence the level of folate [30]. Besides an inadequate folate nutritional intake, deficiency can develop due to malabsorption. Malabsorption can result from a number of disorders in digestive tract, from inflammatory bowel diseases, irritable bowel syndrome, coeliac disease or carcinoma. Inadequate intake from foods is common in elderly, alcoholics and people of low socioeconomic status. Different liver diseases, together with alcoholism can lead to folate deficiency because they intervene with C1 metabolism. In addition, the liver is the main storage organ for folic acid. As already mentioned, an imbalance between folate intake and increased need, as it happens in pregnancy, during lactation, in preterm infants, but also in haemolitic anaemia, inflammatory diseases and psoriasis, can lead to deficiency. A number of drugs also interfere with the metabolism and absorption of folate [11]. Recommended daily intakes and upper limits for folic acid are given in **Table 2** [39].

Nutritional intake of folate is believed to be the most relevant cause of deficiency; therefore, in order to accomplish the recommended intake, the use of supplements is strongly suggested, i.e. the consumption of foods fortified with folic acid. The majority of studies dealing with folic acid are focused on the folic acid deficiency. However, the question is: "Should we be more concerned with the excessive intake of folic acid?" When we observe general population, dietary habits seem to be unfavourable, suggesting the excessive intake of a number of macro and micronutrients. The result of such dietary habits is the ever-increasing rate of morbidity and mortality due to obesity, type 2 diabetes mellitus, cardiovascular diseases, and cancers. The questions of under- and over-nutrition elicit persistent attention in scientific community. The argument is especially acute when the use of supplements is on the table. As shown by the latest report on the supplements use among American adults, the prevalence range fluctuated from 64% to 69% in the period from 2007 to 2011 and the percent of regular users increased from 28% to 36% [40]. Multivitamins are the most commonly used supplements. For 58% of the study respondents, the main reason for supplement use are overall health and wellness, while for 42% of them it is the will to fill nutrient gaps in the diet [40]. These findings highlight one important fact. Despite the number of strong evidence from recent studies [19, 20], general public considers supplements almost as a crucial, key component of a daily diet, such as proteins, fats or folic acid.

In spite of the general belief that nutrition during pregnancy improves [41], compiling data show inadequate intake of energy as well as a wide range of nutrients throughout pregnancy [29, 42]. Nutrition of pregnant women is considered to be the most important external, environmental factor affecting the growth and development of foetus [43]. These external, environmental factors determine the final outcome of pregnancy and infant's birth weight by even 30% [1]. Some studies have shown that primaparae are highly motivated to change their nutritional habits for better during pregnancy [42, 44]. On the other hand, younger women, with lower education, low income, from rural areas, with more children and higher body mass index have nutrition of worse quality during pregnancy [42, 44]. Banjari et al. [29] have found that nutrition quality of pregnant women from Eastern Croatia shows the intake well below recommendations for most macro and micronutrients. In their randomized observational research on pregnant women from Czech Republic, Hronek et al. have shown that they have low intake of dietary folate, representing about 40% of the recommended daily intake [45].

Table 2. Daily need for intake of folic acid (μg/day) [39]
Tabela 2. *Dnevna potreba za unos folne kiseline (μg/day) [39]*

Age *Starost*	Infants (RDI) *Odojče*	Infants (UL) *Odojče*	Adults (RDI) *Odrasli*	Adults (UL) *Odrasli*	Pregnant women (RDI) *Trudnice*	Pregnant women (UL) *Trudnice*	Lactating women (RDI) *Dojilje*	Lactating women (UL) *Dojilje*
0 – 6 months/*meseci*	65	n/a	–	–	–	–	–	–
7 – 12 months/*meseci*	80	n/a	–	–	–	–	–	–
1 – 3 years/*godina*	150	300	–	–	–	–	–	–
4 – 8 years/*godina*	200	400	–	–	–	–	–	–
9 – 13 years/*godina*	300	600	–	–	–	–	–	–
14 – 18 years/*godina*	400	800	–	–	600	800	500	800
> 19 years/*godina*	–	–	400	1000	600	1000	500	1000

RDI – recommended daily intake, UL – upper limit, n/a – not defined
RDI – preporučeni dnevni unos, UL – gornja granica, n/a -nedefinisano

The emerging findings show that epigenetic plasticity may extend beyond the early development and include periods in the life course associated with rapid physiological change such as puberty and aging [26, 46]. In other words, we could modulate our predisposition for certain diseases gained *in utero* by specific nutritional interventions later in life. Gender differences, genotype, target gene, as well as timing of the exposure and the magnitude of the intervention all need to be taken into consideration for such nutritional interventions [26, 30, 34, 46]. A planned, specific supplementation is one of the examples.

Food Sources

Folate can be found in the variety of plant and animal foods. Liver, yeast, mushrooms and green leafy vegetables are the best food sources of folate. Vegetables like asparagus and green leafy vegetables are the excellent source of folate from food. Cereals (fortified breakfast cereals, whole grain products), meat (liver, eggs) and legumes (beans, sunflower seeds), and fruits (oranges, strawberries, melons) are good sources, whereas milk, yoghurt, cheese, fats and oils are considered poor sources of folate [9, 47, 48]. Some of the best food sources of folate are listed in **Table 3**.

Consumption of folate-poor diet is the main cause of folate deficiency. Similarly to vitamin B_{12} deficiency, lack of folate was noticed in poor regions around the world where food availability is restricted [48, 49]. Still, some types of diets have shown to be favourable in terms of folate intake both in pregnancy and in general conditions as well. Timmermans et al. [43] and Sotres-Alvarez et al. [50] have shown that the Mediterranean diet, which includes high intake of vegetables and plant oils, fibres and complex carbohydrates, moderate consumption of fish, poultry and alcohol, low intake of meat and high intake of unsaturated fats, is positively related to different characteristics of foetal growth. The Mediterranean diet also correlates to higher folate intake, as shown by Monteagudo et al. Their research included three different age groups of women from Spain and based on the Mediterranean Diet Score they have shown that women with a diet that shows a higher compliance to the Mediterranean dietary pattern have higher folate intake [51]. It is important to note that B complex vitamins, including folic acid, show better effectiveness with the Mediterranean diet because of the lower consumption of saturated fats, trans-fats and cholesterol [43, 50].

Folate Stability and Bioavailability

The majority of nutritional folate is easily oxidized and therefore they are not stable towards oxidation and aerobic conditions during storage and processing. Under these conditions (especially with additional presence of heat, light and/or metal ions), the physiologically inactive components can be formed due to complete or partial oxidation. In the presence of light, vitamin B_2 catalyses these reactions [9, 47].

Food preparation can significantly affect availability of food folate. Cooking can reduce the content folate by 50%, because it stays in water (overall loss of folate varies from 22% for asparagus to 84% for cauliflower). In addition, extreme pH leads to folate loss during food preparation [21, 47].

Bioavailability of food folate depends on numerous factors, including conditions of deconjugation in bowel, instability of several labile forms during digestion, cell structure of food itself and presence of specific compounds that can improve the stability of folate during digestion. Bioavailability of dietary folic acid is 25 to 50%, 100% from folic acid supple-

Table 3. The best food sources of folic acid [39]
Tabela 3. *Najbolji izvori folne kiseline u hrani [39]*

The best food sources/*Najbolji izvori u hrani*	Average amount (μg/100 g of food)/*Prosečan iznos*
Liver (raw)/*Jetra (sirova)*	141
Liver (cooked)/*Jetra (kuvana)*	1070
Tuna (canned)/*Tunjevina (konzervisana)*	15
Orange juice/*Sok od narandže*	220
Oranges/*Narandže*	24
Bananas/*Banane*	28
Broccoli (raw)/*Brokoli (sirov)*	169
Broccoli (cooked, without water)/*Brokoli (kuvan bez vode)*	65
Egg yolk (hard-boiled)/*Žumance (tvrdo kuvano)*	140
Bread (wholemeal)/*Hleb (integralni)*	54
Tomato (canned)/*Paradajz (konzervisan)*	43
Cabbage (raw)/*Kupus (sirov)*	30
Milk (fresh, cow's)/*Mleko (sveže, kravlje)*	5 – 12
Cheddar cheese/*Sir tvrdi*	20

ments (if taken on an empty stomach), and almost 85% from fortified foods [48]. This disproportion between the consumption and bioavailability can be more easily estimated via the so called dietary folate equivalent (DFE). DFE is adjusted with 50% worse use of folate from natural sources (foods) and synthetic folic acid from supplements and fortified foods (e.g. 1 μg of dietary folic acid = 0.6 μg of folic acid from fortified foods or supplements taken with foods; 1 μg of natural dietary folic acid = 0.5 μg of folic acid from supplements taken on an empty stomach). In other words, 1 μg of DFE equals to 1 μg of dietary folic acid, i.e. 0.6 μg of folic acid from fortified foods, i.e. 0.5 μg of folic acid from supplements [48].

Folic Acid Supplements and Food Fortification

In Europe, more than 40% of all pregnancies are not planned; therefore, prophylactic intake of folic acid is crucial for the successful prevention of neural tube defect. Socioeconomic conditions substantially influence availability of foods that are a significant source of folate. This kicked up food fortification programmes in foods widely consumed [8, 22, 23], so a large number of countries have introduced obligatory (mandatory) measures of food fortification with folic acid [8, 23, 52]. Mandatory fortification for cereal products such as wheat flour and bread is effective in 72 countries around the world, in both low- and high-income countries [53]. Croatia has no such programme in effect, but several years ago, wheat flower enriched with folic acid, some other B complex vitamins and iron was introduced into the market [4].

Studies have shown that not even general population has the recommended daily intake of 400 μg in most of the countries where fortification is not obligatory [8, 21, 22, 27]. Despite these findings, the main reason for controversy about fortification of foods widely consumed comes from the fact that folate, due to its interrelation to vitamin B_{12} metabolism, can mask deficiency of the latter. This is of special concern in elderly, who may develop severe neurological disorders caused by vitamin B_{12} deficiency [8, 21, 23, 54]. For example, the amount of folic acid used to fortify foods in the United States of America (USA) and Canada is balanced in a way that no one consumes more than 1 mg per day, that being the upper limit for daily intake. Still, a large part of scientific circles emphasize that this may not be the case in practice; the risk of potentially excessive intake does exist [21, 23, 30, 54].

National directives on food fortification intended for wide public consumption still differ significantly among the countries of the European Union. Voluntary fortification was implemented for several years in some member countries (the United Kingdom, Ireland, Spain, Portugal and Austria) as well as in Switzerland. In other member countries (Denmark, Finland and Sweden), voluntary fortification is restricted or forbidden. In Ireland, after stopping fortification of breakfast cereals, folate level in erythrocytes decreased by 111 nmol/L during 12 weeks, thus impressively showing what may be the consequences of such measures [8, 22, 23, 47].

The amount of folic acid used for fortification in the USA and Canada is 140 μg on 100 g of grain of cereal, assuming that this will lead to an increase in folate intake by approximately 100 μg a day in women of reproductive age. Fortification with folic acid significantly improved folate status in the USA. Data from the National Health and Nutritional Examination Surveys which had compared year 1999 with period from 1988 to 1994 showed an increase in the mean concentration of folate in serum of women of reproductive age, who did not take folic acid supplements, from $10x10^{-7}$ nmol/L on $28x10^{-6}$ nmol/L [8, 55].

Emphasis should also be put on the findings that the consumption of folic acid supplements can improve iron status significantly in pregnancy, thus partially compensating the lack of iron through gestation [56], and diminishing a possible adverse impact of iron deficiency anaemia on pregnancy outcomes [4, 12]. Christian et al. conducted a controlled intervention study on pregnant women from Nepal and they have shown that folic acid (in form of a supplement) in combination with iron increases haemoglobin level and reduces anaemia by 54%. On the other hand, the combination of folic acid, zinc, and iron resulted in 48% reduction, while the combination of folic acid, zinc, iron and additional 11 micronutrients led to a 36% reduction in anaemia. The same effect was found for food fortification. Ganji and Kafai [52] performed an analysis on the population level for the USA after the folic acid food fortification had been implemented. They found a significant decrease in the prevalence of iron deficiency anaemia in the periods from 1988 to 1994 and from 1999 to 2004, especially among women [57].

According to Mallard and Houghton [58], food fortification with folic acid is desirable because all social groups could achieve adequate intake of folic acid in that way. Studies have shown that women who are aware of the importance of folic acid before their pregnancy are more likely to use folic acid supplements. All the above mentioned strongly suggest the great necessity of education on this issue, which should be directed towards young women of lower educational background, lower income, primaparae and with unhealthy habits. In addition, women who happen to be single during their pregnancy or those with unplanned pregnancy should be encompassed as well [25]. All these women have been shown to have an inadequate folate status during pregnancy, inadequate intake of foods rich in folate, enriched foods or folic acid supplements [22, 25, 41, 45, 51, 53].

Conclusion

When observing pregnancy as the „critical window“ in foetal programming, or in other words as a milestone in setting up basis for a healthy life, additional intake of folic acid from supplements should

be a standard. Still, more effort should be put into the promotion of prophylactic use of folic acid by education or intervention programmes on the national level, which would eventually encourage women to plan their pregnancy. The final outcome of such politics would result in healthier population. On the other hand, if a person has no increased demand for folic acid, diet abundant with fresh fruits and vegetables can satisfy the need for folate. However, considering the aspect of fortified foods and the trend of wide supplement use among the general public, there is a level of caution. Demographic indicators on age of the population show a growing proportion of the population over 65 years of age and they are especially susceptible to the possible masking of vitamin B_{12} deficiency due to intake of folate enriched foods and/or folic acid from supplements.

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