Folate intake and folic acid supplementation in the periconceptional period: recommendations and real intake

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"*Knowing is not enough; we must apply.*
Willing is not enough; we must do." Goethe

Este trabalho é dedicado à HUMANIDADE em geral,

e às GRÁVIDAS em particular.

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Abbreviation	Meaning
DFE	Dietary Folate Equivalents
DRI	Dietary Reference Intakes
DNA	Deoxyribonucleic Acid
D-A-CH	Deutschland-Austria-Confoederatio Helvetica reference values
EAR	Estimated Average Requirements
EU	European Union
FD	Food Diary
FFQ	Food Frequency Questionnaire
MTHFR	5,10-Methylenetetrahydrofolate Reductase
NTD	Neural Tube Defects
NNR	Nordic Nutrition Recommendations
RDA	Recommended Dietary Allowances
THF	Tetrahydrofolate
UL	Tolerable Upper Intake Level
USA	United States of America
WHO	World Health Organization

ABSTRACT

Introduction

Folic acid supplementation in the periconceptional period is associated with reduced risk of neural tube defects. But most supplementation happens after the desirable period, having the supplementation some limitations in the prevention of neural tube defects, contrary to fortification. After an initial enthusiasm regarding folic acid fortification, new concerns raised in relation to potential adverse effects of fortification and supplementation of high doses of folic acid, namely in pregnancy outcomes, early and later in life, both in mother and child.

In what concerns to diet, it has been shown that dietary patterns and the method of cooking have a clearly significant role in population folate intake, being a "healthy diet" more rich in this nutrient.

From this *state of the art,* some questions deserve clarification such as: What are the recommendations in many countries worldwide concerning folic acid in the periconceptional period? Can we predict maternal folate status from their folic acid supplementation and their dietary folate intake? Do we really need to recommend folic acid periconceptionally or simply promote healthy dietary patterns? Could be valuable to measure biomarkers before advice? What is the real intake and the recommendations in Portugal? Which are the consequences for the new-born and for the mother from high supplementation?

This study aims to answer to two specific objectives. The objectives, methods, results and conclusions for each one are described separately.

Study 1

Aim 1: To summarize the recommendations on folate and folic acid intake in the periconceptional period provided by official health organizations in different countries worldwide.

Methods 1: Observational descriptive analysis of data collected from official national health organizations websites of 36 countries worldwide (25 European, including 22 European Union countries; 4 BRICS; 3 Asian Tigers; all The Group of Eight; and Australia) and from the World Health Organization website, concerning folate and folic acid recommendations in the periconceptional period.

Results 1: Recommendations differed between countries, although the majority recommended 400 µg/day of folic acid at least one month before conception until three months of pregnancy. In Portugal there are no official recommendations regarding the dose. There is a consensus in the recommendation of a healthy diet, naturally rich in folate. The daily recommended folate intake for pregnant women could be summarised mainly in three different recommendations: 500 µg of folate, 550 µg of dietary folate equivalents (DFE) or 600 µg of DFE. For women in childbearing age the recommendation ranges from 300 µg/day of DFE to 400 µg/day of DFE

or folate. Some countries emphasize the importance of a healthy diet with no need of folic acid supplementation. By contrast, others advice high supplementation plus mandatory fortification.
Conclusion 1: Folic acid recommendations worldwide are predominantly of 400 µg of folic acid in the periconceptional period, independently of the national epidemiology of intake and disease. Regardless the lack of a quantitative recommendation, in Portugal the only commercialized doses of folic acid are of 5 mg, also prescribed to low risk women. High intakes of folic acid have being shown through pregnancy outcomes studies, both in mother and child, early and later in life, to have adverse effects. Accurate recommendations are needed in Portugal.

Study 2

Aim 2: To estimate the association between folate intake and folic acid supplementation in the periconceptional period and folate status in early pregnancy.
Methods 2: Cross-sectional analysis of data collected from 84 pregnant women, in one public hospital in Porto, with dietary assessment by a semi-quantitative food frequency questionnaire and food diaries, serum and erythrocyte folate status assessment, socio-demographic and lifestyle variables, past medical history, health status during pregnancy, and use of vitamin and mineral supplements.
Results 2: Intake of folate from diet was below the recommended level for pregnancy in 91.7% pregnant women. Although, the median *folate plus folic acid* intake was nearby 6 mg DFE, six-folder higher than the recommendations for folate intake plus supplementation of folic acid for prevention of NTD. The folic acid intake alone was 12-folder higher, without distinction from high risk women. Only 4.8% were classified as folate deficiency using serum folate assessments, but 13.1% through erythrocyte folate assays. It was identified a modest association between folic acid supplementation and maternal folate status. Being a planned pregnancy was the main determinant of using folic acid supplements before pregnancy.
Conclusion 2: The study identified a modest association between folic acid supplementation and folate status. It was shown a low intake of folate during pregnancy and a late initiation of the supplementation, indicating that guidelines are unlikely to be effective. Only being a planned pregnancy was the main determinant of starting folic acid supplementation in the preconception, as desirable.

General conclusion

Recommendations differed between countries, although the majority recommended 400 µg/day of folic acid at least one month before conception until three months of pregnancy. The recommended folate and folic acid vs. the real intake, differs greatly in the Portuguese population. The folate intake from diet is below recommendations and the folic acid

supplementation is 12-folder above international guidelines, and the supplementation happens frequently after the desirable period. High doses are associated with unmetabolized folic acid in the body, fact that is more and more associated with adverse effects in current researches. In Portugal new guidelines are needed, including quantitative recommendations regarding folic acid supplementation and emphasizing a healthy dietary pattern.

Keywords

Folate, periconceptional folic acid supplementation, neural tube defects, folic acid fortification, national recommendations for folic acid supplementation, women of childbearing age, folate status, folate intake assessment.

RESUMO

Introdução

A suplementação em ácido fólico no período periconcecional está associada à redução do risco de malformações do tubo neural. Mas em muitos casos esta suplementação ocorre após o período desejado, apresentando esta suplementação algumas limitações em termos de eficácia, ao contrário do que acontece com a fortificação dos alimentos em ácido fólico. Contudo, após um entusiasmo inicial no que concerne à fortificação em ácido fólico, novas preocupações surgiram em relação aos potenciais efeitos adversos decorrentes da fortificação massiva de alimentos e a suplementação com elevadas doses de ácido fólico, nomeadamente na ocorrência de efeitos adversos na gravidez com reflexos, a curto e a longo prazo, na saúde da mãe e do filho.

Relativamente à alimentação, tem sido demonstrado que quer o padrão alimentar como os métodos de confeção condicionam as quantidades de folato ingeridas, sendo o padrão alimentar dito saudável mais rico neste nutriente.

Partindo destes pressupostos, surgem algumas questões, tais como: O que está a ser recomendado em termos de ingestão de folato e de suplementação em ácido fólico no período periconcecional, em diferentes partes do mundo? Poderemos prever os níveis de folato maternos a partir da sua ingestão alimentar e da sua toma de suplementos? Precisaremos mesmo de recomendar a toma de suplementos no período periconcecional ou bastará fazer um correto aconselhamento alimentar? Será útil medir os níveis sanguíneos de ácido fólico antes de fazer o aconselhamento? Qual é a realidade portuguesa em termos de ingestão de folato e de suplementação de ácido fólico no período periconcecional e quais as recomendações existentes? Quais as consequências de uma elevada suplementação para as mães e para os filhos?

Este estudo pretendeu responder a dois objetivos específicos. Os objetivos, métodos, resultados e conclusões são descritos em seguida, separadamente.

Estudo 1

Objetivo 1: Sistematizar as recomendações oficiais relativas à ingestão de folato e de ácido fólico no período periconcecional, veiculadas por organizações nacionais de saúde de diferentes países do mundo.

Métodos *1:* Análise descritiva dos dados oficiais recolhidos a partir dos *websites* de organizações nacionais de saúde de 36 países de todo o mundo (25 países europeus, incluindo os 22 países da União Europeia; 4 países pertencentes ao BRICS; 3 países incluídos nos "Tigres Asiáticos"; os 8 países do G8; Austrália) e do *website* da Organização Mundial de Saúde, relativamente às recomendações para a ingestão de folato e de ácido fólico no período periconcecional.

Resultados 1: As recomendações diferem entre países, contudo a maioria recomenda 400 µg/dia de ácido fólico pelo menos um mês antes da conceção e durante os primeiros três meses de gravidez. Em Portugal não existem recomendações relativamente à dose de suplemento. Existe o consenso de que uma alimentação saudável é naturalmente rica em folato. A dose diária recomendada para a ingestão de folato durante a gravidez, reportada pelos diferentes países, pode ser resumida em três recomendações: 500 µg de folato, 550 µg de equivalentes de folato (EF) ou 600 µg de EF. Para mulheres em idade fértil as recomendações variam de 300 µg/dia de EF a 400 µg/dia de EF ou folato. Alguns países enfatizam a importância de uma alimentação saudável sem necessidade de suplementação em ácido fólico. Por oposição, outros aconselham elevadas doses de suplementação acrescidas de fortificação obrigatória.

Conclusão 1: As recomendações para ácido fólico em diferentes partes do mundo incidem predominantemente nos 400 µg de ácido fólico no período periconcecional, independentemente da situação epidemiológica do país em termos de ingestão e padrão de doença. Apesar da falta de uma recomendação quantitativa, em Portugal apenas são comercializados suplementos de ácido fólico de 5 mg, prescritos inclusivamente em mulheres com baixo risco de malformações congénitas. Elevadas ingestões de ácido fólico têm sido associadas a efeitos adversos na gravidez com reflexos, a curto e a longo prazo, quer para a mãe, quer para o filho, na saúde. São necessárias recomendações mais precisas em Portugal.

Estudo 2

Objetivo 2: Estimar a associação entre a ingestão de folato e a suplementação em ácido fólico, durante o período periconcecional, com os níveis maternos de folato.

Métodos 2: Análise transversal de dados relativos a 84 mulheres grávidas, num hospital público do Porto, incluindo avaliação da ingestão alimentar através de um questionário semi-

quantitativo de frequência alimentar e de diários alimentares, doseamentos séricos e eritrocitários de folato, recolha de dados sociodemográficos e de estilos de vida, história de saúde, estado de saúde na gravidez e toma de suplementos vitamínicos e minerais.
Resultados 2: A ingestão de folato proveniente da alimentação encontrava-se abaixo dos níveis recomendados para 91,7% das mulheres. No entanto, a mediana de ingestão do folato dietético acrescido do ácido fólico proveniente dos suplementos situava-se perto dos 6 mg de EF, seis vezes superior às recomendações para a prevenção das malformações do tubo neural. Considerando somente as recomendações para a suplementação em ácido fólico, a ingestão era 12 vezes superior, independentemente de se tratar de mulheres de elevado risco ou não. Somente 4,8% das mulheres apresentavam níveis baixos de folato sérico, mas 13,1% apresentavam níveis deficientes de folato eritrocitário. Observou-se uma fraca associação entre ingestão de folato e ácido fólico com os níveis sanguíneos maternos de folato. O facto de se tratar de uma gravidez planeada foi o principal determinante da suplementação na preconceção.
Conclusões 2: Foi identificada uma fraca associação entre suplementação em ácido fólico e os níveis maternos de folato. Encontrou-se uma baixa ingestão de folato na gravidez e um início de suplementação tardio para muitas mulheres, sugerindo que as recomendações existentes não estão a ser efetivas. O principal determinante para o início da suplementação na preconceção, como desejado, foi o facto de se tratar de uma gravidez planeada.

Conclusões gerais

As recomendações para ácido fólico em diferentes partes do mundo incidem predominantemente nos 400 µg/dia de ácido fólico no mês anterior à conceção, prolongando-se durante os primeiros 3 meses de gravidez. As recomendações para folato e para ácido fólico diferem bastante do que é praticado pelas mulheres portuguesas. A ingestão dietética de folato encontra-se abaixo das recomendações e a suplementação é cerca de 12 vezes superior às recomendações internacionais, ocorrendo frequentemente após o período no qual a suplementação apresenta maior benefício. Elevadas doses de suplemento associam-se a elevadas doses de ácido fólico não metabolizado no organismo, que parecem associar-se a efeitos adversos, segundo a investigação atual. Em Portugal são necessárias novas recomendações, incluindo orientações quantitativas relativamente à suplementação em ácido fólico e enfatizar a importância da prática de uma alimentação saudável.

Palavras-chave

Folato, suplementação periconcecional em ácido fólico, defeitos do tubo neural, fortificação em ácido fólico, recomendações para a suplementação em ácido fólico, mulheres em idade fértil, níveis séricos de folato, avaliação da ingestão de folato.

INTRODUCTION

1. Folic acid in the periconceptional period: benefits and potential risks

The findings, in 1991, by the British Medical Research Council(1), and, in 1992, by the Hungarian National Institute of Hygiene, a World Health Organization (WHO) Collaborating Center for the Community Control of Hereditary Diseases, about the effect of folic acid supplementation in the periconceptional period in reducing the risk of Neural Tube Defects (NTD)(2), were revolutionary and it maintains consensual nowadays(3).

In the British study was carried out a randomised double-blind prevention trial at 33 centres in seven countries, which results showed that folic acid supplementation of 4 mg, from before conception until 12 weeks of pregnancy, reduced the risk of recurrence of NTD by 72%(1). Regarding serum folate, at last visit before becoming pregnant (median 7 days) the value was substantially higher in the folic acid supplemented groups (varying from 23 to 194 ng/ml from percentile 10 to percentile 90) than the unsupplemented groups (varying from 3 to 9 ng/ml, respectively). The erythrocyte folate results were similar, so compliance was good(1). In the end of the study, besides the recommendations of folic acid supplementation for preventing recurrence of NTD, was recommended that public health measures should be taken to ensure that the diet of all childbearing women contains an adequate amount of folate(1).

In the Hungarian study, the folic acid supplementation of 0.8 mg for at least one month before conception and until the date of the second missed menstrual period (approximately 8 weeks of pregnancy) decreased by 58% the incidence of a first occurrence of neural tube-defects(2). Furthermore, folic acid seems to prevent other congenital anomalies (cleft lip(4, 5) and cardiovascular malformations), but the results were not completely consistent(2, 6-8).

In order to prevent NTD, and considering that neural tube closes by day 28(9) (4 weeks after conception – conceptional age – corresponding to the 6 weeks of pregnancy – gestational age), when most women do not know they are pregnant(8), even in the planned pregnancies, folic acid supplementation should occurs in the periconceptional period(3, 10). Periconceptional period is commonly defined generically as occurring around the time of conception, from before conception to early pregnancy, but some authors establish a more specific definition, as a 5-6 months period in women embracing oocyte growth, fertilization, conceptus formation and development to week 10 of gestation (coinciding with the closure of the secondary palate in the embryo) (Figure 1)(11).

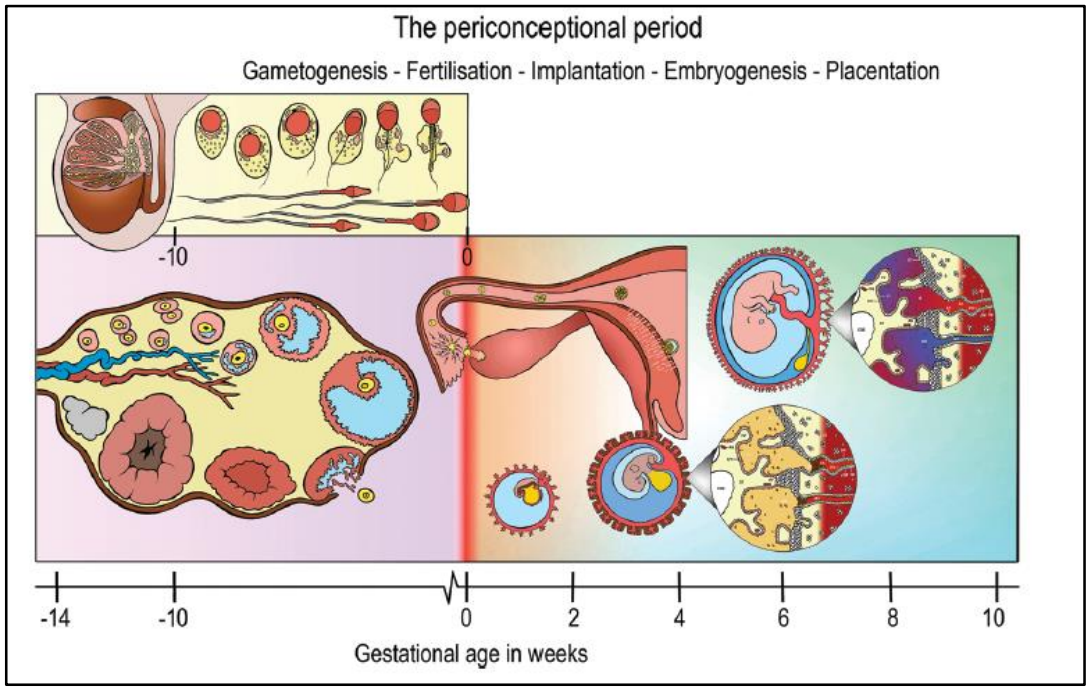

Figure 1. The periconceptional period in humans, depicting the five stages of reproductive development against their time course in weeks (adapted from Steegers-Theunissen et al. 2013 (11)).

For that reason, supplementation occurs frequently after the desirable period(12, 13). Moreover, there is not only one recommendation regarding dosage of folic acid supplementation, it is variable from different countries and different entities (Chapter 1). But real intake and supplementation commonly do not reflect recommendations (Chapter 2).

High blood levels of folate were associated with a lower risk of cardiovascular disease and cancer, not confirmed by randomised trials(14). And folic acid supplementation has been associated with a lower risk of stroke(15, 16). Additionally, folic acid due to its water-soluble character was traditionally considered safe(17).This motivated some countries to opt for folic acid fortification in cereal products, namely flour. Nowadays, flour fortification with folic acid is mandatory in 47 countries, mainly in the continents of America and Oceania, in the Middle East and few in Africa(18, 19).

After mandatory fortification, many questions arise, regarding individual liberty (to choose fortify or not fortify foods) and the concern about feasible monitoring of potential hazards of the fortification.

In the Dietary Reference Intakes (DRI), the only adverse effect considered that may result from excess intake of folic acid was the onset or progression of neurological complications in people with vitamin B_{12} deficiency(3). However, it was emphasized that the studies that evaluated the periconceptional use of supplemental folic acid (in doses of approximately 0.4 to 5.0 mg) to prevent NTD, were not specifically designed to assess adverse effects(8), and the recent studies are inconclusive, so more studies are considered needed(20).

Regarding folic acid in fortified foods (folic acid is added to refined cereals to a level that doubles the amount lost during the refining process(21)), no proven adverse effects have been associated with until now, although more studies are needed to analyse new data(20). However, it has been discussed the possible carcinogenicity of folic acid(8, 22), supported by biologically plausible mechanisms - folate is essential in biological methylation reactions and nucleotide synthesis and impairment of these processes are thought to be involved in cancer development(14). Some researchers suggested that could exist a link between high folic acid intake and cancer incidence(23, 24), but this is not consensual(14, 25, 26).

It is known that through exposure to mandatory food fortification and vitamin supplement use, large populations have an unprecedented high folic acid intake(27). And that the capacity of the body to convert folic acid to 5-methyl-THF is limited(28). Unmetabolized folic acid has been detected in the systemic circulation following folic acid supplementation and ingestion of fortified foods at oral doses above 260 μg(28), exposing body tissues to a form of the vitamin not encountered before(29). Another research shown that the absorption and biotransformation process of folic acid is saturated at a maximum dose of 400 μg (not at 100 μg neither 200 μg)(30).

In other study it was observed that plasma concentrations of unmetabolized folic acid remained low even though they were consuming higher doses (5 mg) and that hepatic metabolic capacity is highly variable(31). Approximately 40% of older adults in USA have unmetabolized folic acid(32). And it was found an inverse relation between the presence of unmetabolized folic acid in plasma and natural killer cell cytotoxicity, but further studies on immune function and health are needed(27). Given the possibility that excessive folic acid exposure may relate to cancer risk, monitoring the long-term effect should be warranted(32-34) especially considering that the vast majority of the population are not at risk of NTD(32).

It can be considered that high doses of folic acid may act as a xenobiotic and not a nutrient(35). In one animal study (mice), it was shown that methyl supplements (including folic acid) have strong effects on DNA methylation and phenotype and are likely to affect long-term health, so optimum, rather than minimal, maternal dietary supplements for the health and longevity of offspring should be intensively investigated(36).

The biochemical properties of the folate and folic acid modelled pathway of a theoretical study suggest that there is an optimum folate concentration above which folate-reaction velocities decline(37). This requires confirmation in experimental studies, yet illustrates how an interdisciplinary approach can help understand different aspects of this complicated pathway(37).

One recent meta-analysis showed no benefit but a borderline significant increase in incidence of overall cancer in the folic acid group compared to controls(38). When analysing site-specific cancers, prostate cancer was the only cancer type where an increase in risk was shown for

folic acid supplements(38). The study did not show any evidence for augmented cancer risk for fertile women who are recommended folic acid periconceptionally in order to reduce risk of NTD(38). The limited time of follow-up of most randomized control trials was a limitation of that review because the time frame for cancer development might exceed the follow-up time in many randomized control trials(38). The highly selected population in which most of the studies were conducted limits the applicability to the general population and women of childbearing age taking folic acid periconceptionally(38).

Many robust studies about this topic have emerged. A recent a meta-analysis of randomized clinical trials concluded that folic acid supplementation has no significant effect (independently of the dose and the duration of the supplementation) on total cancer incidence, colorectal cancer, prostate cancer, lung cancer, breast cancer or haematological malignancy, but reduces the risk of melanoma(39). Concerning supplemented women, a study suggested that folic acid supplements of 400 µg or more per day, in postmenopausal women, may increase the risk of breast cancer(40).

In 2007 the World Cancer Research Fund consider that the epidemiological data on the relationship between folic acid fortification or supplementation and cancer risk were too inconsistent or limited(41). Concerning folate, the conclusion was different: foods containing folate (but not folic acid supplements) probably protect against pancreatic cancer(41).

Some authors consider that safe and effective amounts of folic acid fortification need to be scientifically determined by using relevant animal, experimental, and clinical models(34). The potential cancer-promoting effect of folic acid fortification in the vast majority of the population of countries with mandatory fortification, who are not at risk of NTD but have been unintentionally exposed to high amounts of folic acid, is a legitimate public health concern and needs careful monitoring(34).

No adverse effects have been reported with the consumption of excess folate from foods(29).

Recently, a new concern arises regarding the potential adverse effects of universal maternal supplementation on adverse pregnancy outcomes (results of conception and ensuing pregnancy(42)), early or later in life, both in mother and child. Studies has been associated maternal folic acid supplementation with an increased birthweight(43-46), insulin resistance in children(47), and asthma in children(48, 49).

Regarding birthweight, there is strong evidence of an inverse association between blood folate concentrations and risk of low birthweight(43-46). Folic acid supplementation increased birthweight in studies in Africa and India, being this is an important issue for developing countries(44). In a review, folic acid at high doses (5 mg) was associated with reduced risk of low birthweight(43). In a meta-analysis and systematic review of randomized controlled trials studying the effect of supplementation (with minimum duration of 12 weeks) during the second and third trimesters (the general supplementation on this period is not consensual), was found

a significant dose–response relationship between *folate plus folic acid* intake and birth weight (2% increase in birth weight for every two-fold increase in *folate plus folic acid* intake)(45). In another recent review, folic acid supplementation during pregnancy was associated with improvements in the mean birthweight(46).

In what concerns insulin resistance, in a study of pregnant women in India, maternal erythrocyte folate status during pregnancy was associated positively with insulin resistance in children at 5 years(47). Based on the supposing that folic acid may be involved in the health programming on the fetus and that *in utero* development may have a rule in metabolic syndrome (due to nutritional, hormonal and metabolic changes) it was done a study in animals (female rats) to investigate if folic acid supplementation in high doses during periconceptional period interfere in the health programming of the fetus to develop metabolic syndrome in the postnatal life(50). The results showed that the periconceptional folic acid supplementation in high doses may produce an impaired metabolic function in the new-born (during post natal life: more weight gain; higher glycaemic values; impaired glucose tolerance as referred before; higher insulin values; higher leptin values; lower adiponectin values) and in the mother (more weight gain during the pregnancy)(50). According to the author, this finding should be take into account to change actual public health and nutrition politics related to folic acid(50).

Another animal study (with rats) suggested that folate status in pregnancy can influence cardiovascular function in the mother during pregnancy and the developmental origins of cardiovascular disease (lower risk) in the offspring in the long term(51). It is a good example of how a micronutrient supplement (folic acid) can ameliorate the adverse effects of macronutrient imbalance (in this study, a low-protein diet) in pregnancy(51).

Although the folate metabolism of animals differs from that in humans(52), they are indispensable for a better understanding of nutrient-gene interactions in normal pregnancies, as well as in those affected by metabolic diseases, such as diabetes and obesity, allowing nutritional supplementation to become more specifically targeted(53).

On the topic of childhood asthma, a recent revision concluded that maternal folic acid intake (both in early and later pregnancy) was associated with a short-term increased incidence of allergy-related respiratory impairment in children(48). In one prospective birth cohort study from Australia, maternal folic acid supplementation between 30 and 34 weeks of pregnancy (median daily intake: 300.0 µg; range 27.4–5,895.4 µg) was associated with a 26% increase in the relative risk of physician-diagnosed asthma at 3.5 years, but not at 5 years, while there was no association with folic acid supplementation before 16 weeks of pregnancy (median 658.3 µg; range 42.9–5,500.0 µg)(49). Similarly to these results related to first trimester, a recent meta-analysis did not support the association between periconceptional folic acid supplementation and increased risk of asthma in children, but additional research was considered needed(54). By contrast, a study taken in the Norwegian Mother and Child Cohort

(a large population based pregnancy cohort following more than 100,000 pregnant women and their offspring) found that exposure to folate supplements in the first trimester of pregnancy may be associated with increased risk of wheeze and lower respiratory tract infections up to 18 months of age(55). They concluded that early childhood respiratory health may be affected by possible epigenetic influences of methyl donors in maternal diet during pregnancy(55). Some years later, in the same cohort, through a nested case-control study, was found that higher levels of plasma folate measured during the second trimester of pregnancy were modestly associated with increased risk of asthma at age 3(56). The authors consider that even if this association is confirmed in additional studies, they do not negate the value of folic acid supplementation in pregnancy. In the end they emphasize that additional studies might delineate levels below which adverse effects are unlikely and help to fine tune public health recommendations to maximize benefits(56).

Others pregnancy outcomes, such as placental abruption, placental weigh or gestational age remains inconclusive(43-46). There are some studies describing an inverse association between blood folate concentrations during pregnancy and the risks of placental abruption and preterm delivering or small-for-gestational-age infants(44). In contrast, one recent review found that folic acid supplementation has no impact on pregnancy outcomes such as preterm delivery, and stillbirths/neonatal deaths(46). In another review, folic acid supplementation was not associated with any difference in mean placental weight or gestational age(43). Similar results were found in a recent meta-analysis (the exposure measured was folic acid supplementation in second and third trimesters)(45). In a recent study, in the already mentioned cohort (Norwegian Mother and Child Cohort), was not found a protective effect of dietary folate intake or folic acid supplementation on spontaneous preterm delivery(57). Preconceptional folic acid supplementation starting more than eight weeks before conception was associated with an increased risk of preterm delivery, but the results require further investigation(57).

In a study about folic acid and deoxyribonucleic acid (DNA) methylation, was concluded that periconceptional maternal folic acid use was associated with epigenetic changes in the child that may affect *in utero* programming of growth and development with consequences for health and disease throughout life(58). In another study, folic acid use after 12 weeks of gestation influences offspring repeat element and imprinted gene methylation, but further studies are needed to understand these epigenetic effects(59).

It seems that the relationship between maternal folic acid supplementation and pregnancy outcomes display a U-shaped association, with adverse effects both with low and high dosages.

In order to supply new recommendations and to define the adequate range of intake, more investigation is needed(48) and a crucial issue is the accurate measure of *folate plus folic acid* intake (folate by diet, folic acid by fortified foods and supplements).

2. Folate and folic acid

2.1 Definition and sources

Folic acid was coined in 1941 from Latin *folium* (leaf) plus *ic* (origin)(60), because of the abundance of folate in green leaves.

Folate, a water-soluble vitamin B complex (B9), is a group of approximately 150 compounds, typically in the form of pteroylpolyglutamates(61) (Figure 2), naturally found in a wide range of vegetal (e.g., yeast, leafy green vegetables, pulses, whole cereals, fruits, nuts) and animal foods (e.g., liver, eggs), as it can be observed in Table 1. Data available simultaneously in the Portuguese(62) and the American(63) food composition tables shows, with few exceptions, similar folate content.

Folic acid, a pteroylmonoglutamic acid, is the synthetic form used in vitamin supplements and in fortified cereal products(3).

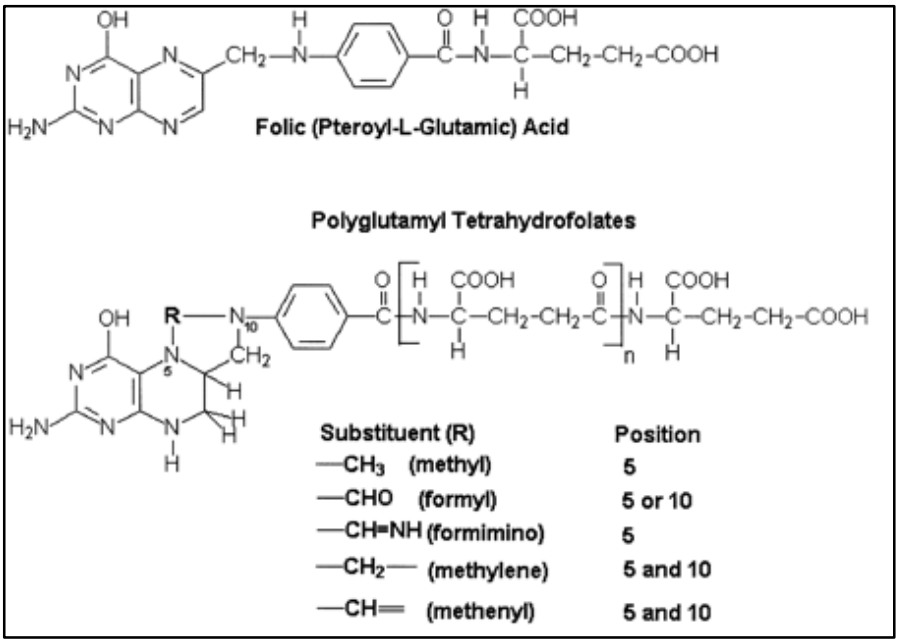

Figure 2. Chemical structures of common folates (adapted from Gregory 2001(64)).

Table 1. Folate content in raw foods, by food groups, from two different food composition tables.

		Portuguese Food Composition Table(62)	American Food Composition Table(63)
Food group	Food item, raw	Folate, DFE* (µg/100 g)	
Unspecified	Yeast, baker's, active dry	2500	2340
Vegetables	Watercress	200	9
	Asparagus	175	52
	Savoy cabbage	150	80
	Spinach	150	194
	Brussels sprouts	135	61
	Beet	109	109
	Broccoli	90	63
	Cabbage sprouts	90	-
	Leeks	87	64
	Green bean	80	33
	Kale	78	31
	Portuguese cabbage	78	-
	Artichoke	74	68
	Cauliflower	66	57
	Lettuce	55	38
	Mushrooms	44	17
	Celery	16	36
Pulses	Cowpeas, dried	630	633
	Butter bean (lima bean), dried	360	395
	Soybean, dried	328	375
	White beans, dried	300	388
	Chickpeas, dried	180	557
	Broad beans (fava beans), dried	145	423
	Lentils, dried	110	479
	Peas, fresh	62	65
Cereals, cereal products, and potatoes	Wheat, germ	-	281
	Chestnut kernels, peeled	61	58
	Oats, rolled	56	32
	Rice, Brown	55	20
	Biscuits whole wheat cereal, Weetabix	50	46
	Potato	35	18
	Spaghetti, dry, unenriched	34	18
	Bread, wheat	29	99
	Corn, dry	26	19
	Rice, white	19	9
Fruits	Strawberry	47	24
	Kiwifruit	42	25
	Mango	36	43
	Raspberry	33	21
	Orange	31	34
	Tangerine	21	16
	Banana	14	20
Nuts	Peanut kernels	110	240
	Walnut, kernels	66	98
	Pistachio	58	51
	Pine nut, kernels	57	34
	Almond, kernels with skin	49	50
Milk and dairy products	Cheese, camembert	50	62
	Cheese, *flamengo*, 45% fat	39	-
	Yogurt, flavoured, semi-skimmed	6	9
Meat, fish, seafood and eggs	Beef, liver	330	290
	Egg, whole, fresh	50	47
	Meat, Beef	16	5
	Poultry, Chicken	13	9
	Fish, Salmon	10	18
Fats and oils	Olive oil	0	0

- Blank mean that no information was available. *DFE: Dietary Folate Equivalents (µg) = food folate (µg) + (1.7 × folic acid (µg))(3, 21). Note: to build this table it was chosen the folate richest foods of each food group according to the Portuguese Food Composition Table(62).

2.2 Intake and availability

Folate, being a group of compounds in the reduced form, is easily oxidized(61) becoming inactive, opposed to folic acid, a stable fully oxidised molecule(65). The degree of loss can be influenced by several factors, including extreme shifts on pH, heat, light(66), and also oxygen content, metal ion concentrations, antioxidant levels, duration and product:water ratio(65). Folate losses are also highly dependent both on the food (e.g., more losses in vegetal than animal origin food (Table 2), more in bigger surface area vegetables(67), and method of cooking (e.g., soaking(68); more on boiling vegetables than steaming – although folate loss from boiling vegetables is mainly as a result of leaching, and not as a result of degradation, being folate preserved on the cooking water(65, 69)). Losses of 50% to 90% may occur during storage, cooking, or processing at high temperatures(61). Moreover, the amount of folate available in processed and stored foods can be significantly lower than in raw products. In a recent study was observed that pre-treatment and freezing technology significantly decreased folate content only in vegetables with the largest degree of fragmentation and the smallest size, and that folate content decreased with the time of frozen storage(70). For example, in frozen cauliflower, the folate loss exceeded 95% compared to the fresh product just after the third month of frozen storage, and in green and yellow beans, significant folate losses (at the level of 75% and 95%, respectively) were observed no earlier than after nine months of frozen storage(70).

For this reason it is recommended that public health efforts should incorporate practical advice on storage and cooking to increase folate intake, helping to optimise folate status(65).

Table 2. Folate content of a healthy nutrition plan (*prudent dietary pattern*) compared to an unhealthy plan (*Western dietary pattern),* with 2000 kcal, built with raw vs. cooked foods

				Prudent dietary pattern* (2000 kcal)			***Western dietary pattern***** (2000 kcal)		
Food group	One food portion Raw food	Folate, DFE (µg/***per portion) Raw food	Nutrient Retention Factor**** Cooked food	Number of food portions per day	Folate, DFE (µg/day) **Raw food**	Folate, DFE (µg/day) **Cooked food**	Number of food portions per day	Folate, DFE (µg/day), **Raw food**	Folate, DFE (µg/day), C**ooked food**
Vegetables	180 g	79 (n=30)	70	4.0	316	221	2.0	158	111
Pulses	25 g dried	70 (n=11)	45	1.5	105	47	0.2	14	6
Cereals, cereal products, potatoes	35 g dried	11 (n=28)	75	7.0	77	58	5.8	64	48
Fruits	160 g	16 (n=30)	50	4.0	64	32	2.0	32	16
Nuts	15 g	10 (n=6)	80	-	-	-	-	-	-
Milk and dairy products	250 ml of milk	10 (n=39)	80	2.0	20	16	2.0	20	16
Meat, fish, seafood, and eggs	30 g	3 (n=57)	80	2.8	8	6	7.0	24	19
Fats and oils	10 ml of oil	0 (n=15)	-	1.8	0	0	4.8	0	0
Total			65*****		**590**	**381**		**312**	**216**
Total reheated			95			**362**			**205**
Total with raw fruit						**413**			**232**
Comparison						**≈ 50% less of folate in the unhealthy plan**			

DFE: Dietary Folate Equivalents.

*According to the Food Guide for the Portuguese Population(71). The value of 2000 kcal was obtained through the estimated calorie needs for groups(21), based on the Estimated Energy Requirements, considering the female group in the childbearing age (from 14 to 50 years(3)), moderate physical active. The food portions were used according to the Food Guide for the Portuguese Population(71). The folate content of the portions was calculated based on the Portuguese Food Composition Table(62).
**Characterized by higher consumption of the food groups Meat and Fats and lower of Fruits and Vegetables(72, 73). The number of food portions were obtained considering the proportions of the Portuguese Food Balance(73).
***Median folate content per portion (n = number of varieties(62) included per food group).
****Median Nutrient Retention Factor. True retention is defined as the measure of the proportion of the nutrient remaining in the cooked food in relation to the nutrient originally present in the raw food(74, 75). The resulting values account for the nutrient content retained in a food after losses due to heating or other food preparation steps (e.g., baked, boiled with water drained, boiled with water used, broiled, dried in some fruit, fried, poached, simmered, steamed, roasted)(75).
*****The global Nutrient Retention Factor for folate in the healthy nutrition plane was calculated (total folate cooked ÷ total folate raw x 100).

Apart from cooking losses, the folate intake also depends on the dietary pattern. Through the analysis of the Table 2, it is possible to conclude that the folate content of the healthy nutrition plan (*prudent dietary pattern*) is clearly superior to the unhealthy plan (*Western dietary pattern*), with a value of 413 µg of Dietary Folate Equivalents (DFE) (considering cooked food, except fruit), that is similar to the Recommended Dietary Allowances (RDA)(3) for women in the childbearing age. Therefore, a *prudent dietary pattern* gives the appropriate amount of folate, as it is expected for every nutrient. The value could be even higher if the richest folate foods of each food group were included in a higher proportion and if the methods of preparing and cooking were only the best to minimize cooking losses (e.g., cutting the vegetables in big pieces, steaming, boiling for few time and using the water).

These results seem to be consistent with other research which showed that dietary patterns have clearly a significant rule in folate intake(72). A *prudent dietary pattern* (Table 2) (compared to a *Western dietary pattern* and other patterns), even in the era of fortification, may decrease the risk of NTD and some heart defects in folic acid nonusers(72), representing in United States of America (USA) 60% of women of childbearing age(76). It was concluded that in preconceptional counselling should be emphasized the need to make healthy dietary choices to optimize not only pregnancy outcomes but also maternal health(72).

A different way of rising folate status is trough concentrate capsules of fruit and vegetables, a more natural source compared to folic acid supplements, and without shown side effects. In a double-blind placebo controlled study, the results showed that the supplementation with a concentrate of fruit and vegetable juice in capsules (provided approximately 420 µg folate), in the absence of dietary modification, increased serum folate by 174.3% (from 8.16 to 22.4 ng/ml, in average), that was correlated with a decrease in plasma homocysteine of 19.9%(77).

Another important issue is the folate bioavailability. It is accepted that it could range greatly between 25 to 50%(3), depending on the chemical structure(3, 64, 78), the presence of inhibitors and binders(61), and the person's nutritional status (e.g., deficiencies of iron and vitamin C can impair folate use)(61). In some vegetables and citrus fruits it can reach 60% to 98% of bioavailability(79). An additional factor is the diet composition, but this is a theoretical problem, since we cannot quantify this effect(8, 64).

Regarding folic acid (the already mentioned synthetic form) it appears to be highly bioavailable, but is affected by the circumstances of the intake: nearly 100% taken on an empty stomach, and about 85% when added to foods(3, 78).

Trying to adjust the referred differences of bioavailability, the Institute of Medicine defined the concept of DFE that places all sources of folate and folic acid on a comparable basis [DFE (µg) = folate (µg) + 1.7 × folic acid (µg)], to establish the RDA(3). The factor 1.7 is the ratio between the bioavailability of folic acid consumed with food (85%) and the bioavailability of folate (assumed to be 50%). This factor may change in the future, when more accurate estimates arise(64).

2.3 Absorption, Transport, Storage, Metabolism and Excretion

After intake, folate is firstly hydrolysed to monoglutamate forms in the gut and then absorption occurs by active transport mainly in the jejunum(61). When folic acid is used, since it is already in a monoglutamate form, it also can be absorbed by passive diffusion(3, 8). In the intestinal mucosal cell folate is reduced to tetrahydrofolate (THF), the active form of folate(61). Folate is taken up from the portal circulation by the liver, where it is metabolized and retained or released into the blood or bile(3). About half of total body folate is stored in the liver, as polyglutamates of 5-methyl-THF and 10-formyl-THF(61). Approximately two-thirds of folate in plasma is bound to protein. A part of the folate is excreted in the urine, bile, and faeces(80). Some reports indicate that daily folate excretion on a normal diet ranges from 5 to 40 µg/day(8, 81). A study of eight healthy pregnant women, not on supplementary folic acid, found a substantial increase in folate excretion from a non-pregnant mean of 3.5 µg/day to a mean of 14.0 µg/day during pregnancy(82).

THF (with its bound components) acts as a co-enzyme in many synthesis reactions in the metabolism of amino acids and nucleotides by donating or accepting single carbon units (e.g., formation and maturation of red and white blood cells; *de novo* synthesis and repair of DNA; synthesis of methionine from homocysteine (Figure 3) – this conversion also requires vitamin B12, which deprivation can lead to a functional secondary folate deficiency – both having essential role in gene stability)(8, 61).

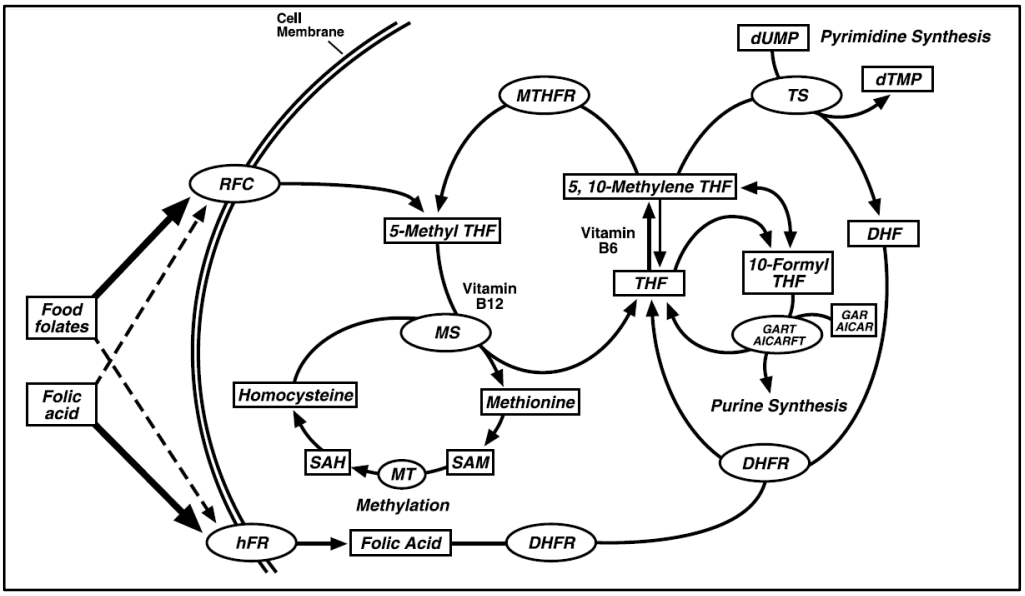

Figure 3. Folate and folic acid metabolism (adapted from Ulrich et al. 2006(37)).

THF: tetrahydrofolate; DHF: dihydrofolate; RFC: reduced folate carrier; hFR: human folate receptor; MTHFR: 5,10-methylenetetrahydrofolate reductase; DHFR: dihydrofolate reductase; GART: glycinamide ribonucleotide transformylase; AICARFT: 5-amino-imidazole-4-carboxamide ribonucleotide transformylase; AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide; GAR: glycinamide ribonucleotide; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine, SHMT: serine-hydroxy-methyltransferase; MS: methionine synthase; TS: thymidylate synthase; MT: methyltransferases.

There is evidence that folate may interact with several substances – alcohol (in a chronic intake), tobacco, drugs (e.g., nonsteroidal anti-inflammatory, in very large therapeutic doses; anticonvulsant, in chronic use; anti-cancer and anti-autoimmune diseases methotrexate, in chronic therapy; antibiotic trimethoprim; diuretic triamterene; antimalarial pyrimethamine; anti-inflammatory bowel diseases sulfasalazine) – by different mechanisms (e.g., substance associated with low intake, impair intestinal absorption and hepatobiliary metabolism, increase renal excretion, antifolate activity), increasing folate requirements(3). Oral contraceptives containing high levels of oestrogens were suggested to have adverse effects on folate status, but its use has not been reported to influence folate status in large-scale population surveys or in metabolic studies in which dietary intake was controlled(8).

About dietary interactions related to dietary fibre, a suggestive evidence of a positive association between dietary fibre intake and folate status in humans was reported, and in animals, evidence indicated that synthesis of folate by intestinal bacteria influences folate status(8).

Folate and its derivatives are involved in numerous biochemical reactions that are catalysed by many different enzymes, consequently, folate metabolism is under genetic control, and as expected, genetic heterogeneity(8, 20), but not yet well understood(44). Polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) may be involved in this heterogeneity, as well as in the methylenetetrahydrofolate dehydrogenase (MTHFD1)(20). Also polymorphisms in the reduced folate carrier gene seems to be a NTD risk factor, in one studied on Chinese population(83).

Folate requirements are increased in specific physiological (pregnancy and lactation) and pathological conditions. During pregnancy occurs a marked acceleration in single-carbon transfer reactions, due to uterine enlargement, placental development, expansion of maternal erythrocyte number, and foetal growth(8, 9, 84).Additionally, folate is excreted by urine to a greater extent(85). Higher folate requirements are also well documented in chronic use of some therapeutic drugs, burns, hepatitis, infection, inflammatory diseases, cancer, surgery, dialysis, haemolytic anaemia, and malabsorption states (e.g., celiac disease, Crohn's disease)(86).

In summary, factors considered when estimating the folate requirement include the bioavailability of folic acid and folate, nutrient interactions (coexisting Iron or vitamin B12 deficiency may interfere with the diagnosis of folate deficiency), interactions with other food components, smoking, folate-drug interactions, and genetic variations(8).

2.4 Folate status assessment

The more consensual biomarker for folate status is its assessment in the erythrocytes, sometimes in conjunction with plasma homocysteine concentrations(61).

Erythrocyte folate concentration is an indicator of long-term status, because uptake of folate occurs mainly during erythropoiesis and the erythrocyte lifespan is about 120 days(8). Another possibility is the determination of the serum (or plasma) folate concentration, however it is a short-term status indicator of dietary folate intake (decreases in intake may be reflected in serum levels within 1 to 3 weeks(8). For that reason, in population surveys it is generally assumed that measuring alone serum levels does not differentiate between what may be a transitory reduction in folate intake or a chronic folate deficiency accompanied by depleted folate stores and functional changes(8). It may be a useful diagnostic test if interpreted correctly in conjunction with other folate status indices(8).

Another possibility could be urinary folate, however this biomarker is not a responsive indicator of folate status, because excretion continued even in the face of advanced folate depletion(8).

Regarding the cut off points, an erythrocyte folate < 305 nmol/l (140 ng/ml) and a serum folate < 7 nmol/l (3 ng/ml) indicates a negative folate balance(8). The concentrations suggested by WHO for defining folate deficiency, based on metabolic indicators, are slightly different: < 340 nmol/l (151 ng/ml) for erythrocyte folate and < 10 nmol/l (4 ng/ml) for serum folate(44). The conversion factor used to transform the units (nmol into ng) was 2.265(44).

Moreover, for assessing folate status in population in all age groups, using macrocytic anaemia as a haematological indicator, WHO establish the following value for erythrocyte/red blood cell folate: deficiency < 226.5 nmol/l (< 100 ng/ml). And values for serum/plasma folate: deficiency < 6.8 nmol/l (3 ng/ml); possible deficiency 6.8-13.4 nmol/l (3-5.9 ng/ml); normal range 13.5-

45.3 nmol/l (6-20 ng/ml); elevated (based on the assay's upper limit capabilities without dilutions, and not on biological implications for health) > 45.3 nmol/l (> 20 ng/ml)(87).
An average decline in serum folate of 10 nmol/l (from 20 to 10 nmol/l) during the 40 weeks of gestation, may represent a physiologic response to pregnancy, but the mechanism is unknown(88). The pattern of changes in erythrocyte folate varies, with a decline observed in early pregnancy followed by a slight increase in mid-pregnancy. The most plausible causes for the declines in blood folate include increased folate demand for the growth of the fetus and utero-placental organs and low folate intake(88).

Another important topic is the analytical method. Universal highly accurate method does not exist, being more difficult to establish universal cut-off points(8), but it has been developed(20). Erythrocyte is the more time-consuming and costly test because before analysis requires sample pre-treatment – and this introduces additional variation into results from different laboratories(89). Others analytical issues that influence results are reductions in the oxygen saturation of haemoglobin and decreased haematocrit, both increase the results(89). The other biomarker, serum folate, more frequently showed the higher correlation with homocysteine, a sensitive marker of deficiency(89). Similarly, both plasma and erythrocyte folate were shown to increase in response to folic acid supplementation and fortification(89). However, plasma folate generally gave the greater response at both short- and long-term follow-up and was able to distinguish different supplementation doses(89).
Studies have shown that very few patients would have their clinical outcome altered by the measurement of erythrocyte folate in addition to serum folate(89). However, serum folate is the main marker in patients with vitamin B_{12} deficiency(89). In contrast, after haemodialysis sessions erythrocyte folate gives the better assessment of folate status(89).
Both markers could assess risk of a current pregnancy being affected by a NTD(89). However, between pregnancies erythrocyte folate appears to be the better risk indicator(89). Studies looking for an association of erythrocyte folate with NTD risk based on estimating erythrocyte folate levels in blood samples taken early in pregnancy are preferred because maternal folate status is likely to change during pregnancy and postpartum(8). MTHFR polymorphisms may alter folate concentrations in populations with low folate status Both serum and erythrocyte folate are able to identify these changes(89). Overall then, as a routine test of folate status, serum folate appeared to offer the best combination of test cost and clinical information, because it is cheaper and faster to perform than erythrocyte folate, is influenced by fewer analytical variables and provides an assessment of folate status that may be better than erythrocyte folate(89).
In an original study, folate status of Japanese women of childbearing age was marginally deficient, with inadequate concentrations of serum folate (the mean concentrations were

5.4±2.1 ng/ml; it was low in 12.1% of the subjects (< 3.0 ng/ml) and was marginal in 58.6% (3.0-5.9 ng/ml)), largely due to insufficient folate intake (the mean folate intake was 262±111 µg/day)(90). The level at which folate deficiency (serum folate, < 3.0 ng/ml) disappeared, corresponded to a daily intake of folate exceeding 349 µg/day(90).

In a study done with Belgium pregnant women, the median erythrocyte folate concentration was 436 ng/ml among first trimester and 496 ng/ml among third trimester(12). Few had an erythrocyte folate concentration below 140 ng/ml, indicating depletion of folate stores(12). In the first trimester, 39% of women had an erythrocyte folate concentration below 400 ng/ml, whereas 15% of the first trimester women had an erythrocyte folate concentration below 300 ng/ml(12). Among women in the first trimester, 69.1% reported taking folic acid supplements of which 41.2% started taking them before pregnancy(12). For third trimester, these percentages were 76.2 % and 21.9%, respectively(12). In both trimesters, folate status increased significantly with education level and was significantly higher among women who planned the pregnancy and who did not smoke(12). Another study shows that the conjunction of *folate with folic acid* intake significantly increased the folate concentration levels in serum and in erythrocytes by 47% and 23%, respectively, and lowered the levels of plasma homocysteine by 7%(91). This dose-response result may in future be applied for deriving the intake dose necessary to achieve the optimal level of a folate biomarker for women of childbearing age, as well as for pregnant and lactating women(91).

Aims of the study

These study aimed:

1. To summarize the recommendations on folate and folic acid in the periconceptional period provided by official health organizations in different countries worldwide (Chapter 1);
2. To estimate the association between folate intake and folic acid supplementation in the periconceptional period and serum folate and erythrocyte folate levels in early pregnancy (Chapter 2).

CHAPTER 1 – FOLATE INTAKE AND FOLIC ACID SUPPLEMENTATION IN THE PERICONCEPTIONAL PERIOD: RECOMMENDATIONS FROM OFFICIAL HEALTH ORGANIZATIONS IN 36 COUNTRIES WORLDWIDE

INTRODUCTION

Folate intake worldwide

The population reference intake set by the Scientific Committee on Food of the European Union (EU) is 200 µg/day for adults, and 400 µg/day in pregnancy(92). In European countries the average folate intake in adults was found to be remarkably similar, around 300 µg/day in men, and 250 µg in women(93).

This values are satisfactory regarding adult population, but clearly insufficient for pregnant women and women intended to get pregnant(29). For the last ones an intake higher than 400 µg/day is considered desirable, namely to protect against NTD(29). More than 90% of women in the childbearing age range dietary folate intakes below this optimal level(29).

More recent assessments, detailed in the European Health Report of 2009, showed that the mean intake of folate, expressed in DFE, was between 203 and 494 µg/day in men and between 131 and 392 µg/day in women(94), therefore the reference value by the German language countries (D-A-CH: an acronym used to represent the dominant states of the German language *Sprachraum*, related to reference values for nutrient intake by German Society of Nutrition, the Austrian Society of Nutrition, the Swiss Society of Nutrition Research and the Swiss Association of Nutrition, which are applied also in Hungary, Slovenia and the Czech Republic) of 400 µg/day DFE(95) was not met by any country(94), Nordic countries also established specific own recommendations (NNR: Nordic Nutrition Recommendations, by Denmark, Finland, Iceland, Norway, and Sweden(96)): 300 µg/day folate for women in general, and 400 µg/day folate for women 18-30 years and for women in childbearing age. Comparing to the average intake, a similar result was obtained for women in childbearing age: the recommendations were not met by any country(94).

The Portuguese mean daily intake for women was 304±144 µg/day(94). Some researchers explained this high intake with the relative high fruits and vegetables quantities available for Portuguese population, of 313 kg/year and per capita, contrasting with only 105 kg/year and per capita in Bulgaria, for instance(97).

When comparing countries, it is very important to consider meticulously the methodology. Different methods were applied across Europe to estimate the adequacy of micronutrient intake, which led to different prevalence estimates of micronutrient inadequacy(98).

Criteria to determine Dietary Reference Intakes in pregnancy for folate

The RDA for folate was based on the amount of folate (in DFE) needed to maintain erythrocyte folate, having been also in account data on plasma homocysteine and plasma folate concentrations(3).

During pregnancy the RDA is 600 µg DFE, which, according to the DFE equivalences[1], corresponds to 600 µg food folate = 360 µg folic acid from fortified food or as a supplement consumed with food = 300 µg folic acid from a supplement taken on an empty stomach(3). Data from the controlled metabolic study support a RDA of 600 µg/day of DFE based on maintenance of normal erythrocyte folate concentrations and agree with the findings from the series of population studies that 600 µg/day of DFE is adequate to maintain normal folate status in groups of pregnant women(3).

Risk reduction for NTD was not considered as a basis for establishing the RDA for pregnant women(3, 8) A separate recommendation was presented and discussed by the Institute of Medicine, USA, as all women capable of becoming pregnant should consume 400 µg/day of folic acid from supplements, fortified foods, or both in addition to consuming folate from a varied diet(3, 8).

The Tolerable Upper Intake Level (UL) was established only for folic acid (from fortified foods, supplements, or both), being 800 µg for 14 to 18 years and 1,000 µg above 18 years, by the Institute of Medicine(3, 8). This values derived from the Lowest-Observed-Adverse-Effect Level based on the precipitation or exacerbation of neuropathy in patients with vitamin B_{12} deficiency – considering that excessive intake of folic acid may mask the diagnosis(3). In childbearing age women, the consumption of folic acid at or above the UL is improbable to produce the adverse effects here considered, as the prevalence of vitamin B_{12} deficiency is generally very low (3, 8). Although, adverse effects are possible in women at risk of vitamin B_{12} deficiency, like vegans, older adults with atrophic gastritis, those with pernicious anaemia and bacterial overgrowth of the gut(3).

In Europe, the Scientific Committee on Food set a similar UL: 1 mg/day for adults(29).

Inadequate folate intake and congenital anomalies

Inadequate folate or folic acid intake leads to: a decrease in plasma folate concentration, a decrease in erythrocyte folate concentration, a rise in homocysteine concentration, megaloblastic changes in the bone narrow and other tissues with high cellular turnover(3) (e.g., erythrocytes, leukocytes and epithelial cells of the stomach, intestine, vagina, and uterine

[1] 1 µg DFE = 1 µg folate = 0.6 µg folic acid from fortified food or as a supplement consumed with food = 0.5 µg folic acid supplement taken on an empty stomach. 1 µg folate = 1 µg DFE; 1 µg folic acid from fortified food or as a supplement consumed with food = 1.7 µg DFE; 1 µg folic acid supplement taken on an empty stomach = 2 µg.

cervix(61)) – because lack of folate impair biosynthesis of DNA and consequently reduces cell division. This makes it particularly important in embryogenesis(61).

Those changes ultimately lead to macrocytic anaemia, at first evidenced by a low erythrocyte count and eventually by a low haematocrit and haemoglobin, as well(3).

During pregnancy, some studies support that NTD are associated with altered status of vitamin B_{12}, folate, or both(8). Furthermore, folate seems related with other types of congenital anomalies, specifically orofacial clefts and cardiovascular malformations, but the results were not yet consistent(6, 8). A study in Norway concluded that folic acid supplements use during early pregnancy reduced the risk of isolated cleft lip by about a third(4). On the other hand, in Canada, the prevalence of orofacial clefts did not change after food fortification with folic acid (1.15 per 1,000 before and 1.21 per 1,000 after)(99).

More recent studies emphasized the role of diet as a whole in the congenital anomalies occurrence. In one investigation, was concluded that the use of the maternal *Western diet* increases the risk of offspring with a cleft lip or cleft palate approximately two fold(100). In other study, it was observed that high intakes of vegetable protein, fibre, ascorbic acid, iron, and magnesium decreased orofacial cleft risk. Authors concluded that a high preconceptional intake of nutrients predominantly present in fruits and vegetables reduces the risk of offspring affected by orofacial cleft(101).

Congenital abnormalities represent the first cause of infant mortalities, with an increasing proportion (more than 25%) in both developed and developing countries. In the world, more than 10% of infant mortalities secondary to congenital abnormalities are caused by nervous system abnormalities(102).

NTD are the most common (from less than 1 to approximately 9 per 1,000 total births) major congenital malformations of the central nervous system, constituting an important public health problem in terms of mortality, morbidity, social cost, and human suffering(8).

Another studies, stated that the incidence NTD ranges from 0.5 to 14 per 1,000 live births (52). The prevalence in Portugal of NTD was of 9.35 per 10,000 live births in 1997-1999(80).

They are heterogeneous malformations – anencephaly, meningomyelocele (spina bifida aperta), meningocele, and craniorachischisis(8). Techniques have been established for prenatal screening of NTD, through assessments in maternal plasma and amniotic fluid.

The prevalence of NTD has declined considerably during the past three decades due to advances in the refined resolution of ultrasonography for *in utero* foetal examination, the clinical availability of serum alpha-fetoprotein measurements, termination of affected pregnancies, and folic acid supplements being widely consumed by women in the reproductive age group(103).

The incidence of myelomeningocele in Seattle, USA, was 0.5 per 1,000 births in 1981–1982, which then declined to 0.05 in 2001(104). The prevalence of NTD in England and Wales was

3.80 per 1,000 live births in 1965, which steadily declined to 0.14 in 1997, that is, a large reduction of 96%(105). Conversely, the estimated number of terminations increased considerably from 0.04 per 1,000 live births in 1970 to 1.50 per 1,000 live births in 1997(105). Contrary to the declining tendency in occidental countries, in Japan was reported that the prevalence of spina bifida has barely altered in the past two decades; that is, the prevalence of spina bifida per 10,000 live births and stillbirths was 4.3 in 1985, 4.3 in 1995, and 4.7 in 2005(106). The following factors are likely related to the unaltered prevalence of NTD in Japan: first, failure to impart adequate information regarding the important role of folic acid in preventing NTD; second, reluctance and/or misunderstanding of the Japanese public toward taking supplements during pregnancy; and third, neglect of the traditional Japanese diet that is rich in vegetables, fish, and rice, combined with an increasing willingness toward consuming fast food(106).

Among the G8 (The Group of 8: Canada, France, Germany, Italy, Japan, Russia, United Kingdom, and USA), the latest prevalence rate between 2000 and 2004 of spina bifida was approximately 3 to 4 per 10,000 live births, and stillbirths with or without termination of pregnancy, where Germany and Japan exceed this value at 7.41 and 5.32, respectively. It is possible that the actual prevalence rate of the latter may be higher because the number of pregnancies terminated due to spina bifida was not included in the report(103).

Neurulation is the first organogenetic process to be initiated and completed. It begins in the human at approximately 21 days post fertilization and is complete by 28 days. Thus, neurulation is ongoing at the time that a woman may first recognize her pregnancy by a missed menstrual period(8).

Evidence from epidemiological studies indicates that heredity is a major contributor (about 60%)(8). Mutations in the gene for MTHFR would account for approximately 15% of NTD cases(8). Indeed, the recurrence risk in a sibling birth is 3% to 5%(8). About 95% of the women who deliver infants with NTD have not previously delivered infants with these defects(2). For the most cases the inheritance is believed to be polygenic(8). Another cause of NTD (for less than 0.1%) are teratogenic drugs, including folate antagonists (specifically aminopterin, previously used as an antitumor agent), carbamazepine and valproate (commonly used antiepileptic drugs); and retinoids(8). Nutrients have been investigated to have a role in the prevention of NTD(8).

The possibility that folate might be involved in NTD was first raised by Hibbard in 1964(8). Data indicate a statistically significant decreasing risk of NTD with increasing dietary folate in unsupplemented women, being the median dietary folate in the control group approximately 300 μg/day(8). A reduced risk of NTD (about 35% to 70%) has been observed for women who took a folic acid supplement of 360 to 800 μg/day (being 400 μg the most usual dose) in

addition to a dietary folate intake of 200 to 300 µg/day(8). The optimal timing for supplementation seems to be during the 4 weeks before and after conception(8). After the first month of pregnancy folic acid will not prevent NTD, however, it will contribute to other aspects of maternal and foetal health(9). Supplemental folic acid in a dose of 4.0 mg/day promote a 71% decreased in NTD incidence(8). Although, because NTD represent a heterogeneous group of congenital malformations, there are cases not preventable even by large doses of folate(8). The mechanism by which folate could reduce NTD risk remains unknown(8).
An erythrocyte folate concentration greater than 870 nmol/l (400 ng/ml) is associated with a significant reduction in NTD risk. Erythrocyte folate concentrations improved significantly only in the groups taking folic acid supplements or food fortified; there was no increase in the group provided extra food folate or dietary advice(8). It is certainly conceivable that, if taken in adequate quantity, food folate will be shown to be as effective as folic acid, but it remains to be demonstrated(8).

The recommendation of folic acid for women able of becoming pregnant (400 µg folic acid daily from supplements, fortified foods, or both in addition to consuming folate from a varied diet) is for intake that exceeds the RDA for folate(8).Explaining, 400 µg folic acid as DFE is
400 × (1 ÷ 0.6) = 670 > 600 µg (RDA for pregnant). And adding the folate from the diet the value become even higher.
Many population-based studies confirmed that approximately 300 µg folic acid/day consumed in conjunction with a low-folate diet prevented folate deficiency in pregnant women(107). Reduced risk of NTD (about 35% to 70%) has been observed for women who took a folic acid supplement of 360 to 800 µg/day (being 400 µg the most usual dose) in addition to a dietary folate intake of 200 to 300 µg/day(8).
Folate intake is positively associated with erythrocyte folate concentration, and NTD risk is inversely associated with both folate intake and erythrocyte folate concentration(8).
Uncertainties still exist about the relationships among folate intake, erythrocyte folate, and NTD risk and about the extent to which the effect of food folate should be distinguished from the effect of folic acid from supplements or fortified foods (in some countries fortification is mandatory(19), in others is voluntary or neither exist), but the evidence is judged sufficient to support a specific recommendation to reduce risk of NTD(8).
Many countries in the EU recommend women who might become pregnant take folic acid supplements in order to reduce risk of NTD occurrence, but public health campaigns promoting this advice have been unsuccessful(14, 108).

Folic acid fortification and NTD

One of the possibilities to increase folic acid intake is through food fortification. This possibility is special interesting, considering that about 41% of worldwide pregnancies are not planned(109), so voluntary supplementation in that cases is virtual impossible. This proportion is even higher in developing regions (58% to 64%), and was also high in North America (48%), exceeding the proportions in Europe's northern, southern and western regions (39% to 42%)(109).

Voluntary fortification is permitted in most European countries, but any has mandatory fortification(14). Recently the EU introduced new rules to regulate voluntary food fortification – Regulation (EC) 1925/2006(14).

Countries like USA and Australia implemented mandatory folic acid fortification, respectively in years 1998(8) and 2009(33).

In Australia, in the year after mandatory fortification there was a 77% reduction in the prevalence of low plasma folate levels in all samples tested and an 85% reduction in the prevalence of low erythrocyte folate levels, showing a significantly reduction in the prevalence of folate deficiency(110). The effectiveness of mandatory folic acid fortification programmes in the USA and Canada have resulted in significant declines in the occurrence of some NTD by 28%(111) to 46%(112), respectively.

However, this enthusiastic results were haunted with the possibility of link between foetal folate exposure and cognitive decline, cancer or vascular disease occurrence later in life, due to changes in offspring phenotype via epimutations(113).

Moreover, in a recent review, authors defended that women of childbearing age may not yet be adequately targeted, while the general population may be over fortified with folic acid(114). It remains uncertain the minimum effective folate intake and status required for NTD prevention, and the safe upper folate level(114). Besides folate, several other lifestyle and environmental factors as well as genetic variations may influence NTD development, possibly by affecting one-carbon metabolism and thus epigenetic events(114). Although mandatory folic acid fortification plays a significant part in the reduction of NTD prevalence, but possibly at a cost and with a portion of NTD remaining, more effective preventive strategies require better understanding of the aetiology of this group of birth defects(114).

The same point of view is defended by other authors. Countries with mandatory fortification achieved a significant increase in folate intake and a significant decline in the prevalence of NTD, being this was also true for supplementation trials(115). However, the prevalence of NTD at birth declined to approximately 5 cases at birth per 10,000 births and 7-8 cases at birth or abortion per 10,000 births(115). This decline was independent of the amount of folic acid administered and apparently reveals a ‘floor effect’ for folic acid-preventable NTD(115). This clearly shows that not all cases of NTD are preventable by increasing the folate

intake(115).The relative decline depends on the initial NTD rate(115). Countries with NTD prevalence close to the observed floor may have much smaller reductions in NTD rates with folic acid fortification(115). Additionally, potential adverse effects of fortification on other vulnerable population groups have to be seriously considered(115). Policy decisions concerning national mandatory fortification programmes must take into account realistically projected benefits as well as the evidence of risks to all vulnerable groups(115).

Folic acid supplementation and incidence of NTD

Periconceptional folic acid supplementation lowers the incidence of NTD significantly, by 20% to 60%(52). A Cochrane review that included 4 studies with a total of 6425 women showed that periconceptional folic acid supplementation substantially reduces the incidence of NTD (relative risk 0.28, 95% confidence interval 0.13 to 0.58) (52). A meta-analysis showed that folate supplementation can prevent recurrent NTD in 85% to 100% of cases(52).

In a retrospective cohort study of births monitored by 13 birth defect registries from 1988 to 1998 in Norway, Finland, Northern Netherlands, England and Wales, Ireland, France (Paris, Strasbourg, and Central East), Hungary, Italy (Emilia Romagna and Campania), Portugal, and Israel, cases of NTD were ascertained among liveborn infants, stillbirths, and pregnancy terminations (where legal)(108). Main outcome measures included incidences and trends in rates of NTD before and after 1992 (the year of the first recommendations) and before and after the year of local recommendations (when applicable)(108). The issuing of recommendations on folic acid was followed by no detectable improvement in the trends of incidence of NTD(108). The results were intriguing. It was concluded that recommendations alone did not seem to influence trends in NTD up to six years after the confirmation of the effectiveness of folic acid in clinical trials. New cases of NTD preventable by folic acid continue to accumulate(108). Authors concluded that a reasonable strategy would be to quickly integrate food fortification with fuller implementation of recommendations on supplements(108). Another study obtain a similar result: no detectable improvement in the trends of incidence of NTD in France, after recommendations on folic acid supplementation(116).

There is no doubt that women at high risk of NTD benefit from higher doses of folic acid supplementation. These group includes women with certain folate-enzyme genotypes(117), or that smoke(117), alcohol abuse(118), malabsorption disorders(117, 118) or gastric bypass(118), haemolytic anaemia(118), liver disease(118), renal failure(118), those who take antifolate medications(117)(valproic acid, carbamazepine, trimethoprim, phenobarbital, primidone, diphenylhydantoin, oxcarbamazepine, sulphonamides, and methotrexate)(118), personal(117, 118) or family history of NTD(118), obesity(103, 117, 118), pre-gestational diabetes(103, 117, 118), epilepsy(118).

They should take 5 mg/day of folic acid for the 2 months before conception and during the first trimester(117). Other authors consider 4 mg/day(1).

In a European study (with 22 925 women, aged 15–49 years, from 18 countries) was concluded that, of the respondents, 58% had at least one biological child, and of these 38% reported that their first pregnancy was not planned(119). Nearly 60% of women who planned their pregnancy indicated that they had stopped using their method of contraception without first consulting a physician or another health care professional(119). Overall, 70% reported that they had heard of folic acid and 40% stated that they knew the benefits of folic acid(119). However, when prompted to indicate which diseases and/or birth defects folic acid can protect against, only 17% knew that folic acid can reduce the risk of NTD/spina bifida(119).
Regarding Portuguese situation, in a study about supplement use by pregnant women (from two major public hospitals in the North of Portugal, nearby 1998), it was concluded that a high proportion of women reported the use of folic acid (81.9%), iron (55.4%), and multivitamins (76.2%) as supplements during pregnancy(120). Higher schooling was also associated with increased use of folic acid. Use of folic acid was less prevalent in single women and during unplanned pregnancies(120). In a more recent study (nested within Geração XXI, a Portuguese birth cohort, who delivered in 2005-2006), 97% of women reported folic acid supplements use during the first trimester of pregnancy, although it is important to note that the median gestational age at initiation was 6.5 weeks (interquartile range 5-9)(13).
In a study in Portugal, 77.5% of women aged 24-44 years knew about folic acid, but only 20% were able to describe folic acid as an effective method to reduce NTD and 15.4% of all respondents knew that supplementation with folic acid should begin before conception(80)
In 2005, it was found an increase in the proportion of women taking folic acid prior to pregnancy since 1998. In 2005, 23.9% of women of the study took folic acid prior to pregnancy. There was a significant association between appropriate intake and the pregnancy being planned. In one survey, 54.5% of all women surveyed who had ever been pregnant reported having consulted a physician while preparing for their last pregnancy(121).
Although the role of folic acid in NTD prevention is clear, many other questions arise. In consequence, the establishment of recommendations at a population level are not straightforward.

Aim of the study

This study aimed to summarize the recommendations on folate and folic acid in the periconceptional period provided by official health organizations in different countries worldwide.

METHODS

Research about folate and folic acid recommendations for women in the periconceptional period was conducted in national health official websites of 46 countries worldwide: 33 European[2], including all 28 EU countries; 3 missing countries belonging to G8[3]; all 5 BRICS[4]; all 4 Asian Tigers/ Asian Dragons[5]; and Australia. Developed countries were chosen due to the easiest access to data. Despite of that, data were unavailable or not found for 10 countries: 8 European countries, being 6 from EU (Croatia, Cyprus, Czech Republic, Greek, Lithuania, Slovakia) and 2 not (Liechtenstein, Turkey); 1 BRICS (India); 1 Asian Dragon (South Korea). As a result, the total information was available for 36 countries (78%). The recommendations from the WHO were also investigated.

For European countries the web addresses were obtained through the European Commission website, on the page entitled *Public Health – Trustworthy websites on "Population groups"*(122). The keywords used for researching in each country were the following: folic acid, folate, pregnancy, nutrition. The keywords and the information found were translated on *Google® Translate*, from English to the official languages and vice-versa. When information was unavailable at the referred website (it was the case of Ireland; Lithuania has no mention to any website; Denmark was missing) the research was made on Google®, adding another keywords: ministry of health, neural tube defects.

From the websites of each country were registered recommendations for women in the periconceptional period concerning the following topics: folic acid supplementation for NTD risk reduction (dose, begin, end, upper limit, dose for high risk of NTD), folate recommended intake (measured in folate or in DFE), healthy diet and folate rich food, the website address, and the name of the official entity.

For all countries e-mails were sent for validation of the information (59% were validated). In the validation process, the information was confirmed, corrected, and/or completed. From the 36 countries with data, 26 countries validated the information (72%) and 10 did not (France, Latvia, Romania, Japan, Brazil, China, Russia, South Africa, Singapore, Taiwan). From the others 10 countries without data, only one (Liechtenstein) validated the (missing) information. The WHO have not validated data. Data without official validation were equally considered for results and analysis, after "internal validation" by beginning the researching process and conforming the data from the websites at least three times (corresponding to three different days).

[2] EU: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom. Some other European: Iceland, Liechtenstein, Norway, Switzerland, Turkey.

[3] G8: Canada, Japan, United States of America, and the other 5 countries mentioned both in EU (France, Germany, Italy, United Kingdom) and in BRICS (Russia).

[4] BRICS: Brazil, Russia, India, China and South Africa.

[5] Asian Tigers/ Asian Dragons: Hong Kong, Singapore, South Korea, Taiwan.

Websites were chosen instead of searching scientific articles with original information or revision of the official guidelines or instead of sending e-mails to each health country representative, because the revisions found didn't have all the countries desired and because the propose was to know which information is available in the current year both for health professionals (guidelines) and for general public (Appendix 3). In the era of information, many people consult health recommendations trough the World Wide Web(123-125).

Data were collected from May to October of 2013.

All statistical analyses were performed with the IBM SPSS Statistics for Windows, Version 21.0 (Released 2012. Armonk, NY: IBM Corp.).

RESULTS

Initially, it was tried to summarise information for 46 countries. However, for 10 countries (21.7%) information did not exist or it was not found. Data were available for 36 countries. The recommendations gathered are systematised in Tables 1 to 6 from countries and in Table 7 from WHO. The majority (83.4%) of the analysed countries had some information in their national health official websites, about both folic acid supplementation and folate rich diet/healthy diet. Only few refers to supplementation alone (8.3%) or to diet alone (8.3%).

In a general overview, from this studied were obtained the following main data related to folic acid supplementation for NTD risk reduction (higher percentages achieved are shown):

- Dose: 400 µg/day (77.8%), discuss with healthcare provider (5.6%);
- Begin: any period before conception (88.9%): at least 4 weeks (41.7%), at least 12 weeks (11.1%), when planning a pregnancy (27.8%), when there is a chance of becoming pregnant (16.7%), before conception (11.1%), before stopping contraceptive (5.6%), when the contraceptive is stopped (5.6%);
- End: first trimester (75.0%): 12 weeks (69.4%), 10 or 8 to 10 weeks (5.6%); throughout pregnancy (5.6%);
- Upper limit: 1 mg/day (44.4%), not exceed recommendations (5.6%);
- Dose for high risk of NTD: higher doses (58.3%): 5 mg (30.6%), 4 mg (13.9%), to be discussed with physician (8.3%).

And data related to folate (higher percentages obtained are shown):

- Is healthy diet and folate rich food recommended? Yes (80.6%): healthy diet may be enough (no need of supplementation) (13.9%);
- Folate recommended intake: Nordic Nutrition Recommendations (16.7%): pregnant 500 µg/day, women 18-30 years or childbearing age 400 µg/day, women 300 µg/day.
- DFE recommended intake: DRI (16.7%): pregnant 600 µg/day, women 400 µg/day; German language D-A-CH (8.3%): pregnant 550 µg/day; women 300 µg/day; higher

intake of folate only after first trimester (5.6%): pregnant second and/or third trimester 600 µg/day, women 400 µg/day.

It was observed that the recommendations differ between countries, although the majority (63.9%) recommend 400 µg of folic acid at least one to three months before conception until three months of pregnancy. In Portugal there are no official recommendation of the dose that should be taken. There was a consensus in the recommendation of a healthy diet, naturally rich in folate.

Worldwide, the daily folate recommended intake for pregnant women could be summarised in three different recommendations: 500 µg of folate (from Nordic Nutrition Recommendations), 550 µg of DFE (from D-A-CH recommendations – dominant states of the German language *Sprachraum*), and 600 µg of DFE (from Institute of Medicine). Two countries recommend only a higher intake of folate after the first trimester of pregnancy, being 400 µg for women of childbearing age and pregnant women in the first trimester. For high risk women, recommendations are high, mostly 4 mg and 5 mg.

Some countries emphasize the importance of a healthy diet naturally rich in folate with no need of folic acid supplementation. By contrast, others advice supplementation (one on very high doses) plus mandatory folic acid fortification. The upper limit of folic acid intake is considered 1 mg by many countries. Many guidelines consider that recommendations may change with new research, to meet the optimum level and not supraphysiologic levels that may have adverse effects.

In what concerns the USA different recommendations were found (in one it was referred that folic acid could be taken from supplements or fortified foods, another should be from supplements; one referred a dosage of 400 µg/day, another 400-800 µg/day). Out of this general pattern were Canada and China (400 µg/day of folic acid supplements in the periconceptional period, but also recommends supplementation throughout pregnancy), and Brazil (recommended a dosage of 5 mg, particularly in women with history of NTD). The others countries (25.0%) – Ireland, Portugal, Romania, Slovenia, Japan, Russia, South Africa, Singapore, Taiwan –, have at least one missing data in the mentioned characteristics. BRICS was the only group completely out of the general pattern (out or missing data), all the others (Europe, EU, Asian Tigers, G8, and Australia) had at least one country in the general pattern. Concerning WHO, it almost fits the general pattern, being missing the advice for a healthy diet/folate rich diet, mentioned only in the topic for high risk women (advised to increase their food intake of folate and to take 5 mg of folic acid daily). However, considering another available document of the same institution, made with the purpose of improving pregnancy outcomes and reduce maternal anaemia, it is out of the pattern (400 µg/day of folic acid supplements periconceptionally and throughout pregnancy).

Some particularities were observed in some countries, described below.

From the analysed countries, five had mandatory fortification.

The only country that recommended doses between 400 µg/day and 800 µg/day was USA.

Brazil was not completely clear in the recommendation about dose and about the target: recommended 5 mg/day especially in women with history of NTD.

At least one country (Denmark) changed the designation about the period to begin folic acid supplementation, from "*when planning"* to "*when start thinking about getting pregnant"* or *"before conception"* or "*when there is a chance of becoming pregnant"*, because almost half pregnancies are not planned.

The countries that specially emphasized healthy diet (where the need of supplementation was not generalized) were: Finland, France, Sweden, Singapore, and Taiwan.

Only one country (Singapore) mentioned, in information for general population, the importance of folate status, which should be achieved by a healthy diet and gave examples of folate rich foods. Also recommended to check with the healthcare provider the need of taking supplements containing folate.

Table 1. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some EU countries (n=22).

Country	Folic acid supplementation for NTD risk reduction					Is healthy diet and folate rich food recommended?	Folate recommended intake	DFE recommended intake	Website ‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Austria	400 µg/day	At least 4 weeks/ 1 month before conception	12 weeks/ 3 months of pregnancy	1 mg/day for adults (D-A-CH 2013)	Higher doses (>400 µg/day)/ to discuss with physician (D-A-CH 2013)	Yes	-	Pregnant 550 µg/day Women 300 µg/day (D-A-CH 2013)	Federal Ministry of Health – Public health portal
Belgium – Flanders	400 µg/day	When the contraceptive is stopped	12 weeks/ 3 months of pregnancy	1 mg/day for adults	4 mg/day	Yes	-	-	Flemish government – Child and Family Support Centre for Policy Research – Welfare, Health and Family
Bulgaria	400 µg/day	At least 12 weeks/ 3 months before conception	12 weeks/ 3 months of pregnancy	-	-	Yes	-	-	The official Bulgarian patient organization Zachatie/ Conception Bulgarian portal *
Denmark	400 µg/day	When start thinking about getting pregnant **	12 weeks/ 3 months after conception or 8 weeks/ 2 months (for high risk of NTD)	Not exceed recommendation	5 mg prescribe by the physician	Yes	Pregnant 500 µg/day Women 18-30 years or childbearing age 400 µg/day Women 300 µg/day (NNR 2012)	-	Danish Health and Medicines Authority Nordic Nutrition Recommendations
Estonia	400 µg/day	At least 12 weeks/ 3 months before conception	12 weeks/ 3 months of pregnancy	1 mg/day for adults	4 mg/day	Yes	Pregnant 500 µg/day Women 18-30 years or childbearing age 400 µg/day Women 300 µg/day	-	National Institute for Health Development Health Information Network Treatment Guide

‡The corresponding web addresses are listed in Appendix 3. .- Blank mean that no information was available.

EU: European Union. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents. D-A-CH: reference values for nutrient intake by German Society of Nutrition, the Austrian Society of Nutrition, the Swiss Society of Nutrition Research and the Swiss Association of Nutrition. NNR: Nordic Nutrition Recommendations (by Denmark, Finland, Iceland, Norway, and Sweden).

* The referred article is just an article on the site and nothing more. The information on our site (which is the site of the official Bulgarian patient organization Zachatie) is a collection of Bulgarian and translated articles, approved by our medical editor. These are not medical standards. Therefore, the information contained within may vary (information received by e-mail, 2013-10).

** Early we said “when you plan to get pregnant” but not all pregnancies are planned (information received by e-mail, 2013-10).

Table 1. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some EU countries (n=22) (continuation).

Country	Folic acid supplementation for NTD risk reduction					Is healthy diet and folate rich food recommended?	Folate recommended intake	DFE recommended intake	Website‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Finland	400 µg/day for woman with an unhealthy diet *** / ****	When the contraceptive is stopped / for those who plan to become pregnant	12 weeks/ 3 months of pregnancy	1 mg/day for adults	4 mg/day	Yes *** / ****	Pregnant 500 µg/day Women 18-30 years or childbearing age 400 µg/day Women 300 µg/day (NNR 2012)	-	Ministry of Social Affairs and Health Nordic Nutrition Recommendations
France	400 µg/day may be prescribed by healthcare provider *****	At pregnancy project	12 weeks/ 3 months of pregnancy	-	5 mg/day	Yes *****	-	-	National Institute for Prevention and Health Education National Nutrition and Health Program
Germany	400 µg/day	At least 4 weeks/ 1 month before conception	12 weeks/ 3 months of pregnancy	1 mg/day for adults	To discuss with physician	Yes	-	Pregnant 550 µg/day Women 300 µg/day (D-A-CH 2013)	Federal Centre for Health Education – Family planning German Society for Nutrition
Hungary	400 µg/day	At least 4 weeks/ 1 month before conception	12 weeks/ 3 months of pregnancy	-	-	Yes	-	-	National Centre for Food Safety and Nutrition
Ireland	400 µg/day	12 weeks/ 3 months before conception / all women of childbearing age	12 weeks/ 3 months of pregnancy	-	5 mg/day	-	-	-	Health Service Executive
Italy	400 µg/day	Before conception	12 weeks/ 3 months of pregnancy	-	5 mg/day ******	Yes	-	-	Ministry of Health
Latvia	400 µg/day	While trying to get pregnant	12 weeks/ 3 months of pregnancy	-	-	Yes	-	-	Latvian Health Portal
Luxemburg	400 µg/day	4 weeks/ 1 month before conception	12 weeks/ 3 months of pregnancy	-	Higher doses (>400 µg/day)	Yes	-	-	Ministry of Health

‡The corresponding web addresses are listed in Appendix 3. .- Blank mean that no information was available. EU: European Union.
*** Unhealthy diet means having less than 400 µg of folate. Supplementation is also recommended for women with other problems (e.g., epilepsy, celiac disease, diabetes mellitus insulin dependent). **** Similar to new recommendations that will be launched in 2014. The rationale is that the most critical period for sufficient folic acid intake is during the period when the women actually do not even know that they are pregnant. In Finland healthy diet which includes rye bread, berries and green leafy vegetables which is recommended for all, provides sufficient amount of nutrients. Finland healthy diet provides sufficient amount of nutrients (information received by e-mail, 2013-10). ***** A varied diet may be enough, although, for precaution, the healthcare provider may prescribe a supplement. ****** The dose of 5 mg (instead of 4 mg as suggested by the evidence of efficacy) is indicated because in Italy does not have a formulation of 4 mg of folic acid per tablet.

Table 1. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some EU countries (n=22) (continuation).

Country	Folic acid supplementation for NTD risk reduction					Is healthy diet and folate rich food recommended ?	Folate recommended intake	DFE recommended intake	Website‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Malta	400 µg/day	Women who are planning a pregnancy/ prior to conception	12 weeks/ 3 months of pregnancy	1 mg/day for adults	-	Yes	-	- Women 400 µg	Ministry for Health
Netherlands	400 µg/day or 500 µg/day	4 weeks/ 1 month before conception / when there is a chance of becoming pregnant	10 weeks/ 2 months of pregnancy	-	Higher doses (>400 µg/day or 500 µg/day)	Yes	-	-	National Institute for Public Health and Environment – Ministry of Health, Welfare and Sport Nutrition Centre
Poland	400 µg/day	While trying to get pregnant	12 weeks/ 3 months of pregnancy	-	-	Yes	-	-	Ministry of Health
Portugal	-	At least 8 weeks/ 2 months before stop using contraception	-	-	-	Yes	-	-	Ministry of Health
Romania	400 µg/day	-	-	1 mg/day for adults	5 mg/day	Yes	-	Pregnant 3rd trimester 600 µg/day Women 400 µg/day	Ministry of Health – Romanian Nutrition Society
Slovenia	-	-	-	-	-	Yes	-	Pregnant 2nd and 3rd trimester 600 µg/day Women 400 µg/day (D-A-CH 2004)	Institute of Public Health of the Republic of Slovenia Ministry of Health
Spain	400 µg/day	12 weeks/ 3 months before stop contraception/ 4-8 weeks/ 1-2 months before conception*******	12 weeks/ 3 months of pregnancy	1 mg/day for adults	5 mg/day *******	Yes	-	-	Ministry of Health, Social Services and Equality – National Health System
Sweden	400 µg/day except for women with high folate intake ********	4 weeks/ 1 month before conception / woman who may get pregnant	12 weeks/ 3 months of pregnancy	1 mg/day for adults	-	Yes	Pregnant 500 µg/day Women 18-30 years or childbearing age 400 µg/day Women 300 µg/day (NNR 2012)	-	National Food Agency Nordic Nutrition Recommendations European Food Safety Authority
United Kingdom	400 µg/day	While trying to get pregnant	12 weeks/ 3 months of pregnancy	-	5 mg/day	Yes	-	-	National Health Service

‡The corresponding web addresses are listed in Appendix 3. .- Blank mean that no information was available. EU: European Union.

******* According to new guidelines, in press, 2013. ******** For women with a high intake of folate-rich foods, such as vegetarians and vegans eating large quantities of pulses and vegetables, folate intake from food may happen to be enough. For them there is no need for extra folic acid supplementation.

Table 2. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some others European countries (n=3).

Country	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Iceland	400 µg/day	At least 4 weeks before conception/ all women able to get pregnant	12 weeks/ 3 months of pregnancy	-	5 mg/day	Yes	Pregnant 500/day µg Women 18-30 years or childbearing age 400/day µg Women 300/day µg (NNR 2012)	-	Directorate of Health Nordic Nutrition Recommendations
Norway	400 µg/day	4 weeks/ 1 month before conception / women who are planning to conceive	8-12 weeks / 2-3 months of pregnancy	-	4 mg/day	Yes	Pregnant 500/day µg Women 18-30 years or childbearing age 400/day µg Women 300/day µg (NNR 2012)	-	National Institute of Public Health Nordic Nutrition Recommendations
Switzerland	400 µg/day	Women who wish or may fall pregnant/ At least 4 weeks/ 1 month before conception	12 weeks/ 3 months of pregnancy	1 mg/day for adults (D-A-CH 2013)	Higher doses (>400 µg/day)/ to discuss with physician (D-A-CH 2013)	Yes	-	Pregnant 550 µg/day Women 300 µg/day (D-A-CH 2013)	Federal Office of Public Health * Swiss Society of Nutrition

‡The corresponding web addresses are listed in Appendix 3.

- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents.

D-A-CH: an acronym used to represent the dominant states of the German language *Sprachraum*, and are reference values for nutrient intake by German Society of Nutrition, the Austrian Society of Nutrition, the Swiss Society of Nutrition Research and the Swiss Association of Nutrition.

*According to official information from Swiss Society of Nutrition, the new D-A-CH recommendations will be online in the website, and are already in practice (information received by e-mail, 2013-10).

Table 3. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some G8 countries (n=3).

Country	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Canada +	400 µg/day (by supplements)	At least 12 weeks/ 3 months before conception	Throughout the pregnancy	1 mg/day for adults	Higher doses (1-5 mg/day)	Yes	-	Pregnant 600 µg/day Women 400 µg/day	Health Canada
Japan	-	12 weeks/ 3 months before conception	12 weeks/ 3 months of pregnancy	-	-	-	-	-	Ministry of Health, Labour and Welfare
United States of America +	400 µg/day (by supplements or fortified food) Or 400-800 µg/day (by supplements)	At least 4 weeks/ 1 month before conception/ all women able to get pregnant	During the first few months	1 mg/day for adults	4 mg/day	Yes	-	Pregnant 600 µg/day Women 400 µg/day	Centers for Disease Control and Prevention Institute of Medicine of the National Academies Women's health – Department of Health and Human Service

‡The corresponding web addresses are listed in Appendix 3. + Country with mandatory fortification(19).
- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents.
Note: *The Group of Eight (**G8**) is a forum for the governments of eight of the world's largest national economies as nominal Gross Domestic Product (GDP) with higher Human Development Index (HDI): Canada, France, Germany, Italy, Japan, Russia, United Kingdom, and USA. EU is also represented.*

Table 4. Folate and folic acid recommendations for women in the periconceptional period from national health organization official website of Australia (n=1).

Country	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Australia +	400 µg/day (by supplements)	At least 4 weeks/ 1 month before conception / women planning a pregnancy	12 weeks/ 3 months of pregnancy	1 mg/day for adults	5 mg/day	Yes	-	Pregnant 600 µg/day Women 400 µg/day	National Health and Medical Research Council Food Standards Australia New Zealand

‡The corresponding web addresses are listed in Appendix 3. + Country with mandatory fortification(19).
- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents.

Table 5. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some BRICS countries (n=4).

Country	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Brazil +	5 mg/day especially in women with history of NTD	8-12 weeks/ 2-3 months before conception	-	-	5 mg/day	-	-	-	Ministry of Health
China	400 µg/day	At least 4 weeks/ 3 months before conception	Throughout the pregnancy	1 mg/day for adults	-	Yes	-	Pregnant 600 µg/day Women 400 µg/day	E-health – Shanghai Jing'an District Health Promotion Board – Ministry of Health Chinese Nutrition Society
Russia	-	-	-	1 mg/day for adults	-	Yes	-	Pregnant 600 µg/day Women 400 µg/day	Ministry of Health of the Russian Federation Protection and Human Welfare
South Africa +	-	Women of reproductive age	-	Not exceed recommendation	-	Yes	-	-	The National Department of Health Association for Dietetics in South Africa

‡The corresponding web addresses are listed in Appendix 3. + Country with mandatory fortification(19).
- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents.
Note: ***BRICS*** *is the acronym for an association of five major emerging national economies:* ***B****razil,* ***R****ussia,* ***I****ndia,* ***C****hina and* ***S****outh Africa.*

Table 6. Folate and folic acid recommendations for women in the periconceptional period from national health organizations official websites of some Asian Tigers/ Asian Dragons countries (n=3).

Country	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
Hong Kong	400 µg/day	4 weeks/ 1 month before conception / when planning for pregnancy	12 weeks/ 3 months of pregnancy*	-	-	Yes	-	-	Department of Health The Hong Kong College of Obstetricians and Gynaecologist
Singapore	-** Check with healthcare provider	Before conception	12 weeks/ 3 months of pregnancy	-	-	Yes**	-	Pregnant 600 µg/day Women 400 µg/day	Health Promotion Board – Singapore Government
Taiwan	-***	-	-	1 mg/day	-	Yes***	Pregnant 600 µg/day Women 400 µg/day	-	Ministry of Health and Welfare: Health Promotion Administration; Food and Drug Administration

‡The corresponding web addresses are listed in Appendix 3.
- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents.
Note: the **Asian Tigers** or Asian Dragons is a term used in reference to the highly developed economies of Hong Kong, Singapore, South Korea, and Taiwan.
* *Folic acid supplementation should be continued in women carrying thalassemia trait to prevent folic acid deficiency and maternal anaemia.*
** *It is important to ensure an adequate intake of folate even prior to conception. Keep up your folate status by eating a diet that includes rich sources of folate, such as green leafy vegetables, lentils and fortified products. Also, check with your healthcare provider on the need of taking supplements containing folate.*
*** *In order to ensure that there is sufficient folate for the baby's development, the best method is to consume foods high in folate on a frequent basis, such as green vegetables, fruits, fresh orange juice, liver*. A balanced diet is assumed to be enough to achieve the recommendations.

Table 7. Folate and folic acid recommendations for women in the periconceptional period from World Health Organization website (n=1).

Entity	**Folic acid supplementation for NTD risk reduction**					Is healthy diet and folate rich food recommended?	**Folate** recommended intake	**DFE** recommended intake	**Website**‡
	Dose	Begin	End	Upper limit	Dose for high risk of NTD				
World Health Organization	400 µg/day*	As early as possible* (periconceptionally)	Throughout pregnancy*	-	-	Yes	-	-	World Health Organization
	400 µg/day**	Women trying to conceive at 8 weeks/ 2 months before the planned pregnancy**	12 weeks/ 3 months of pregnancy**	-	5 mg** / ***	Yes for high risk **/***	-	-	

‡The corresponding web addresses are listed in Appendix 3.
- Blank mean that no information was available. NTD: Neural Tube Defects. DFE: Dietary Folate Equivalents. **Purpose: improving pregnancy outcomes and reducing maternal anaemia in pregnancy* (9).
** *Aim: to prevent NTD and other congenital malformations in the fetus* (10). *** For women who have previously had a baby with NTD, who have diabetes or who are under anticonvulsant treatment.
*** High risk: advise to increase their food intake of folate.

DISCUSSION

The association between adequate maternal folate levels during the periconceptional period and the reduction in the congenital defects, especially NTD is completely establish. However, warrant an adequate folate status in this period is a challenge worldwide, according to prior studies (3, 8, 11). To fill this gap, WHO and many national health organizations have been developing recommendations related to folic acid supplementation and folate rich diet. Some countries also implemented mandatory folic acid fortification.

In Public Health it is indispensable to widespread clear and unique messages among health professionals and general public that is more and more interested in to judge the information provided by health professionals.

The question of folate adequacy seeking congenital anomalies prevention is very interesting, because the critic period for that adequacy is the first 6 gestational weeks when the majority of women unknown their pregnancies, even in the planned pregnancies (exceptions would be the pregnancies medical assisted). Ideally, a correct diet would supply adequate intakes, but this is not the reality(13). Supplementation could be a natural solution for this deficiency. However, regarding this special scenario, food fortification in folate appears as a better solution, providing adequate folate intakes, without any voluntary attitude taken by women. So, recommendations should be a clear message regarding at least two of the three possibilities of sources for an adequate folate intake – diet and supplements. Fortification is restricted for countries that opted for this measure.

Nowadays, World Wide Web is available for the majority of the population in many countries worldwide. This is an excellent vehicle for given messages for health professionals and population. For this reason, this source of information was used.

Initially, it is intended to obtain information for 46 countries, but for 10 of them it was impossible to reach any information. For the other 36 countries diverse information was obtained. Obviously that it should have differences among countries regarding different dietary patterns and different diseases prevalence, but probably there is not a clear rational justification for the disparities between countries. Inclusively, differences in the recommendations for the same country (USA) were found. This situation is completely undesirable regarding the doubt that could arise in the population.

A huge amount of work should be done regarding dietary advice. For instance, in the Portugal, a healthy diet was recommended without specify many details. This type of message is clearly insufficient for the population. Detailed recommendations regarding type of foods, methods of cooking and quantities would be very useful. It is important to note that the information about a healthy diet was not in the same document that contains the guidelines for health professionals regarding preconception advices, which mention the time to begin folic acid supplementation (but not to stop, neither dose nor diet). Furthermore, the document about diet

refers a dose of 400 µg/day as a reference for folic acid intake, but this was not a guideline, neither has an official format, nor authors, but can be consulted by Internet users.

The recommendations differ between countries, although the majority (66.7%) recommend *400 µg/day of folic acid supplements in the periconceptional period and a folate rich diet*. Some countries emphasize the importance of a healthy diet naturally rich in folate with no need of folic acid supplementation. According to many studies, mentioned in the introduction(72), this advice should be the main in all recommendations for NTD prevention. By contrast, others advice supplementation and have mandatory folic acid fortification. In Portugal there is no official recommendation of the amount that should be taken.

The current study found that the designations to mention the adequate period for supplementation vary between countries. It can considered the more adequate the ones that included the general designation "before conception" and that refers to "planning plus chance of becoming pregnant", although this needs a specific study to see this information if is correctly interpreted. The only designation that seemed less precise is "reproductive age", because many women may belong to this group but not have "chance to get pregnant", so they may not need to have supplementation. Although this group is the main general target of all countries, is not the specific target.

Comparing folate recommendations, DRI had higher recommendations than D-A-CH or NNR. The last seems more realistic to achieve throw a normal healthy diet, as shown by Table 2 (in the general Introduction).

Apart from recommendations about supplementation, some countries introduced fortification and others not. Surprisingly, in one of the countries with mandatory supplementation (Brazil) were recommended in general high doses of folic acid (5 mg) and not only for high risk of NTD. Although the guideline refers "especially in women with history of NTD", it is not an easy understandable statement. In the research made for Brazil, it was found other recommendations, but not officially from ministry of health, for this reason it was not included in the analysed tables. It was from University Maternity of Rio de Janeiro[6], where it was advised 400 µg/day (by supplements) and 4 mg for high risk of NTD. We also did not find a clear justification for the recommendation of 5 mg of folic acid exclusively for Brazil. But, once again, we cannot warrant that these doses were not practiced in other countries, as it as mentioned. It is not easy to know the real intake of folic acid in different countries, because some scientific studies limit this kind of information, providing only the proportion of supplemented women without refers the dose.

6 http://www.maternidade.ufrj.br/portal/images/stories/pdfs/obstetricia/consulta_pre_concepcional.pdf (information for health professionals).

It is interesting to note that all the mandatory fortified countries also recommended supplementation and any refers that diet may be enough. By contrast, the only two groups (European and Asian) without mandatory fortification in all their analysed countries, are the same that consider diet to be enough (by some countries) and that mentioned the importance to discuss supplementation with the physician or health provider (by some countries).

Apart from recommendations, from this research it is not possible to assume that the recommendations are effectively implemented. Particularly for Portugal, the recommendations from ministry of health do not specify the dose for the supplementation, but from Infomed (126) *website*, we knew that are only commercialized doses with 5 mg of folic acid, for supplements containing only folic acid. The most of multivitamins have 400 µg of folic acid and some combinations of folic acid and iron supply 1 mg of folic acid (recommended for second and third trimesters of pregnancy). The folic acid dose of 5 mg/day is even higher than UL. The 5 mg and 1 mg (in combination with iron) are reimbursed by the government in 37% (126), the others not. We don't know why in Portugal are only commercialized high doses.
Spain and Malta also had had available high doses, but they already changed the posology of the commercialized drugs, to adjust for NTD prevention (and not for megaloblastic anaemia treatment, as this doses were used for). Portugal did not change yet.
Returning Portuguese situation, it was shown by data from INFARMED[7](127) an increased waiver of folic acid in the last 12 years (2000-2012), with a linear tendency, from 215,308 packs in 2000 to 388,213 packs in 2002, representing an increase of 80.3% in this period of time, with an average perceptual change (AVC) of 5.2% per year. But has data is aggregated and cannot be separated by groups of supplements users, no conclusion can be taken. Only discuss that if population didn't change in the same proportion(128, 129) to justify this rise, others factors may explain, being the begging of the supplementation in 1998(130, 131) a possible reason, that needs confirmation. Data can be analysed (some available at Eurocat(121)), and compared incidence and prevalence before and after supplementation.
Accurate health registries would be very useful in order to measure the impact of such recommendations. For instance, regarding congenital anomalies, it would be necessary information regarding miscarriages, medical abortions, stillbirths and neonates with congenital information, information not always easy to aggregate.

[77] Data from Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. The information is from the SIARS (Sistema de Informação da Administração Regional de Saúde / Information System of the Regional Health Authority) and may be subject to alteration. The data refer to the reimbursed medicines and dispensed in an outpatient population covered by the National Health Service in the period from January 1, 2000 to December 31, 2012, in Portugal. In this universe are not included medicines related to hospitalization (information sent by e-mail, 2013-10).

CONCLUSION

Folic acid recommendations worldwide in the periconceptional period are predominantly of 400 µg/day, despite the fact that the epidemiology of the neural tubes defects is different between countries, as folate intake. However, big disparities were seen for the recommendations of some countries – at least one country recommend 5 mg of folic acid and, in contrast, some do not recommend supplementation, only a healthy diet.

In Portugal no dose is officially recommended, although the only commercialized doses of folic acid (alone, not in multivitamins) are of 5 mg, so women with low risk are taken doses above the UL for folate. Accurate and, ideally, evidence-based recommendations are needed for Portugal.

CHAPTER 2 – FOLATE INTAKE AND FOLIC ACID SUPPLEMENTATION IN THE PERICONCEPTIONAL PERIOD: DIFFERENT APPROACHES FOR ITS MEASUREMENT

INTRODUCTION

It is consensual that maternal diet during pregnancy influences maternal and child health(132). Folate deficiency is strongly associated with neural tube defects (NTD)(133), but dietary folate was not clearly associated with the reduction of NTD risk(8), contrasting with folic acid supplementation(3, 134) and, consequently, widely recommended in the periconceptional period(3).

Regarding risk reduction of NTD, folic acid supplementation should be initiated in the pre-pregnancy period(11, 108, 132, 135, 136). According to DRI (3, 8)., women capable of becoming pregnant should take 400 µg of folic acid daily from fortified foods, supplements, or both, in addition to the folate consumption from a varied diet, until their pregnancy is confirmed and their enrolment in the prenatal care (which ordinarily occurs after the critical time for the formation of the neural tube, that closes by day 28 after conception). These recommendations are impossible to be followed in unplanned pregnancies, and even in planned pregnancies, frequently, the supplementation initiated about the week 6 of pregnancy(13, 108).

Concerning DFE, DRI establishes that women in the childbearing age should intake 400 µg/day DFE, increasing to 600 µg/day DFE during pregnancy(3). It is important to note that risk reduction for NTD was not a “cornerstone” in this values definition. A separate recommendation, mentioned above, was presented and discussed(3).

A previous study in Portuguese pregnant women determined that 58.2% of them consumed inadequate quantities of folate previous to conception (median daily intake: 293.5 µg; interquartile range: 239.4-380.1 µg) and this proportion ascended to 90.8% during pregnancy (median daily intake: 314.6 µg; interquartile range: 245.1-400.8 µg), considering the Estimated Average Requirements (EAR) of 320 µg/day and 520 µg/day, respectively, as cut-off points for folate intake alone(13). In the same study, authors documented that although almost all women took folic acid supplements during first pregnancy trimester, the median age at initiation was 6.5 weeks of pregnancy, certainly after the closure of neural tube, when it is a decisive role(13). The inadequacy intake in folate is also described in others populations(137, 138).

In order to determine the inadequacy of folate intake, it is indispensable to have tools that accurately quantify folate intake and, ideally, to know what is the relationship between folate intake in its blood levels. Then we could really associate maternal nutritional status with pregnancy outcomes, in early and later in life, both in child and mother.

There are different tools to access dietary intake, however none is perfect, being into account the complexity of the diet. In the selection of one method, it would be considered the population characteristics and the sample size.

Food frequency questionnaire (FFQ) is one of the tools more frequently used in epidemiological studies involving a large number of participants, due to economical and logistic reasons and for its ability to reflect usual dietary intake of a large period of time(139-145).

A semi-quantitative FFQ was validated to measure dietary intake in Portuguese pregnant women, using as a gold standard food diaries (FD)(146). Another possibility in the validation of dietary questionnaires is the use of biomarkers, in this case serum/plasma and/or erythrocyte folate levels(139, 147, 148).

The advantage of using biochemical markers in dietary assessments is related with the low probability to doubt that their random errors are truly independent of those of food questionnaires(147). Biomarkers retrieve the nutrient quantities available for biological processes and depend on the balance between intake and nutrient's metabolism. This metabolism could range between individuals. For instance, in subjects with the MTHFR 677 T/T genotype folate intake was not correlated with plasma levels(142).

Although some studies have been associated dietary folate intake with respective serum/plasma or erythrocytes levels, few considered both dietary folate and folic acid supplements intake(149, 150). However, regarding pregnancy population, especially in the first pregnancy trimester, this supplementation is very frequent.

Aim of the study

The main objective of this study was to estimate the association between folate intake and folic acid supplementation (measured through a semi-quantitative FFQ, a FD and a questionnaire about vitamins supplements intake) in the periconceptional period and folate status (through serum folate and erythrocyte folate) in early pregnancy. As secondary objective, we aimed to establish socio-demographic determinants of an adequate intake of folic acid supplements in the periconceptional period.

METHODS

This study was developed in Department of Clinical Epidemiology Predictive Medicine and Public Health from the University of Porto with the collaboration of the Obstetrics and Gynaecology Department of Hospital S. João (Porto, Portugal). Women's recruitment occurred between December 2007 and December 2008. The study was submitted and approved by the Ethics Committee of the hospital S. João. In the first contact, participants signed a written informed consent.

Participants

Pregnant women were invited at first trimester ultrasound or in the prenatal visits (gestational age approximately of 12 weeks) at Obstetrics and Gynaecology Department of Hospital S. João, consecutively. During this first contact, all women with diabetes(103, 117, 118) or hypertension or taking anticonvulsants(3) or sulfasalazine(3) were excluded, since all these conditions can affect folate and folic acid metabolism. This situation was carefully explained to women excluded.

For all women who filled inclusion criteria, the aims of the study were explained, the participation was formalized by the written consent and identification data and contacts were collected in this first contact. A 3-days food diary (FD) protocol was given to women, accompanied of the respective instructions for completing. FD should have been filled in the 3 days previous to the following interview, scheduled for 15-16 gestational weeks, preferably in the same day of other appointments in Hospital. Women were instructed to attend fasting, in order to withdraw a blood sample. In the day previous to the start of FD (5 days before the interview), women received a phone call or a written message reminding the beginning of FD and the fasting condition in the interview day.

The interview included these steps: blood sample collection (after that, a breakfast was provided); filling (by the interviewer) a structured questionnaire with demographic and lifestyle variables, past medical history, health status during pregnancy, and use of vitamin and mineral supplements (including details on type of supplementations, and timing of their initiation and cessation); application of a validated semi-quantitative FFQ to access diet; and collection of FD.

Dietary and nutrient intake assessment

In order to assess dietary intake, women answered to a semi-quantitative FFQ, applied by a trained interviewer and filled a 3-days FD.

FFQ applied to access dietary intake comprise eighty-six food or food group items. Frequency of consumption was recorded into nine pre-specified categories from “never or less than once per month” to “six or more times per day”. Pre-specified portion sizes were allocated to each food item. Dietary intake was estimated by multiplying the frequency of intake for any given item by its respective portion size, in grams, and by a seasonal variation factor for foods consumed only in some seasons. The FFQ used had been validated to assess diet among Portuguese pregnant women(146). The FFQ reported usual diet during the first pregnancy trimester.

To the fulfilment of a 3-days FD, a protocol was given to each woman, containing written instructions, an example of one day of record and a page with standardized measures, in order to facilitate the completion. The means nutrients intake of the three days were calculated.

The conversion of foods into nutrients was performed through the Food Processor Plus program, version SQL (ESHA Research, Salem, OR, USA). This database has been supplemented with nutritional composition of Portuguese foods and recipes, using data from Portuguese food composition tables(62, 151) and national(152-155) and international(156-159) publications.

Folate status assessment

An overnight fasting blood sample was collected to determine a complete blood count (CBC), serum folate concentration, and erythrocyte folate concentration. Three tubes were collected: two EDTA (ethylenediaminetetraacetic acid) tubes, one for a complete blood test determination, and another for folate assessment in erythrocytes, and one gel tube was used for serum folate determination. Both tubes intended for folate assessments were protected with foil in order to protect from direct light exposure. The biochemical analysis of erythrocyte folate and serum folate were performed in the Abbott ARCHITECT® Folate based on a chemiluminescent microparticle immunoassay (CMIA) principle(160). For erythrocyte folate, the sample was firstly haemolysed with haemolysis reagent containing ascorbic acid and guanidine hydrochloride(160).

Statistical analysis

The Kolmogorov-Smirnov test was used to check normality of the variables distribution. Continuous variables were described as mean and standard deviation or as median and interquartile range (percentile 25; percentile 75), as adequate. Categorical variables were described as absolute and relative frequencies.

Total duration of folic acid supplementation during pregnancy was multiplied by the quantity of folic acid provided and divided by the pregnancy duration until the evaluation day. Folic acid from supplements was further expressed as DFE: 1 µg of DFE = 1 µg of folate = 0.6 µg of folic acid from a supplement consumed with food(3). After that, final quantities were added to estimates of folate intake by diet in order to quantify total folate intake.

Mean nutrients intake estimated by FFQ and by FD were compared using the paired-samples t-test. Total *folate plus folic acid* intake, considering both dietary methods and supplements, were compared by Wilcoxon test.

Erythrocyte folate and serum folate concentrations were log-transformed in order to assume a normal distribution. Pearson correlation coefficients were computed to estimate the association between folate intake and folic acid supplementation and biomarkers.

All statistical analyses were performed with the IBM SPSS Statistics for Windows, Version 21.0 (Released 2012. Armonk, NY: IBM Corp.).

RESULTS

Participant characteristics

Among the participants, we analysed 84 subjects, corresponding to the information that was computerized. The characteristics of the analysed participants are shown in Table 1. Pregnant women had a mean age of 31.8±5.0 at entry into the study. A third had 9 or less completed years of schooling. Almost all (91.7%) were married or in fact union. About a quarter reported smoking in the 3 months before pregnancy, in a median of 9/day cigarettes (interquartile range: 6-12). From the analysed participants, 23 (27.4%) were overweight and 13 (15.5%) were obese when got pregnant. It was the first delivery for about half (44.0%) of the women and gravidity ranged between 1 and 6 pregnancies (including actual). Three reported congenital anomalies in a previous pregnancy. Over half pregnancies were planned (66.7%) and the first prenatal visit occurred at 7.5±3.1 weeks of gestational age. It occurred before 6 gestational weeks for 21(25.0%) women. Most pregnant women experienced nausea (72.6%) and/or vomiting (61.9%) in the first trimester of pregnancy.

Table 1. Characteristics of the analysed participants (n=84).

Characteristic	Result
Maternal age (years) [mean (SD)]	31.8 (5.0)
Maternal education (years) [n (%)]	
≤ 9 years	28 (33.3)
10 – 12 years	33 (39.3)
> 12 years	23 (27.4)
Marital status [n (%)]	
Married/ fact union	77 (91.7)
Other	7 (8.3)
Maternal smoking habits	
In the 3 months before pregnancy [n (%)]	19 (22.6)
No. cigarettes per day [median (P25; P75)]	8.0 (3.0; 20.0)
During the first pregnancy trimester [n (%)]	14 (16.7)
No. cigarettes per day [median (P25; P75)]	3.5 (1.6; 5.3)
Pre-pregnancy BMI (kg/m^2) [mean (SD)]	25.6 (5.7)
25.0-29.9 [n (%)]	23 (27.4)
≥ 30.0 [n (%)]	13 (15.5)
Gravidity = 1 [n (%)]	37 (44.0)
Pregnancy after assisted reproduction techniques [n (%)]	6 (7.1)
Planned pregnancy [n (%)]	56 (66.7)
Gestational age at first prenatal visit (weeks) [mean (SD)]	7.5 (3.1)
Reported health problems in first trimester [n (%)]	
Nausea	61 (72.6)
Vomiting	52 (61.9)
Diarrhea	14 (16.7)
Constipation	24 (28.6)
Urinary infection	17 (20.2)

SD: standard deviation. P25: percentile 25. P75: percentile 75. BMI: body mass index. No.: number.

Socio-demographic characteristics (maternal age and education, marital status, gravidity, pregnancy after assisted reproduction, planned pregnancy, and gestational age at first prenatal visit) were compared between women by had taken or not had taken folic acid supplements before pregnancy (results not shown). Women who had taken folic acid supplements before pregnancies were more likely to have planned pregnancies (94.3% vs. 46.9%; $P < 0.001$) and to have their first delivery (57.1% vs. 34.7%; $P < 0.05$) compared to unsupplemented women. Others characteristics had no statistical significant difference. The mean age was equal.

Folic acid supplementation

Prevalence of use of folic acid supplements during the three months before pregnancy was 41.7%, increasing to 91.7% during the first pregnancy trimester, as can be seen from the Table 2. The median dose of folic acid supplements used in the first trimester of pregnancy was 3.29 mg (interquartile range: 1.85-5.00), corresponding to 5.58 mg expressed as DFE.

Almost all (33/35) women who took any supplements in the three months before to get pregnant, took supplements containing 5 mg of folic acid per day.

Table 2. Folic acid supplements use (n=84).

Use of folic acid supplements	Result
During the 3 months before pregnancy [n (%)]	35 (41.7)
During the first pregnancy trimester [n (%)]	77 (91.7)
Folic acid (mg) [median (P25; P75)]	3.29 (1.85; 5.00)
Folic acid as DFE (mg) [median (P25; P75)]	5.58 (3.15; 8.50)

DFE: Dietary Folate Equivalents.

Nutrient intake

Absolute daily mean energy intake was 2410±534 kcal and 2042±508 kcal, accessed through FFQ and FD, respectively, as shown in Table 3. Energy, protein and carbohydrates obtained by FFQ and FD were significantly different ($P < 0.05$), but total fat and folate were not. Mean folate intake was 0.35±0.11 mg and 0.30±0.16 mg (by FFQ and FD, respectively). Both values approximates the EAR for women (320 µg/day DFE), but were clearly below the EAR for pregnant women (520 µg/day DFE). According to the first cut-off point, the prevalence of folate inadequacy was 41.7% by FFQ. According to the second cut-off point, the prevalence of folate inadequacy was 95.2% using FFQ data and 91.7% using FD.

Table 3. Absolute daily energy, macronutrients and folate intakes, estimated by the food frequency questionnaire and food diaries, and folic acid supplementation, in the first trimester of pregnancy (n=84).

Nutrient	FFQ	FD	*P*
	Mean (SD)		
Energy (kcal)	2410 (534)	2042 (508)	0.022
Protein (g)	112.6 (23.1)	93.7 (24.1)	0.005
Carbohydrates (g)	303.8 (82.5)	244.6 (69.4)	0.007
Total fat (g)	86.7 (22.1)	78.3 (24.0)	0.209
Folate (mg)	0.35 (0.11)	0.30 (0.16)	0.113
	Median (P25; P75)		
Folate + folic acid as DFE (mg)	5.93 (3.46; 8.75)	5.95 (3.46; 8.71)	0.001

FFQ: food frequency questionnaire. FD: food diary. DFE: Dietary Folate Equivalents.

Folate plus folic acid median intake was nearby 6 mg DFE from both tools of dietary assessment, although the values were considered significantly different ($P < 0.01$).

Folate status

The results obtained from the preliminary analysis of biomarkers are presented in Table 4.

The median and interquartile values for serum folate and erythrocyte folate were in the normal range, considering as folate deficiency values lower than 7 nmol/l for serum folate and lower than 300 nmol/l for erythrocyte folate(107). Only 4.8% of the analysed participants were classified as folate deficiency using the first criteria, but 13.1% considering the second criteria. All pregnant women with low serum folate levels had also low erythrocyte levels.

Table 4. Folate status (n=84).

Biochemical markers	Median (P25; P75)
Serum folate (nmol/l)	14.7 (13.0; 17.0)
Erythrocyte folate (nmol/l)	476.2 (369.6; 660.9)
Erythrocyte count* ($10^6/\mu l$)	3.96 (3.76; 4.23)

P25: percentile 25. P75: percentile 75. *RBC: Red blood cell count.

Associations between nutrient intake and biomarkers

From the analysed participants, were obtained the associations provided by Table 5. Folate intake (both by FFQ and FD) didn't show any association with biomarkers, but folic acid alone or with folate intake showed.

Table 5. Associations between folate intake and folic acid supplementation and folate status (n=84).

Nutrient	Serum folate	Erythrocyte folate
	Pearson correlation coefficients	
Folate by FFQ	-0.075	-0.147
Folate by FD	0.098	0.084
Folic acid as DFE	0.316**	0.251*
Folate by FFQ + folic acid as DFE	0.312**	0.245*
Folate by FD + folic acid as DFE	0.352**	0.263*

FFQ: food frequency questionnaire. FD: food diary. DFE: Dietary Folate Equivalents. * $P < 0.05$; ** $P < 0.01$.

DISCUSSION

Maternal folate intake is clearly below the international recommendations during pregnancy. Considering the more robust biomarker for folate (erythrocyte levels), 13% of women showed low levels. Although supplementation prevalence was very high during pregnancy, the median age at initiation is visibly late, based on the knowledge that neural tube closure occurs about 28 days after conception. More than 60% of pregnancies were planned, however preconception supplementation occurred in only about 40%.

According to this study, being a planned pregnancy was the main determinant of using folic acid supplementation before pregnancy. This finding is consistent with a previous study(120). It is interesting to note that only 33.3% pregnancies were unplanned, below the estimated 41% worldwide(109) and the 43.4% from a Portuguese birth cohort (Geração XXI) nested study(13). The maternal education level seems similar to Portuguese population for this group(129), although with a slightly higher education level compared with the mentioned cohort. Overall, the socio-demographic characteristics analysed had no clear relationship with the use of folic acid supplements.

These differences in relation to others studies, could result from a possible selection bias, led by the fact that women were invited at first trimester ultrasound or in the prenatal visits (gestational age approximately of 12 weeks), where some women do not know yet they are pregnant, which could result in more than the previous obtained percentage of women taking folic acid supplements before pregnancy (41.7% against 18.6% in the referred cohort(13)). During pregnancy the prevalence of folic acid supplementation (91.7%) was, to some extent, similar to others studies (81.9%(120) and 96.8%(13)). The supplementation before pregnancy is consensually considered more effective than only in pregnancy, because most women know they are pregnant after the week 6 of pregnancy, when the neural tube closes.

Another factor that could have risen the prevalence of folic acid supplementation is the high percentage of planned pregnancies. Concerning smoking habits, less (16.7%) than previous found (25% (13)) smoked in the first pregnancy trimester. This factor is associated with a lower folate intake (13), here not analysed.

Most pregnant women (61.9%) reported vomiting, which hypothetically could impair the intake of folate, although it does not modify the previous validation and reproducibility of the FQQ among Portuguese women (146).

From the analysed participants, 23 (27.4%) were overweight, 13 (15.5%) were obese when got pregnant, and the mean body mass index was coincident with the cut-off point for overweigh. So it can be hypothesized that many of them have higher ingestion of calories than their need (from a Western dietary pattern and/or lower physical activity levels), and a lower ingestion of folate rich food (typical from a Western dietary pattern), which can explain the low level of folate intake. Obesity is considered a high risk factor for having low folate status.

Almost all women that used supplements took the same dose: 5 mg of folic acid, which is internationally recommended only for high risk women. None had 400 µg of supplemented folic acid alone as, according to Infarmed (the national authority for drugs and health products), supplements of 400 µg of folic acid alone are not commercialized in Portugal, but only of 5 mg (126).

Concerning folate intake, the mean of 350±110 µg/day and 300±160 µg/day (not significantly different in both FFQ and FD, respectively, being FFQ already validated for Portuguese pregnant women(146)) is consistent with previous findings that showed a mean intake of 300 µg/day(13, 94). The median *folate plus folic acid* intake was nearby 6 mg DFE, greatly higher than the RDA for folate intake expressed in DFE plus supplementation of folic acid for prevention of NTD. Comparing to the best scenario: a high folate diet (600 µg/day) + folic acid intake (400 µg/day) = 600 µg DFE + (400 ÷ 0.6) µg DFE = 1270 µg DFE (approximately 1 mg DFE), the value of this sample is much higher (six-folder) than the “ideal” scenario. Here was considered the DRI values, but other recommendations from other entities could be considered.

Even recommendations seems to be clearly satisfied considering supplementation and being supplementation prevalence quite high, a critical point remains, regarding the beginning of supplementation.

Considering folate status, only 4.8% were inadequate in serum folate, and 13.3% in erythrocyte folate. The median values (P25; P75) were 14.7 (13.0; 17.0) nmol/l for serum folate and 476.2 nmol/l (369.6; 660.9) for erythrocyte folate, all above the cut-off points considered. These high values can be mostly explained by the high supplementation in folic acid (5 mg), present in almost all women. Erythrocyte levels are considered a more robust biomarker, since it reflects the folate status approximately in the previous four months, in opposite to serum folate that reflects only the previous 3-4 days.

Seeking the quantification of the association between folate and folic acid intakes and maternal folate levels, both intake sources were combined. However, we recognized that this combination could have some problems in the serum assays, regarding women that had

stopped supplementation more than week before the blood collection. It also important to emphasize that the physiological blood folate levels of pregnant women are not the same as non-pregnant women (usually lower) and this should be taken in consideration when analysing data(11). Considering the value of 13 nmol for serum folate, the median from this sample is above it.

The results indicate that there is a modest association between folic acid supplementation and folate status, but failed to detect any association between folate intake and folate status in early pregnancy. One of the reasons for the weak moderate associations could be the supraphysiologic doses supplemented. Previous study referred the increase of unmetabolized folic acid when the doses are high.

It is plausible that maternal folate levels are mainly justified by supplements intake. Considering that we selected the sample from an Obstetrics department, during the first pregnancy trimester, we have probably a biased image of the reality of Portuguese pregnant women, where the supplementation prevalence would be lower and in some cases initiated even later. The possibility to performed these blood assays around 4^{th} – 6^{th} gestational weeks would be also interesting, in order to quantify the inadequacy in this crucial period.

CONCLUSION

Maternal folate intake is clearly below the international recommendations during pregnancy. Considering the respective biomarker (erythrocyte folate levels), 13% of women showed low levels. The study identified a moderate association between folic acid supplementation and folate status. It was shown a low intake of folate during pregnancy and a late initiation of the supplementation, indicating that guidelines are unlikely to be effective. Only being a planned pregnancy was the main determinant of starting folic acid supplementation in the preconception, as desirable.

GENERAL CONCLUSION

Regardless the importance of adequate levels of folate during pregnancy, many countries has not clear recommendations regarding the possibilities for warrant this adequacy.

Diet should be the first element considered, namely because a healthy diet should be encouraged independently of the pregnancy condition. Based on the literature review, the folate content of a *prudent dietary pattern* plan was almost double of a *Western dietary pattern* plan, and achieved the RDA for folate for women in childbearing age. Public health efforts should incorporate practical advice on storing and cooking and emphasize the importance of the *prudent dietary pattern* to increase folate intakes.

Disparities in what concerns folate adequacy were seen among different countries. However, the recommendation of 400 µg of folic acid in the periconceptional period is predominant. In Portugal, a quantitative recommendation is not available. Only high levels supplements are commercialized, so the majority of women take this kind of supplements. High intakes of folic acid have being shown through pregnancy outcomes studies, both in mother and child, early and later in life, to have adverse effects. Accurate recommendations are needed in Portugal.

Intake of folate in pregnant women was below the recommended level for pregnancy in 91.7% pregnant women. Although, the median *folate plus folic acid* intake was nearby 6 mg DFE, six-folder higher than the recommendations for folate intake plus supplementation of folic acid for prevention of NTD. The folic acid intake alone was 12-folder higher, without distinction from high risk women, due to use of high doses of folic acid (5 mg) without differentiate low risk women. Concerning folate status, only 4.8% of the analysed participants were classified as folate deficiency using serum folate, but 13.1% through erythrocyte folate.

The study identified a modest association positive association between folic acid supplementation and folate status, but not with folate intake and folate status. It was shown a low intake of folate during pregnancy and a late initiation of the supplementation, indicating that guidelines are unlikely to be effective. Only being a planned pregnancy was the main determinant of starting folic acid supplementation in the preconception, as desirable.

In Portugal new guidelines are needed, including quantitative recommendations regarding folic acid supplementation and emphasizing a healthy dietary pattern.

REFERENCES

1. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet. 1991;338(8760):131-7.
2. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. The New England journal of medicine. 1992;327(26):1832-5.
3. Institute of Medicine. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Otten JJ, Hellwig JP, Meyers LD, editors. Washington, DC: The National Academies Press; 2006.
4. Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Abyholm F, et al. Folic acid supplements and risk of facial clefts: national population based case-control study. BMJ (Clinical research ed). 2007;334(7591):464.
5. Kelly D, O'Dowd T, Reulbach U. Use of folic acid supplements and risk of cleft lip and palate in infants: a population-based cohort study. The British journal of general practice : the journal of the Royal College of General Practitioners. 2012;62(600):e466-72.
6. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Pena-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. The Cochrane database of systematic reviews. 2010(10):Cd007950.
7. Rozendaal AM, van Essen AJ, Te Meerman GJ, Bakker MK, van der Biezen JJ, Goorhuis-Brouwer SM, et al. Periconceptional folic acid associated with an increased risk of oral clefts relative to non-folate related malformations in the Northern Netherlands: a population based case-control study. European journal of epidemiology. 2013.
8. Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington,DC: National Academies Press; 1998.
9. World Health Organization. WHO Guidelines Approved by the Guidelines Review Committee. Guideline: Daily Iron and Folic Acid Supplementation in Pregnant Women. Geneva: World Health Organization; 2012.
10. World Health Organization. Prevention of neural tube defects. Standards for Maternal and Neonatal Care Department of Making Pregnancy Safer. Geneva: World Health Organization; 2006.
11. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Human reproduction update. 2013;19(6):640-55.
12. Vandevijvere S, Amsalkhir S, Van Oyen H, Moreno-Reyes R. Determinants of folate status in pregnant women: results from a national cross-sectional survey in Belgium. European journal of clinical nutrition. 2012;66(10):1172-7.
13. Pinto E, Barros H, dos Santos Silva I. Dietary intake and nutritional adequacy prior to conception and during pregnancy: a follow-up study in the north of Portugal. Public health nutrition. 2009;12(7):922-31.
14. European Food Safety Authority. Folic acid: an update on scientific developments. Uppsala, Sweden: 2009.
15. Huo Y, Qin X, Wang J, Sun N, Zeng Q, Xu X, et al. Efficacy of folic acid supplementation in stroke prevention: new insight from a meta-analysis. International journal of clinical practice. 2012;66(6):544-51.
16. Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. Lancet. 2007;369(9576):1876-82.
17. Butterworth CE, Jr., Tamura T. Folic acid safety and toxicity: a brief review. The American journal of clinical nutrition. 1989;50(2):353-8.
18. United Nations. United Nations Statistics Division 2012 [2013-10]. Available from: http://unstats.un.org/unsd/methods/m49/m49regin.htm.

19. EUROCAT. World map of countries having mandatory fortification of food with folic acid. 2013 [2013-09]. Available from: http://www.eurocat-network.eu/content/EUROCAT-Folic-Acid-Map.pdf.
20. Institute of Medicine. Chapter 4 - Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. In: Suitor CW, Meyers LD, Food and Nutrition Board, editors. Dietary Reference Intakes Research Synthesis Workshop Summary. Washington, DC: The National Academies Press; 2006.
21. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary Guidelines for Americans. 7th ed. Washington, DC: U.S. Government Printing Office; 2010.
22. Food and Agriculture Organization of the United Nations, World Health Organization. FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements. FAO/WHO, editor. Bangkok, Thailand2001.
23. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA : the journal of the American Medical Association. 2007;297(21):2351-9.
24. Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA : the journal of the American Medical Association. 2009;302(19):2119-26.
25. Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, Nickel C, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. Cancer epidemiology. 2011;35(1):2-10.
26. Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals. Lancet. 2013;381(9871):1029-36.
27. Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. The Journal of nutrition. 2006;136(1):189-94.
28. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. The American journal of clinical nutrition. 1997;65(6):1790-5.
29. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate. In: European Commission, editor. Brussels: European Comission; 2000.
30. Sweeney MR, McPartlin J, Scott J. Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. BMC public health. 2007;7:41.
31. Tam C, O'Connor D, Koren G. Circulating unmetabolized folic Acid: relationship to folate status and effect of supplementation. Obstetrics and gynecology international. 2012;2012:485179.
32. Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, et al. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged > or =60 y in the United States. The American journal of clinical nutrition. 2010;92(2):383-9.
33. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients. 2011;3(3):370-84.
34. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? The American journal of clinical nutrition. 2004;80(5):1123-8.
35. Achon M, Reyes L, Alonso-Aperte E, Ubeda N, Varela-Moreiras G. High dietary folate supplementation affects gestational development and dietary protein utilization in rats. The Journal of nutrition. 1999;129(6):1204-8.
36. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. The Journal of nutrition. 2002;132(8 Suppl):2393s-400s.

37. Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(2):189-93.
38. Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Klemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. BMJ open. 2012;2(1):e000653.
39. Qin X, Cui Y, Shen L, Sun N, Zhang Y, Li J, et al. Folic acid supplementation and cancer risk: a meta-analysis of randomized controlled trials. International journal of cancer Journal international du cancer. 2013;133(5):1033-41.
40. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, Johnson KA, Johnson C, Buys SS, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The American journal of clinical nutrition. 2006;83(4):895-904.
41. World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR; 2007.
42. National Center for Biotechnology Information. MeSH - Pregnancy Outcome. USA: U.S. National Library of Medicine; 2013 [2013-10]. Available from: http://www.ncbi.nlm.nih.gov/mesh/?term=pregnancy+outcomes.
43. Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall MH. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. Paediatric and perinatal epidemiology. 2005;19(2):112-24.
44. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food and nutrition bulletin. 2008;29(2 Suppl):S238-44.
45. Fekete K, Berti C, Trovato M, Lohner S, Dullemeijer C, Souverein OW, et al. Effect of folate intake on health outcomes in pregnancy: a systematic review and meta-analysis on birth weight, placental weight and length of gestation. Nutrition journal. 2012;11:75.
46. Lassi ZS, Salam RA, Haider BA, Bhutta ZA. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. The Cochrane database of systematic reviews. 2013;3:Cd006896.
47. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008;51(1):29-38.
48. Burdge GC, Lillycrop KA. Folic acid supplementation in pregnancy: Are there devils in the detail? The British journal of nutrition. 2012;108(11):1924-30.
49. Whitrow MJ, Moore VM, Rumbold AR, Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. American journal of epidemiology. 2009;170(12):1486-93.
50. Keating E. Suplementação com ácido fólico: impacto metabólico transgeracional. FMUP, 2013.
51. Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, Jackson AA, et al. Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. Hypertension. 2006;47(5):982-7.
52. Herrmann W, Obeid R. The mandatory fortification of staple foods with folic acid: a current controversy in Germany. Deutsches Arzteblatt international. 2011;108(15):249-54.
53. Kappen C. Modeling anterior development in mice: Diet as modulator of risk for neural tube defects. American journal of medical genetics Part C, Seminars in medical genetics. 2013;163(4):333-56.
54. Crider KS, Cordero AM, Qi YP, Mulinare J, Dowling NF, Berry RJ. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. The American journal of clinical nutrition. 2013;98(5):1272-81.
55. Haberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. Archives of disease in childhood. 2009;94(3):180-4.

56. Haberg SE, London SJ, Nafstad P, Nilsen RM, Ueland PM, Vollset SE, et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. The Journal of allergy and clinical immunology. 2011;127(1):262-4, 4.e1.
57. Sengpiel V, Bacelis J, Myhre R, Myking S, Pay AD, Haugen M, et al. Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: a prospective observational cohort study. BMC pregnancy and childbirth. 2013;13:160.
58. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, et al. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. PloS one. 2009;4(11):e7845.
59. Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G. Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. The American journal of clinical nutrition. 2013;97(1):94-9.
60. Harper D. Online Etymology Dictionary 2013 [cited 2013-06]. Available from: http://www.etymonline.com/index.php?allowed_in_frame=0&search=folic&searchmode=none .
61. Mahan LK, Escott-Stump S. Krause's Food and Nutrition Therapy. 12 ed. Canada: Saunders; 2008.
62. Martins I, Porto A, Oliveira L. Tabela da Composição de Alimentos. Lisboa: Instituto Nacional de Saúde Dr. Ricardo Jorge; 2006.
63. U.S. Department of Agriculture. National Nutrient Database for Standard Reference: USDA; 2011 [2013-06]. Available from: http://ndb.nal.usda.gov/.
64. Gregory JF, 3rd. Case study: folate bioavailability. The Journal of nutrition. 2001;131(4 Suppl):1376s-82s.
65. McKillop DJ, Pentieva K, Daly D, McPartlin JM, Hughes J, Strain JJ, et al. The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. The British journal of nutrition. 2002;88(6):681-8.
66. Witthoft CM, Forsskn K, Johannesson L, Jagerstad M. Folates - food sources, analyses, retention and bioavailability. Scandinavian Journal of Nutrition. 1999;43:138-46.
67. Malin J. Total folate activity in Brussel sprouts: the effects of storage, processing, cooking and ascorbic content. Journal of Food Technology. 1977;12:623–32.
68. Dang J, Arcot J, Shrestha A. Folate retention in selected processed legumes. Food chemistry. 2000;68(3):295-8.
69. Leichter J, Switzer V, Landymore A. Effect of cooking on folate content of vegetables Nutrition Reports International. 1978;18:475–9.
70. Czarnowska M, Gujska E. Effect of freezing technology and storage conditions on folate content in selected vegetables. Plant foods for human nutrition (Dordrecht, Netherlands). 2012;67(4):401-6.
71. Rodrigues SS, Franchini B, Graca P, de Almeida MD. A new food guide for the Portuguese population: development and technical considerations. Journal of nutrition education and behavior. 2006;38(3):189-95.
72. Sotres-Alvarez D, Siega-Riz AM, Herring AH, Carmichael SL, Feldkamp ML, Hobbs CA, et al. Maternal dietary patterns are associated with risk of neural tube and congenital heart defects. American journal of epidemiology. 2013;177(11):1279-88.
73. Instituto Nacional de Estatística. Balança Alimentar Portuguesa 2003-2008. INE, 2010 [2013-06]. Available from: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_destaques&DESTAQUESdest_boui=83386467&DESTAQUESmodo=2&xlang=pt.
74. Murphy EW, Criner PE, Gray BC. Comparisons of methods for calculating retention of nutrients in cooked foods. Journal of agricultural and food chemistry. 1975;23(6):1153-7.
75. U.S. Department of Agriculture. USDA Table of Nutrient Retention Factors Release 6. Maryland, 2007 [2013-08]. Available from: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/retn/retn06.pdf.
76. Centers for Disease Control and Prevention. Use of Supplements Containing Folic Acid Among Women of Childbearing Age - United States, 2007. In: Morbidity and Mortality Weekly

Report. Atlanta: CDC; 2008 [2013-09]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5701a3.htm.
77. Kawashima A, Madarame T, Koike H, Komatsu Y, Wise JA. Four week supplementation with mixed fruit and vegetable juice concentrates increased protective serum antioxidants and folate and decreased plasma homocysteine in Japanese subjects. Asia Pacific journal of clinical nutrition. 2007;16(3):411-21.
78. Food and Agriculture Organization of the United Nations, World Health Organization. Vitamin and mineral requirements in human nutrition. 2 ed. China: 2004.
79. Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CM, Duran M, et al. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. The Journal of nutrition. 1999;129(6):1135-9.
80. Machado A, Feijóo M. Ácido fólico e anomalias congénitas: conhecimentos da população portuguesa. Rev Port Clin Geral. 2006;22:149-60.
81. Tamura T, Stokstad EL. The availability of food folate in man. British journal of haematology. 1973;25(4):513-32.
82. Hytten FE. The renal excretion of nutrients in pregnancy. Postgraduate medical journal. 1973;49(575):625-9.
83. Pei L, Zhu H, Ren A, Li Z, Hao L, Finnell RH, et al. Reduced folate carrier gene is a risk factor for neural tube defects in a Chinese population. Birth defects research Part A, Clinical and molecular teratology. 2005;73(6):430-3.
84. Mayes P. Struture and function of the water-soluble vitamins. In: Murray R, Granner D, Mayes P, Rodwell V, editors. Harper's Biochemistry. Stamford: Appleton & Lange; 2000. p. 627-41.
85. Beckmann C, Ling F, Barzansky B, Herbert W, Laube D, Smith R. Obstetrics and Gynecology. 6 ed. China: Lippincott Williams & Wilkins; 2010.
86. Escott-Stump S. Nutrição relacionada ao diagnóstico e tratamento. 5 ed. Barueri: Manole; 2007.
87. World Health Organization. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva: WHO, 2012.
88. Tamura T, Picciano MF. Folate and human reproduction. The American journal of clinical nutrition. 2006;83(5):993-1016.
89. Farrell CJ, Kirsch SH, Herrmann M. Red cell or serum folate: what to do in clinical practice? Clinical chemistry and laboratory medicine : CCLM / FESCC. 2013;51(3):555-69.
90. Ihara H, Watanabe T, Aoki Y, Nagamura Y, Totani M, Hashizume N. Dietary folate intake and serum folate status in Japanese women of childbearing age. Journal of Analytical Bio-Science. 2009;32(2).
91. Berti C, Fekete K, Dullemeijer C, Trovato M, Souverein OW, Cavelaars A, et al. Folate intake and markers of folate status in women of reproductive age, pregnant and lactating women: a meta-analysis. Journal of nutrition and metabolism. 2012;2012:470656.
92. Scientific Committee on Food. Report of the Scientific Committee on Food. Nutrition and energy intakes for the European Community. Commission of the European Communities; 1993.
.
93. de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers-Theunissen RP. Folate intake in Europe: recommended, actual and desired intake. European journal of clinical nutrition. 1997;51(10):643-60.
94. European Nutrition and Health Report 2009. Vienna: European Union, 2009.
95. Sieber R, Eyer H. Nährstoffzufuhr: Neue Referenzwerte - Chance für Landwirtschaft. Agrarforschung. 2001;8(2):72-7.
96. Becker W, Lyhne N, Pedersen A, Aro A, Fogelholm M, Þhórsdottir I, et al. Nordic Nutrition Recommendations 2004 - integrating nutrition and physical activity. Scandinavian Journal of Nutrition. 2004;48(4):178-87.
97. Direcção-Geral da Saúde. Portugal - Alimentação Saudável em Números - 2013. Lisboa: 2013.

98. Tabacchi G, Wijnhoven TM, Branca F, Roman-Vinas B, Ribas-Barba L, Ngo J, et al. How is the adequacy of micronutrient intake assessed across Europe? A systematic literature review. The British journal of nutrition. 2009;101 Suppl 2:S29-36.
99. Ray JG, Meier C, Vermeulen MJ, Wyatt PR, Cole DE. Association between folic acid food fortification and congenital orofacial clefts. The Journal of pediatrics. 2003;143(6):805-7.
100. Vujkovic M, Ocke MC, van der Spek PJ, Yazdanpanah N, Steegers EA, Steegers-Theunissen RP. Maternal Western dietary patterns and the risk of developing a cleft lip with or without a cleft palate. Obstetrics and gynecology. 2007;110(2 Pt 1):378-84.
101. Krapels IP, van Rooij IA, Ocke MC, West CE, van der Horst CM, Steegers-Theunissen RP. Maternal nutritional status and the risk for orofacial cleft offspring in humans. The Journal of nutrition. 2004;134(11):3106-13.
102. Rosano A, Botto LD, Botting B, Mastroiacovo P. Infant mortality and congenital anomalies from 1950 to 1994: an international perspective. Journal of epidemiology and community health. 2000;54(9):660-6.
103. Kondo A, Kamihira O, Ozawa H. Neural tube defects: prevalence, etiology and prevention. International journal of urology : official journal of the Japanese Urological Association. 2009;16(1):49-57.
104. Shurtleff DB. Epidemiology of neural tube defects and folic acid. Cerebrospinal fluid research. 2004;1(1):5.
105. Morris JK, Wald NJ. Quantifying the decline in the birth prevalence of neural tube defects in England and Wales. Journal of medical screening. 1999;6(4):182-5.
106. Sumiyoshi Y. How do we react on folic acid deficiency? Brain Spinal Cord 2007;14:1-3.
107. Bailey LB. New standard for dietary folate intake in pregnant women. The American journal of clinical nutrition. 2000;71(5 Suppl):1304s-7s.
108. Botto LD, Lisi A, Robert-Gnansia E, Erickson JD, Vollset SE, Mastroiacovo P, et al. International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? BMJ (Clinical research ed). 2005;330(7491):571.
109. Singh S, Sedgh G, Hussain R. Unintended pregnancy: worldwide levels, trends, and outcomes. Studies in family planning. 2010;41(4):241-50.
110. Brown RD, Langshaw MR, Uhr EJ, Gibson JN, Joshua DE. The impact of mandatory fortification of flour with folic acid on the blood folate levels of an Australian population. The Medical journal of Australia. 2011;194(2):65-7.
111. Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995-2002. Pediatrics. 2005;116(3):580-6.
112. De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, et al. Reduction in neural-tube defects after folic acid fortification in Canada. The New England journal of medicine. 2007;357(2):135-42.
113. Lucock M, Yates Z. Folic acid fortification: a double-edged sword. Current opinion in clinical nutrition and metabolic care. 2009;12(6):555-64.
114. Osterhues A, Ali NS, Michels KB. The role of folic acid fortification in neural tube defects: a review. Critical reviews in food science and nutrition. 2013;53(11):1180-90.
115. Heseker HB, Mason JB, Selhub J, Rosenberg IH, Jacques PF. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. The British journal of nutrition. 2009;102(2):173-80.
116. Stoll C, Alembik Y, Dott B. Are the recommendations on the prevention of neural tube defects working? European journal of medical genetics. 2006;49(6):461-5.
117. Kennedy D, Koren G. Identifying women who might benefit from higher doses of folic acid in pregnancy. Canadian family physician Medecin de famille canadien. 2012;58(4):394-7.
118. Talaulikar VS, Arulkumaran S. Folic acid in obstetric practice: a review. Obstetrical & gynecological survey. 2011;66(4):240-7.

119. Bitzer J, von Stenglin A, Bannemerschult R. Women's awareness and periconceptional use of folic acid: data from a large European survey. International journal of women's health. 2013;5:201-13.
120. Lunet N, Rodrigues T, Correia S, Barros H. Adequacy of prenatal care as a major determinant of folic acid, iron, and vitamin intake during pregnancy. Cadernos de saude publica. 2008;24(5):1151-7.
121. Braz P, Dias C. Report on Periconceptional Folic Acid Supplementation for Portugal. Folic Acid Special Reports - Survey of Folic Acid Policy and Practice in European Countries [Internet]. 2007; (2013-09). Available from: http://www.eurocat-network.eu/preventionandriskfactors/folicacid/folicacidspecialreports.
122. European Comission. Trustworthy websites on “Population groups" Brussels: European Comission; 2013 [2013-10]. Available from: http://ec.europa.eu/health/population_groups/portal/index_en.htm#tab_my_country.
123. Theodosiou L, Green J. Emerging challenges in using health information from the internet. Advances in Psychiatric Treatment. 2003;9:387–96.
124. Mo P. The Use of Internet for Health Education. J Biosafety Health Educ. 2012;1(1):e102.
125. Houston TK, Allison JJ. Users of Internet health information: differences by health status. Journal of medical Internet research. 2002;4(2):E7.
126. Infarmed - Autoridade Nacional do Medicamento e Produtos de Saúde IP. Infomed - Base de dados de medicamentos: Ministério da Saúde; 2013.
127. Infarmed - Autoridade Nacional do Medicamento e Produtos de Saúde IP. Consumos de Substâncias Ativas a Conter Ácido Fólico, no Âmbito do Serviço Nacional de Saúde, em Ambulatório. 2013.
128. Eurostat. Eurostat - Tables, Graphs and Maps Interface (TGM) 2013 [2013-10]. Available from: http://epp.eurostat.ec.europa.eu/tgm/printTable.do?tab=table&plugin=1&language=en&pcode=tps00001&printPreview=true.
129. Instituto Nacional de Estatística. Instituto Nacional de Estatística - Statistics Portugal 2013 [2013-10]. Available from: http://www.ine.pt/.
130. Instituto Nacional de Saúde Dr. Ricardo Jorge. Registo Nacional de Anomalias Congénitas (RENAC). Relatório de 2002-2007. Lisboa: 2010.
131. Direcção-Geral da Saúde. Circular Normativa - Prestação de cuidados pré-concepcionais - N.º2/DSMIA, 18-03-1998. In: Divisão de Saúde Materna Infantil e dos Adolescentes, editor.: Direcção-Geral da Saúde; 1998.
132. Kaiser LL, Allen L. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. Journal of the American Dietetic Association. 2002;102(10):1479-90.
133. Shah D, Sachdev HP. Maternal micronutrients and fetal outcome. Indian journal of pediatrics. 2004;71(11):985-90.
134. Safi J, Joyeux L, Chalouhi GE. Periconceptional folate deficiency and implications in neural tube defects. Journal of pregnancy. 2012;2012:295083.
135. Hall J, Solehdin F. Folic acid for the prevention of congenital anomalies. European journal of pediatrics. 1998;157(6):445-50.
136. Kaiser L, Allen LH. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. Journal of the American Dietetic Association. 2008;108(3):553-61.
137. Aranceta J, Serra-Majem L, Perez-Rodrigo C, Llopis J, Mataix J, Ribas L, et al. Vitamins in Spanish food patterns: the eVe Study. Public health nutrition. 2001;4(6a):1317-23.
138. Bacardi-Gascon M, Ley y de Gongora S, Castro-Vazquez BY, Jimenez-Cruz A. Validation of a semiquantitative food frequency questionnaire to assess folate status. Results discriminate a high-risk group of women residing on the Mexico-US border. Archives of medical research. 2003;34(4):325-30.
139. Cade J, Thompson R, Burley V, Warm D. Development, validation and utilisation of food-frequency questionnaires - a review. Public health nutrition. 2002;5(4):567-87.

140. Ishihara J, Yamamoto S, Iso H, Inoue M, Tsugane S. Validity of a self-administered food frequency questionnaire (FFQ) and its generalizability to the estimation of dietary folate intake in Japan. Nutrition journal. 2005;4:26.
141. Jackson MD, Walker SP, Younger NM, Bennett FI. Use of a food frequency questionnaire to assess diets of Jamaican adults: validation and correlation with biomarkers. Nutrition journal. 2011;10:28.
142. Johansson I, Van Guelpen B, Hultdin J, Johansson M, Hallmans G, Stattin P. Validity of food frequency questionnaire estimated intakes of folate and other B vitamins in a region without folic acid fortification. European journal of clinical nutrition. 2010;64(8):905-13.
143. Pufulete M, Emery PW, Nelson M, Sanders TA. Validation of a short food frequency questionnaire to assess folate intake. The British journal of nutrition. 2002;87(4):383-90.
144. Verkleij-Hagoort AC, de Vries JH, Stegers MP, Lindemans J, Ursem NT, Steegers-Theunissen RP. Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. European journal of clinical nutrition. 2007;61(5):610-5.
145. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. American journal of epidemiology. 1985;122(1):51-65.
146. Pinto E, Severo M, Correia S, dos Santos Silva I, Lopes C, Barros H. Validity and reproducibility of a semi-quantitative food frequency questionnaire for use among Portuguese pregnant women. Maternal & child nutrition. 2010;6(2):105-19.
147. Kaaks RJ. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. The American journal of clinical nutrition. 1997;65(4 Suppl):1232s-9s.
148. Willett W, Lenart E. Reproducibility and Validity of Food-Frequency Questionnaires. Nutritional Epidemiology. 2 ed. New York, 1998.
149. Caudill MA, Cruz AC, Gregory JF, 3rd, Hutson AD, Bailey LB. Folate status response to controlled folate intake in pregnant women. The Journal of nutrition. 1997;127(12):2363-70.
150. West AA, Yan J, Perry CA, Jiang X, Malysheva OV, Caudill MA. Folate-status response to a controlled folate intake in nonpregnant, pregnant, and lactating women. The American journal of clinical nutrition. 2012;96(4):789-800.
151. Ferreira F, Graça M. Tabela de composição de alimentos portugueses. 2 ed. Lisboa: Instituto Nacional de Saúde Dr. Ricardo Jorge; 1985.
152. Amaral C, Sequeira C. Iogurte – composição e valor nutritivo de variedades comercializadas em Portugal. Subsídio para a tabela de composição dos alimentos portugueses. Rev Port Nutr. 1993(3):35–52.
153. Batista I, Bandarra N. Influência de quatro métodos culinários na composição química de várias espécies de peixe. Rev Port Nutr. 1993(3):5-14.
154. Mano M, Meister M, Fontes M, Lobo P. Composição de alguns alimentos cozinhados. Alguns produtos servidos em 'snack-bares'. Rev Port Nutr. 1989(4):19–24.
155. Mano M, Meister M, Fontes M, Lobo P. Composição de sobremesas doces. Rev Port Nutr. 1989(1):16-24.
156. Aro A, Amaral E, Kesteloot H, Rimestad A, Thamm M, van Poppel G. Trans fatty acids in French fries, soups, and snacks from 14 European countries: the TRANSFAIR study. J Food Compost Anal. 1998(11):170–7.
157. Aro A, Antoine J, Pizzoferrato L, Reykdal O, Van Poppel G. Trans fatty acids in dairy and meat products from 14 European countries: the TRANSFAIR study. J Food Compos Anal 1998(11):150–60.
158. Aro A, Van Amelsvoort J, Becker W, Van Erp-baart M, Kafatos A, Leth T, et al. Trans fatty acids in dietary fats and oils from 14 European countries: the TRANSFAIR study. J Food Compost Anal. 1998(11):137–49.
159. Van Erp-baart M, Couet C, Cuadrado C, Kafatos A, Stanley J, van Poppel G. Trans fatty acids in bakery products from 14 European countries: the TRANSFAIR study. J Food Compost Anal. 1998(11):161–9.
160. Abbott. ARCHITECT System Folate. PT Folate REF 1P74: Abbott.; 2010.

APPENDIX

Appendix table of contents

APPENDIX 1. DECLARATION OF THE OBSERVATIONAL INTERNSHIP IN HOSPITAL S. JOÃO – PORTO

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Departamento de Epidemiologia Clínica,
Medicina Preditiva e Saúde Pública

DECLARAÇÃO

Data: Porto, 19 de dezembro de 2012

Assunto: Estágio observacional

Para os devidos efeitos, declara-se que Sandra Cristina da Silva Gomes assistiu a consultas médicas de saúde materna do Serviço de Obstetrícia do Hospital de São João, com a obstetra Dra. Teresa Rodrigues. Teve ainda a oportunidade de assistir a algumas consultas de enfermagem de saúde materna. Teve como objetivo o enriquecimento académico, no âmbito do mestrado em Epidemiologia na Faculdade de Medicina da Universidade do Porto.

O período decorrido totalizou 25 horas, entre 16 de maio e 24 de Setembro de 2012, com uma periodicidade média de uma manhã por mês.

A Obstetra e Professora

Teresa Rodrigues

Alameda Prof. Hernâni Monteiro, 4200-319 Porto Tel. +351 22 5513652 Fax. +351 22 5513653 http://epidemiologia.med.up.pt

APPENDIX 2. DECLARATION OF THE OBSERVATIONAL INTERNSHIP IN USF +CARANDÁ – BRAGA

USF +Carandá
Unidade de Saúde Familiar

Certificado

Assunto: Assistir a consultas médicas e de enfermagem de saúde materna e infantil

Para os devidos efeitos, certifica-se que Sandra Cristina da Silva Gomes assistiu a consultas médicas e de enfermagem de saúde materna e infantil na USF + Carandá, com o objetivo de enriquecimento académico no âmbito do mestrado em Epidemiologia na Faculdade de Medicina da Universidade do Porto. Teve ainda oportunidade de assistir a sessões do curso de preparação para o parto.
O período totalizou 95 horas, entre 9 de maio e 19 de dezembro de 2012, com uma periodicidade média de uma manhã ou de uma tarde por semana.

Braga, 19 de dezembro de 2012

A Coordenadora

Maria Antónia Miguel

APPENDIX 3. WEB ADDRESSES FROM OFFICIAL HEALTH ORGANIZATIONS WEBSITES (CHAPTER 1)

Table a1. Web addresses and links from official health organizations websites of 46 countries worldwide and from World Health Organization.

Country/ Entity	Website	Web address
Australia	National Health and Medical Research Council	http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n55_australian_dietary_guidelines_0.pdf [a] http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n35.pdf [a]
	Food Standards Australia New Zealand	http://www.foodstandards.gov.au/consumer/generalissues/pregnancy/folic/Pages/default.aspx [b]
Austria	Federal Ministry of Health – Public health portal	https://www.gesundheit.gv.at/Portal.Node/ghp/public/content/Ernaehrung_SchwangerschaftLN.html [b] https://www.gesundheit.gv.at/Portal.Node/ghp/public/content/Folsaeure_Folat_KH.html [b]
Belgium – Flanders	Flemish government – Child and Family Support Centre for Policy Research – Welfare, Health and Family	http://www.kindengezin.be/zwangerschap-en-geboorte/zwanger/voeding/foliumzuur/ [b] http://www.kindengezin.be/img/foliumzuursuppletie.pdf [a]
Brazil	Ministry of Health	http://www2.ghc.com.br/GepNet/publicacoes/atencaosaudedagestante.pdf [a] http://189.28.128.100/dab/docs/publicacoes/geral/caderno_atencao_pre_natal_baixo_risco.pdf [a]
Bulgaria	The official Bulgarian patient organization Zachatie/ Conception Bulgarian portal	http://www.zachatie.org/index.php?option=com_content&view=article&id=498:folacid----&catid=164:---sp-429&Itemid=135&highlight=WyJcdTA0NDRcdTA0M2VcdTA0M2JcdTA0MzhcdTA0MzVcdTA0MzJcdTA0MzAiLCJcdTA0M2FcdTA0MzhcdTA0NDFcdTA0MzVcdTA0M2JcdTA0MzhcdTA0M2RcdTA0MzAiLCJcdTA0NDRcdTA0M2VcdTA0M2JcdTA0MzhcdTA0MzVcdTA0MzJcdTA0MzAgXHUwNDNhXHUwNDM4XHUwNDQxXHUwNDM1XHUwNDNiXHUwNDNiwiI0= [a]
Canada	Health Canada	http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/pubs/folate-fra.pdf [a] http://www.hc-sc.gc.ca/fn-an/nutrition/prenatal/fol-qa-qr-fra.php [a] http://www.phac-aspc.gc.ca/fa-af/pdf/brochure_f.pdf [b] http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/nutrition/dri_tables-eng.pdf [a]
China	E-health – Shanghai Jing'an District Health Promotion Board – Ministry of Health	http://www.ejiankang.org/articles/12 [b] http://www.ejiankang.org/articles/5 [b] http://www.ejiankang.org/articles/225 [a]
	Chinese Nutrition Society	http://www.cnsoc.org/cn/nutrition.asp [a]
Croatia	Health Site	http://zdravlje.hzjz.hr/index.php
	Ministry of Health	http://www.zdravlje.hr
Cyprus	Ministry of Health	*http://www.moh.gov.cy/*
Czech Republic	Health Magazine	http://www.magazinzdravi.cz/kyselina-listova-dokaze-snizit-riziko-porodn [b]
Denmark	Danish Health and Medicines Authority	http://www.sst.dk/publ/Publ2010/CFF/English/SundeVaner_en.pdf [b] http://sundhedsstyrelsen.dk/da/sundhed/graviditet-og-spaedboern/anbefalinger-til-gravide/folsyre,-jern,-d-vitamin-og-eventuelt-kalk [b]
	Nordic Nutrition Recommendations	http://www.norden.org/en/publications/publikationer/nord-2013-009 [a]
Estonia	National Institute for Health Development	https://intra.tai.ee/images/prints/documents/130311768374_Eesti_toitumis_ja_toidusoovitused_est.pdf [a] http://www.kliinikum.ee/attachments/article/522/rasedate_ja_imetavate_emade_toitumis_ja_toidusoovitused.pdf [a]
	Health Information Network	http://www.terviseinfo.ee/et/component/search/?searchword=foolhape&x=-1383&y=-52&Itemid=131 [b]
	Treatment Guide	http://www.ravijuhend.ee/ravijuhendikasutajale/patsiendijuhendid/20/Foolhape%20%E2%80%93%20mis%20ja%20milleks?%20Foolhappe%20osat%C3%A4htsus%20terve%20beebi%20s%C3%BCndimisel [b]
Finland	Ministry of Social Affairs and Health	http://www.stm.fi/c/document_library/get_file?folderId=28707&name=DLFE-3555.pdf&title=Lapsi__perhe_ja_ruoka_fi.pdf [a] http://www.slv.se/en-gb/Startpage-NNR/NNR5-News/A-draft-proposal-for-NNR-20121/ [a]
	Nordic Nutrition Recommendations	http://www.norden.org/en/publications/publikationer/nord-2013-009 [a]
France	National Institute for Prevention and Health Education	http://www.inpes.sante.fr/cfesbases/catalogue/pdf/1059.pdf [b] http://www.inpes.sante.fr/70000/dp/04/dp041209.pdf#xml=http://search.atomz.com/search/pdfhelper.tk?sp_o=2,100000,0 [a]
	National Nutrition and Health Program	http://www.mangerbouger.fr/pour-qui-242/future-maman/manger-de-facon-adaptee/avant-la-grossesse/les-folates.html [b]
Germany	Federal Centre for Health Education – Family planning	http://www.familienplanung.de/lexikon/folsaeure-prophylaxe/ [b]
	German Society for Nutrition	http://www.dge.de/pdf/ws/Referenzwerte-2013-Folat.pdf [a]
Greek	Ministry of Health	http://www.moh.gov.gr/
Hong Kong	Department of Health	http://www.fhs.gov.hk/english/health_info/files/an_5.pdf [b]
	The Hong Kong College of Obstetricians and Gynaecologist	http://hkcog.obg.cuhk.edu.hk/docs/college_guidelines/Guidelines_on_Antenatal_Care_(Part_1)_2008.pdf [a]
Hungary	National Centre for Food Safety and Nutrition	http://oeti.hu/download/taplalkozasi_ajanlasok_varandos_es_szoptato_anyaknak.pdf [a] http://www.oeti.hu/download/kartyafolsav.pdf [b]
Iceland	Directorate of Health	http://www.landlaeknir.is/servlet/file/store93/item2792/version7/3252.pdf [b] http://www.landlaeknir.is/servlet/file/store93/item2350/version4/2046.pdf [a]
	Nordic Nutrition Recommendations	http://www.norden.org/en/publications/publikationer/nord-2013-009 [a]
India	Ministry of Health and Family Welfare	http://mohfw.nic.in/
Ireland	Health Service Executive	http://www.hse.ie/eng/services/Publications/services/Children/Unit_7_Food_and_Nutrition.pdf [a]

[a] Information for health professionals. [b] Information for general population. - Blank mean that any website was available/found.

Table a1. Web addresses and links from official health organizations websites of 46 countries worldwide and WHO (continuation).

Country/ Entity	Website	Web address
Italy	Ministry of Health	http://www.salute.gov.it/servizio/galleria.jsp?lang=italiano&id=543&dad=s&men=campagne07&label=genit [b] http://www.salute.gov.it/imgs/C_17_opuscoliPoster_126_allegato.pdf [a]
Japan	Ministry of Health, Labour and Welfare	http://www.mhlw.go.jp/shingi/2009/05/dl/s0529-4u.pdf [a]
Latvia	Latvian Health Portal	http://www.medicine.lv/raksti/folijskabe-grutniecibas-laika [b] http://www.medicine.lv/raksti/gudras-uzturvielas-sievietei [b]
Liechtenstein	Land administration	http://www.llv.li/
Lithuania	-	-
Luxemburg	Ministry of Health	http://www.sante.public.lu/publications/rester-bonne-sante/alimentation/alimentation-saine-grossesse/alimentation-saine-grossesse-2012-pt.pdf [b]
Malta	Ministry for Health	https://ehealth.gov.mt/HealthPortal/chief_medical_officer/healthinfor_research/registries/birth_defects.aspx [a]
Netherlands	National Institute for Public Health and Environment – Ministry of Health, Welfare and Sport	http://toolkits.loketgezondleven.nl/toolkits/?page_id=1754#link_5487 [b]
	Nutrition Centre	http://www.voedingscentrum.nl/nl/mijn-kind-en-ik/zwanger/0-3-maanden-zwanger.aspx [b]
Norway	National Institute of Public Health	http://www.fhi.no/eway/default.aspx?pid=239&trg=Content_6496&Main_6157=6263:0:25,6269&MainContent_6263=6496:0:25,6277&Content_6496=6178:68328::0:6276:41:::0:0&5637=6276:2 [a] http://www.fhi.no/eway/default.aspx?pid=239&trg=List_6212&Main_6157=6263:0:25,6665&MainContent_6263=6464:0:25,6667&List_6212=6218:0:25,6685:1:0:0:::0:0 [a]
	Nordic Nutrition Recommendations	http://www.norden.org/en/publications/publikationer/nord-2013-009 [a]
Poland	Ministry of Health	http://www.mz.gov.pl/wwwfiles/ma_struktura/docs/polzdrow_broszciaza_20120523zal12.pdf [b]
Portugal	Ministry of Health	www.dgs.pt [a] (Circular Normativa n.º 02/DSMIA, 16-01-2006) http://www.plataformacontraaobesidade.dgs.pt/ResourcesUser/Institucional/Projectos%20ARS/Madeira/Alimentacao_Mulher.pdf [b]
Romania	Ministry of Health – Romanian Nutrition Society	http://www.ms.ro/documente/Ghid1_8318_6022.pdf [a]
Russia	Ministry of Health of the Russian Federation	https://www.rosminzdrav.ru/docs/mzsr/analytics/2 [a] http://www.takzdorovo.ru/pitanie/zdorovoe-pitanie/folievaya-kislota [b]
	Protection and Human Welfare	75.rospotrebnadzor.ru [a]
Singapore	Health Promotion Board – Singapore Government	http://www.hpb.gov.sg/HOPPortal/health-article/3826 [b] http://www.hpb.gov.sg/HOPPortal/health-article/2740 [b] http://www.hpb.gov.sg/HOPPortal/article?id=2652 [a]
Slovakia	Ministry of Health	*http://www.health.gov.sk*
Slovenia	Institute of Public Health of the Republic of Slovenia	http://www.ivz.si/prehrana?pi=5&_5_Filename=attName.png&_5_MediaId=6551&_5_AutoResize=false&pl=8-5.3 [a]
	Ministry of Health	http://www.mz.gov.si/si/delovna_podrocja/javno_zdravje/sektor_za_krepitev_zdravja_in_zdrav_zivljenjski_slog/prehrana/publikacije_in_druga_gradiva/ [a]
South Africa	The National Department of Health	http://www.doh.gov.za/docs/policy/humangenetics.pdf [a]
	Association for Dietetics in South Africa	http://www.nutritionweek.co.za/pregnancy/31food.html [b]
South Korea	Ministry of Health and Welfare	http://english.mw.go.kr/front_eng/index.jsp
Spain	Ministry of Health, Social Services and Equality – National Health System	http://www.msssi.gob.es/organizacion/sns/planCalidadSNS/ENSSRIntro.htm [a]
Sweden	National Food Agency	http://www.slv.se/sv/grupp1/Mat-och-naring/Kostrad/Rad-om-folsyra/ [b] http://www.slv.se/en-gb/Group1/Food-and-Nutrition/Recommendations/Advice-about-food-for-you-who-are-pregnant/ [b] http://www.slv.se/en-gb/Startpage-NNR/NNR5-News/A-draft-proposal-for-NNR-20121/ [a]
	Nordic Nutrition Recommendations	http://www.norden.org/en/publications/publikationer/nord-2013-009 [a]
	European Food Safety Authority	*http://www.efsa.europa.eu/en/home/publication/folicacid2009.htm?WT.mc_id=EFSAHL01&emt=1* [a]
Switzerland	Federal Office of Public Health	http://www.bag.admin.ch/themen/ernaehrung_bewegung/05207/05212/index.html?lang=fr [b]
	Swiss Society of Nutrition	http://www.sge-ssn.ch/fr/toi-et-moi/les-denrees-alimentaires/valeurs-de-reference/#fl [a] http://www.sge-ssn.ch/media/medialibrary/2012/06/feuille_d_info_alimentation_de_la_femme_enceinte_2011.pdf [a]
	German Society for Nutrition	http://www.dge.de/modules.php?name=Content&pa=showpage&pid=3&page=1 [a]
Taiwan	Health Promotion Administration – Ministry of Health and Welfare	http://www.hpa.gov.tw/BHPNet/Web/HealthTopic/Topic.aspx?id=200712250002 [b]
	Food and Drug Administration – Ministry of Health and Welfare	https://consumer.fda.gov.tw/Pages/List.aspx?nodeID=636 [a] https://consumer.fda.gov.tw/Files/eweekly/%E7%AC%AC213%E6%9C%9F%E6%A0%B8%E5%AE%9A%E7%A8%BF.doc [b]
Turkey	Ministry of Health	http://www.saglik.gov.tr/
United Kingdom	National Health Service	http://www.nhs.uk/conditions/pregnancy-and-baby/pages/vitamins-minerals-supplements-pregnant.aspx#close [b]
United States of America	Centers for Disease Control and Prevention	http://www.cdc.gov/ncbddd/folicacid/documents/ganda_english.pdf [b] http://www.cdc.gov/ncbddd/folicacid/recommendations.html [a]
	Institute of Medicine of the National Academies	http://www.iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/2_%20RDA%20and%20AI%20Values_Vitamin%20and%20Elements.pdf [b]
	Women's health – Department of Health and Human Service	http://www.womenshealth.gov/publications/our-publications/fact-sheet/folic-acid.html [b]
World Health Organization	World Health Organization	http://apps.who.int/iris/bitstream/10665/77770/1/9789241501996_eng.pdf [a] http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/neural_tube_defects.pdf [a]

[a] Information for health professionals. [b] Information for general population. - Blank mean that any website was available/found.