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ORIGINAL CONTRIBUTION

Plasma folate concentrations after a single dose ingestion of whole and skimmed folic acid fortified milks in healthy subjects

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Abstract
Background Since mandatory folic acid fortification of grains and cereals was introduced in order to prevent neural tube defects, the number of products that are being fortified with folic acid is growing, especially milk and dairy products. However, the effectiveness of this action remains controversial.
Aim of the study To investigate the efficiency of skimmed milk as a vehicle for folic acid fortification by the determination of the acute absorption from low-fat fortified milk compared to fortified and unfortified whole milk in healthy subjects.
Design A single-dose bioavailability study was performed using three commercially available milks (whole and skimmed milk fortified with folic acid and unfortified whole milk). Healthy volunteers (3 women, 2 men) were administered a single dose of 430 ml of each milk, at 1-week intervals between test days. Plasma total folate concentrations, at baseline and hourly from 1.5 up to 6.5 h after ingestion, were measured.
Results Plasma folate concentration was significantly increased, when compared to baseline values, 1.5 h after ingestion of skimmed fortified milk, and 2.5 h after whole fortified milk, and remained significantly higher than baseline values for up to 6.5 h after both treatments. The highest plasma folate concentration (20.9 ± 3.1 nmol/l) was obtained 6.5 h postprandial in response to skimmed fortified milk. The acute absorption of folic acid, calculated on the basis of area under the plasma folate concentration curve, was significantly higher from skimmed fortified milk compared to fortified and unfortified whole milk.
Conclusions The absorption of folic acid from fortified skimmed milk is faster than the absorption of folic acid from fortified whole milk, and it renders significantly higher plasma folate concentration when compared to whole milk. These findings indicate that skimmed milk could be considered an efficient food matrix for folic acid fortification.

Keywords Folic acid · Fortification · Skimmed milk · Plasma folate · Bioavailability

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Introduction

It is 12 years since mandatory folic acid fortification was introduced in the United States and Canada in order to prevent neural tube defects (NTD). Doubtless, this fortification programme has achieved a great success in its main goal [1–4].

However, Universal folic acid fortification is also controversial, since additional folic acid may pose unexpected adverse effects in some population groups not initially targeted for fortification (e.g. children and elderly). It has been postulated that, in humans, the presence of unmetabolized folic acid could affect the normal homeostatic regulation of folate, as folic acid is metabolized differently from reduced folates [5]. Moreover, high blood concentrations of folic acid may be related to decreased natural killer cell cytotoxicity [6]. In the elderly, a combination of high fastened plasma folate concentration and low vitamin B_{12} status may be associated with an increased risk of cognitive impairment and anaemia [7]. Folate and extra folic acid may also have a dual effect on cancer, preventing from cancer initiation but enhancing the development and progression of already existing, undiagnosed premalignant and malignant lesions [8–10]. Thus, the classic idea of "folic acid is considered as safe" is nowadays under reconsideration, since high folic acid intake may be harmful for some people.

It has been therefore proposed that nations considering fortification should be cautious and stimulate further research to identify the effects, beneficial *vs.* negative, derived from high folic acid intakes from fortified foods or dietary supplements [5]. This need for research becomes specially relevant since mandatory fortification in the United States of America, Canada and other countries, was implemented and standardised only for flour and cereal grains (140 µg folic acid/100 g cereal product) [11], but the number of products that are being fortified nowadays is growing, especially milk and dairy products. We have recently described more than 250 fortified foods in Spain, including cereals and derivatives, dairy products, spreads, non-alcoholic beverages, dietetic products and infant formula. Most of these foodstuffs lacked a target population for their consumption (37%), and, surprisingly, only 2% targeted women at a childbearing age [12]. There are very few studies (in Spain there are no studies) evaluating whether these new folic acid fortified products can enhance folate status in humans, and to what extent. In fact, and equally important, there is still uncertainty regarding precise data on folate bioavailability from different food matrixes [13]. This need for accurate data on folate bioavailability from food sources is of particular concern in those countries without mandatory policies. Food folate fortification on a voluntary basis could be a helpful tool to achieve an optimal status for women at childbearing age, while safety concerns for the rest of the population are clearly defined.

Research on folate bioavailability has focused on folate-rich vegetables and citric fruits and on deconjugation of polyglutamates as a potential limiting step in digestion and absorption [14]. Dairy products should also be considered as an interesting food category for folate absorption studies as a potential matrix for folate fortification. In this sense, milk is a basic food which has not been traditionally considered as a good source of folates, compared to folate-rich food products. However, it is widely consumed in many Western countries, and as a result of its high consumption, dairy products provide 10–15% of the daily folate intake in such countries, especially among the younger population [15].

Research studies on bioavailability of folic acid from fortified milk showed that semi-skimmed pasteurised and ultra-high-temperature (UHT) milk are suitable carriers for fortification. In a human study with 69 volunteers, an additional 200 µg of folic acid/day over 4 weeks substantially increased folate status and decreased plasma total homocysteine [16]. In vitro studies with a dynamic gastrointestinal model demonstrated that folic acid and 5-methyltetrahydrofolate in fortified milk were easily released from the milk matrix and highly available for absorption (60–70%) [17].

In Spain, skimmed milk consumption represents 26.7% of total milk consumption. Moreover, milk is the most consumed dairy product in Spain (*ca* 214 g/d) [18]: the volume of milk consumed represents approximately 2.5 times the aggregate volume of other dairy products including yoghurt, cheese, butter, creams and related. Skimmed milk is typically fortified with vitamins A and D, and more recently, its current fortification profile includes folic acid. However, the effectiveness of this option has never been determined.

The aim of the present study was to explore the feasibility of skimmed milk, when compared to whole milk, as a vehicle for folic acid fortification, by comparing plasma folate concentrations following a single-dose ingestion of fortified and unfortified milks in healthy subjects.

Methods

Study design, subjects, and types of milk

The study consisted of a single-dose pharmacokinetic assay involving three types of commercially available milk: whole milk (3.6% fat, 5 µg folates/100 ml), whole milk fortified with folic acid (3.6% fat, 30 µg folates/100 ml (0.679 µmol/l)) and skimmed milk fortified with folic acid (0.3% fat, 30 µg folates/100 ml (0.679 µmol/l)). The three milks were of the same commercial brand (with a high market share), and the fortified milks also contained vitamins A, D, E, calcium and phosphorus (Table 1).

Healthy volunteers were enrolled in the present study. Inclusion criteria were: 20–31 years of age, body mass index of 20–25 kg/m^2, normolipidic, and normal hematocrit. Exclusion criteria included consumption of vitamin and mineral supplements, habitual use of drugs or oral contraceptives, dieting, pregnancy, lactation, high-density exercise, and chronic or metabolic diseases. A total of five volunteers (3 women, 2 men) were enrolled in the study.

The volunteers were asked to consume a folic acid-free diet (with the aid of a list of folic acid supplements and fortified foods that was given to them) over the 24 h previous to each assay, in order to reduce possible interferences from previous meals. After an overnight fast, the volunteers were cannulated and blood samples collected before breakfast (baseline). For the three assays, a breakfast consisting of 430 ml of milk plus 10 unfortified biscuits was provided. The volunteers were asked to consume the breakfast within 10 min, after which blood samples

Table 1 Nutrition content in unfortified whole milk and folic acid fortified whole and skimmed milk

Nutrient (per 100 ml)	Whole milk	Fortified whole milk	Fortified skimmed milk
Calories (Kcal)	64	71	41
Proteins (g)	3.0	3.9	3.9
Carbohydrates (g)	4.8	5.7	5.7
Fat (g)	3.6	3.6	0.3
Calcium (mg)	120	160	160
Total folate (μg)*	5	30	30
Vitamin A (μg)	na	120	120
Vitamin D (μg)	na	0.8	0.8
Vitamin E	na	1.5	1.5
Phosphorus	na	120	120

Values provided by the manufacturer as "nutrition facts label"

na data not available

* Total folate content: native folate for unfortified milk and native folate plus added synthetic folic acid for fortified milk

were taken 90 min later and hourly for 5 h. A total of six post-dose plasma samples were obtained for each volunteer. Volunteers remained fasting until the end of the study. All the subjects were involved in the three assays (whole milk, fortified whole milk, and fortified skimmed milk) and the different assays took place 1 week apart, each time on the same weekday. The order of administration of the different types of milk for each volunteer was randomly assigned.

The volunteers were also asked to complete a Food Frequency Questionnaire (FFQ) [adapted from 19] at screening, in order to assess their previous vitamin intakes. The FFQ referred to previous food intake on a yearly basis, it includes a total of 118 food items and its reproducibility and validity has been tested in Spanish women [19]. The FFQ does not specifically address folic acid fortified products, except for multivitamin-fortified breakfast cereals, because up to now there are no reliable data on folic acid-fortified products available in Spain, where folic acid fortification is only voluntary. Food composition data were obtained from Spanish Food Composition Tables [20]. Folate data in these tables refers to total natural food folates.

The study procedures were performed in accordance with the Ethical Committee for Clinical Investigation of Hospital Universitario Puerta de Hierro, where the studies took place, and according to the protocols previously reported [21]. Subjects were informed about the study and gave their written consent.

Analytical procedures

Plasma obtained within 20 min of blood collection in EDTA 7.5% was stored at −20 °C until analysis. Plasma folate levels were measured by using the Abbot IMx folate assay, an enzyme-immunoassay technique, as previously reported [22, 23]. Samples were analysed in duplicates.

Statistical analysis

Data are expressed as means ± SDs. The AUCs for plasma folate concentration were calculated by using the trapezoidal rule for the time points from 0 to 6.5 h, after correction for baseline concentrations. Differences in postprandial folate levels after the ingestion of the three types of milk were estimated by a one-way ANOVA plus *post hoc* Tukey test. Vitamin intake (folate, vitamin B12 and vitamin B6) was determined from the Food Frequency Questionnaire by using the software *Alimentación y Salud*, 2.0 (Granada, Spain). All statistical analyses were carried out using SPSS (15.0).

Results

Plasma folate concentration at baseline and at different time points during the 6.5-h test period after treatments is shown in Fig. 1. Plasma folate concentration at baseline was not significantly different among the three dietary treatments (12.1 ± 2.5 nmol/l; 10.8 ± 1.1 nmol/l; 11.4 ± 1.6 nmol/l, $p = 0.6$, one–way ANOVA). Compared to baseline, plasma folate concentration was significantly increased (from 11.4 ± 1.6 to 17.6 ± 4.3 nmol/l, $p < 0.05$) 1.5 h after the ingestion of fortified skimmed milk and 2.5 h after the ingestion of fortified whole milk (from 10.8 ± 1.1 to 17.4 ± 3.5 nmol/l, $p < 0.05$). Both fortified milks induced increased plasma folate concentrations, which remained significantly higher from their respective baseline values up to the end of the study (6.5-h post single dose ingestion). When compared to baseline, a significant increase in plasma folate concentration was obtained 1 h earlier after the ingestion of fortified skimmed milk than after the ingestion of the same level of fortification in whole milk (1.5 vs. 2.5 h postprandial). These results are in contrast with unfortified whole milk, which induced a slight increase in plasma folate concentration only at the end of the study period (17.4 ± 1.4 nmol/l at 6.5 h vs. 12.1 ± 2.5 nmol/l at baseline, $p < 0.05$). In all three treatment groups, plasma folate concentrations did not return to baseline values by 6.5 h. The kinetic profile of the plasma folate concentration curves show that the Cmax value is probably not reached within 6.5 h.

The comparison of the response to the ingestion of the different types of milk shows that fortified skimmed milk resulted in significantly higher plasma folate levels from 2.5 h up to the end of the study, when compared to whole

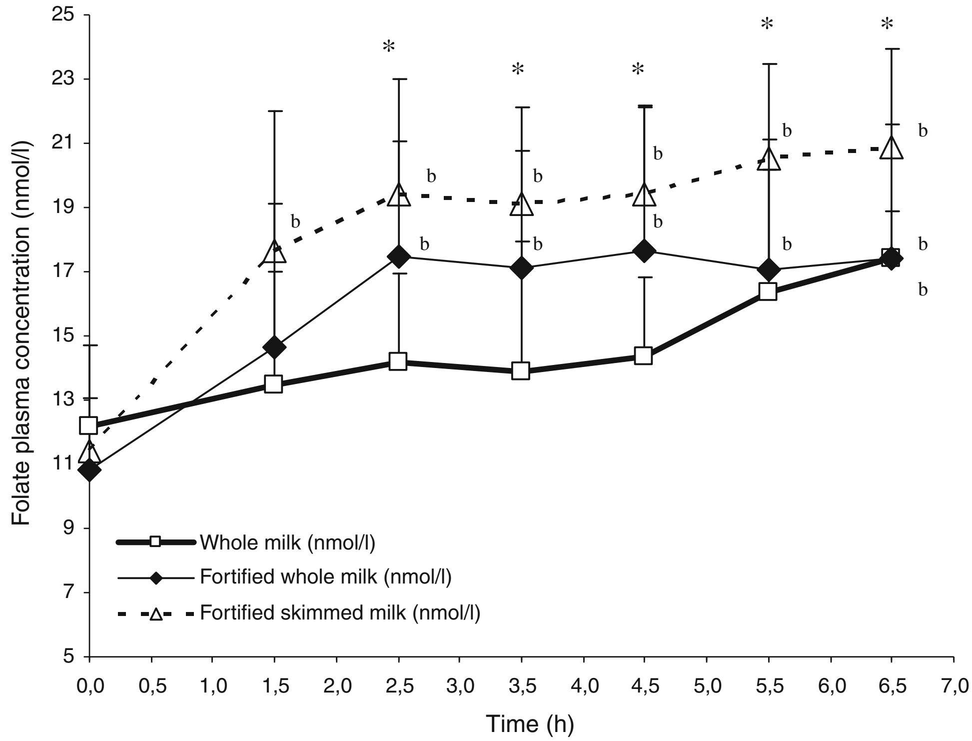

Fig. 1 Mean (±SD) plasma folate concentrations at baseline and up to 6.5 h after the ingestion of 430 ml of whole milk (5 μg folates/100 ml), fortified whole milk (30 μg folates/100 ml) and fortified skimmed milk (30 μg folates/100 ml). Number of volunteers: $n = 5$. Samples were analysed in duplicates. Values are means ± SD. $^{*}P \leq 0.05$ fortified skimmed milk *vs* whole milk, for the same time point. ^{b} $P < 0.05$ *vs.* baseline values within the same treatment

milk (19.3 ± 3.6 vs. 14.1 ± 2.7 nmol/l at 2.5 h for fortified skimmed milk and whole milk, respectively). The highest plasma folate concentration (20.9 ± 3.1 nmol/l) appeared after consumption of fortified skimmed milk, after 6.5 h (Table 2). Fortified skimmed milk produced an increase in plasma folate concentration of 6.2–9.5 nmol/l (1.5–1.8 times baseline concentration) that was statistically evident 1.5 h after ingestion and up to the final sampling point (6.5 h). It is important to note that fortified milks provided 6 times more folic acid than unfortified milk. However, fortified whole milk did not result in significantly higher plasma folate concentration when compared to unfortified whole milk.

The AUC for plasma folate concentrations calculated up to 6.5 h postprandial was found to be significantly higher after the intake of fortified skimmed milk compared to whole milk and fortified whole milk (Table 2). AUC was smallest for unfortified whole milk.

Folate, vitamin B12 and vitamin B6 daily intakes, as assessed by Food Frequency Questionnaire, are shown in Table 3. Volunteers' usual intake for the three vitamins was well above Spanish recommended nutrient intakes. Mean folate intakes were 104% of present recommendations, whereas vitamin B_{12} intake exceeded recommendations more than 10 times (1,209%). Vitamin B_6 reached 174% of the recommended intakes.

Discussion

Folate bioavailability from different foods is considered to be dependent of a number of factors, including the food matrix, the conditions of cooking, the intestinal deconjugation of polyglutamyl folates, the instability of certain labile folates during digestion, and the presence of certain dietary components that may enhance folate stability

Table 2 AUC and highest concentration for plasma folate after the ingestion of 430 ml of whole milk (5 μg folates/100 ml), fortified whole milk (30 μg folates/100 ml) and fortified skimmed milk (30 μg folates/100 ml)

Milk type	AUC (h*nmol/l)	Highest concentration (nmol/l)	Time (h)
Whole milk (3.6% fat)	19.07 ± 13.82	17.4 ± 1.4	6.5
Fortified whole milk (3.6% fat)	29.58 ± 12.51	17.6 ± 3.6	4.5
Fortified skimmed milk (0.3% fat)	51.62 ± 9.21*	20.9 ± 3.1*	6.5

Values are means ± SD ($n = 5$). $^{*}P < 0.05$ *vs.* whole unfortified milk

AUC area under the plasma concentration curve

Table 3 Daily dietary intakes of folate, vitamin B_{12} and vitamin B_6 as assessed by Food Frequency Questionnaire. Adequacy to the Spanish Recommended Nutrient Intakes (RNI)

Vitamin	Intake	% RNI
Folate (μg/day)	416.2 (362.0–532.6)	104 (91–133)
Vitamin B_{12} (μg/day)	24.2 (13.4–38.2)	1,209 (670–1,909)
Vitamin B_6 (mg/day)	2.9 (2.6–3.5)	174 (144–223)

Values are expressed as means (min–max), ($n = 5$)

during digestion [24]. There is general agreement among experts that the bioavailability of natural food folates is incomplete when compared with the synthetic vitamin folic acid, as found in fortified foods and supplements [13]. For the purpose of public health recommendations, and the establishment of Folate Dietary Equivalents, the bioavailability of folic acid from fortified products and supplements has been assumed to be approximately 1.7 and 2 times higher, respectively, than that of natural folate from food products [25, 26]. Nonetheless, there is still uncertainty regarding precise data on folate bioavailability from foods. For example, the trap of natural folate in the food matrix could impair folate bioavailability, because of its influence on folate release and availability for absorption, i.e. bioaccessibility [14]. The consumption of minced or liquefied spinach led to a higher plasma folate response than consumption of whole leaf spinach [27]. Using an in vitro gastrointestinal model, folate was found to be highly bioaccessible from liquid foods and 10% less bioaccessible from vegetables, thus indicating the impact of the food matrix (although limited) on the release of folate from food products during gastrointestinal passage [14].

Moreover, estimates of the degree of lower relative bioavailability of food folates compared with folic acid show great variation among human studies, ranging between 10 and 98%, depending on the methodological approach used [24].

The lack of accurate data on folate bioavailability is of particular concern, especially in those countries where the governments have decided against implementing population-based mandatory policies, because of safety concerns. In these cases, there is great dependence on native food folates and/or voluntarily added folic acid, as means to "optimise folate status".

In this context, milk is a basic food which has not been traditionally considered as one of the main folate-rich foods, but recently its current fortification profile includes folic acid. However, the efficacy of folic acid fortification of skimmed and whole milk in the improvement of folate status has never been determined.

Research studies on bioaccessibility and bioavailability of folic acid from fortified milk have mainly evaluated the effect of folate binding proteins (FBP) [16, 28–30] and the differences between added synthetic folic acid or natural occurring 5-methyltetrahydrofolate [17, 30]. Taken together, these studies show that both pasteurised milk, in which folate occurs bound to folate-binding proteins (FBP), and ultra-high-temperature (UHT) milk, where FBP is partly denatured, are suitable matrices for fortification [16, 17, 28–30]. De Jong et al. [16] clearly showed that milk fortified with folic acid enhances folate serum and red blood cell concentrations and decreases plasma total homocysteine concentration within 4 weeks in healthy subjects. No significant effect of endogenous FBP on bioavailability of folic acid was found in this intervention study [16]. In healthy ileostomy volunteers, relative folate absorption, as determined using post-dose plasma concentration curves, was highest for yeast polyglutamates (from desert creams), followed by 5-methyltetrahydrofolate from fermented milk without FBP, and folic acid from bread [30]. In vitro bioaccessibility studies with a dynamic gastrointestinal model showed that folic acid and 5-methyltetrahydrofolate in fortified milk were easily released from the milk matrix, and highly available for absorption (60–70%) [17]. It has also been reported that there is a small but statistically significant difference in the bioaccessibility of folic acid (60%) and 5-methyltetrahydrofolate (70%) added to milk [17], presumably because folic acid remains bound to FBP after gastric passage [28]. Both in vitro and in vivo studies show that FBP should not be added to milk products, because this can inhibit the bioaccessibility of folic acid [17, 29] and the bioavailability of 5-methyltetrahydrofolate [30].

The aim of the present study was to compare plasma folate response following a single ingestion of folic acid fortified whole or skimmed milk in healthy subjects. Our results reveal that plasma folate concentration was significantly increased for up to 6.5 h (the final sampling point) in response to fortified skimmed milk, and the highest concentration (20.9 ± 3.1 nmol/l, almost 2-fold baseline values) was also induced by fortified skimmed milk, when compared with whole fortified milk and whole unfortified milk. Therefore, our results suggest that absorption of folic acid from fortified skimmed milk is faster, and thus its potential bioavailability could be different. Moreover, when AUC was compared between treatments, a significantly higher response was observed for folic acid-fortified skimmed milk, compared with the other treatments. The AUC for fortified whole milk was 58% of that obtained for fortified skimmed milk, and the AUC for unfortified whole milk was only 37%. Nonetheless, we cannot assure from our results that the bioavailability of folic acid in skimmed milk is higher, since the final sampling point was 6.5 h and it is not clear, from the kinetic profile of the plasma concentration curve, that Cmax is effectively reached. The interpretation of these results should take this fact in consideration.

These findings indicate that skimmed milk could be considered a feasible vehicle for folic acid fortification. Fortification of fat-reduced milks with folic acid has only been evaluated in terms of physicochemical characteristics. Achanta et al. [31] showed that fortification can be accomplished without adversely affecting product characteristics. Addition of folic acid to low-fat milks before or after pasteurisation did not affect flavour, appearance, or texture/mouthfeel scores. However, to our knowledge no other study has examined the bioavailability of folic acid from fortified skimmed milk.

De Jong et al. [16] described that semi-skimmed milk is a suitable matrix for fortification to enhance folate status in humans. In an intervention study, 69 volunteers consumed daily, and for 4 weeks, 500 ml of fortified milk, pasteurised or UHT treated. The adults participating in this study had a relatively low natural dietary folate intake, and the consumption of fortified semi-skimmed milk increased their folate concentration in serum and in red blood cells by 6.6–7.0 and 32–36 nmol/l, respectively.

Our results are also in accordance with that obtained by Pentieva et al. [32] with fortified low-fat spreads. These authors reported that the absorption of folic acid from fortified low-fat spread, although lower than absorption from a tablet, is effective, also suggesting that low-fat spreads, typically associated with fat-soluble vitamin fortification, may be considered reasonable as vehicle for folic acid fortification.

Volunteers were asked to consume a folic acid-free diet 24 h before the beginning of the assay, but not a natural folate free diet. We wanted to test the plasma concentration response to folic acid-fortified products in individuals regularly consuming natural food folates. Nonetheless, volunteers were asked to fast overnight before the baseline blood collection in order to provide a feasible accurate estimate of the “routine” folate status of the individuals [33].

In our current study, the reason for the statistically similar folate plasma concentration at different time points after unfortified whole milk and fortified whole milk ingestion is unclear. However, fortified whole milk ingestion induced a significant plasma folate increase, when compared to baseline, from 2.5 h and up to the end of the study (6.5 h). Conversely, unfortified whole milk did not increase plasma folate concentration, when compared to baseline, until 6.5 h after ingestion. Whole milk, because of the higher energy and lipid contents, could delay gastric emptying and thus slow down folate absorption. This could explain the differences obtained in plasma folate concentration after whole fortified and unfortified milks when compared to skimmed fortified milk. From our data, however, we cannot conclude whether this effect could lower folic acid bioavailability in whole milk. Conversely, whole milk, because of fat, may have stimulated bile acid secretion into the gut, a known reabsorption route for folates [34]. Therefore, the slight increase in plasma folate seen in response to unfortified whole milk ingestion at the end of the sampling period (6.5 h) may be the result of the reabsorption of excreted bile folate. This increase could partially come close to the levels obtained by folic acid fortification, especially at the end of the study, thus not leading to statistical differences between treatments.

In summary, this study shows that the absorption of folic acid from fortified skimmed milk is faster than the absorption of folic acid from fortified whole milk, and furthermore, postprandial folate concentration is higher after the ingestion of skimmed fortified milk when compared to whole fortified milk. This could render a different bioavailability of folic acid in fortified whole or skimmed milk. Further studies are needed in order to confirm the effectiveness of folic acid-fortified milks in terms of improving long-term folate status.

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References

1. Olney RS, Mulinare J (2002) Trends in neural tube defect prevalence, folic acid fortification, and vitamin supplement use. Semin Perinatol 26:277–285
2. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong L-YC (2001) Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA 285:2981–2986
3. Centers for Disease Control, Prevention (2004) Spina bifida and anencephaly before and after folic acid mandate-United States, 1995–1996 and 1999–2000. MMWR Morb Mortal Wkly Rep 53:362–365
4. De Wals P, Tairou F, Van Allen MI et al (2007) Reduction in neural tube defects after folic acid fortification in Canada. N Engl J Med 357:135–142
5. Smith AD, Kim YI, Refsum H (2008) Is folic acid good for everyone? Am J Clin Nutr 87:517–533
6. Troen AM, Mitchell B, Sorensen B et al (2006) Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 136:189–194
7. Smith AD (2007) Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12. Am J Clin Nutr 85:3–5
8. Sohn KJ, Stempack JM, Reid S, Shirwadkar S, Mason JB, Kim YI (2003) The effect of dietary folate on genomic and p53-specific DNA methylation in rat colon. Carcinogenesis 24:81–90
9. Kim YI (2004) Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 80:1123–1128
10. Kim YI (2008) Folic acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev 17:2220–2225
11. Food and Drug Administration (1996) Food standards: amendment of standards of identify for enriched grain products to require addition of folic acid. Final rule. 21 CFR Parts 136, 137 and 139. pp 87818807

12. Samaniego Vaesken L, Alonso-Aperte E, Varela-Moreiras G (2009) Alimentos fortificados con ácido fólico comercializados en España: tipo de productos, cantidad de ácido fólico que proporcionan y población a la que van dirigidos. Nutr Hosp 24(4):445–452
13. McNulty H, Scott JM (2008) Intake and status of folate and related B-vitamins: considerations and challenges in achieving optimal status. Br J Nutr 99(Suppl 3):S48–S54
14. Finglas PM (2004) Folate bioaccessibility (in vitro studies). In: Folate: from food to functionality and optimal health. Final report. pp 115–137. Project's www address: http://www.ifr.ac.uk/folate/Publications.htm Accessed: May 2009
15. Forssén KM, Jägerstad MI, Wigertz K, Witthöft CM (2000) Folates and dairy products: a critical update. J Am Coll Nutr 19:100S–110S
16. De Jong RJ, Verwei M, West CE, van Vliet T, van den Berg H, Castenmiller JJM (2005) Bioavailability of folic acid from fortified pasteurised and UHT-treated milk in humans. Eur J Clin Nutr 59:906–913
17. Verwei M, Arkbåge K, Havenaar R, van den Berg H, Witthöft C, Schaafsma G (2003) Folic acid and 5-methyltetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic in vitro gastrointestinal model. J Nutr 133:2377–2383
18. Ministerio de Medio Ambiente y Medio Rural y Marino (MARM) (2008) Panel de consumo alimentario: Base de datos. http://www.mapa.es/es/alimentacion/pags/consumo/BD/consulta.asp Accessed April 2009
19. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, Willett WC (1993) Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol 22(3):512–519
20. Moreiras O, Carbajal A, Cabrera L, Cuadrado C (2007) Tablas de Composición de Alimentos, 11th edn. Ediciones Pirámide, Madrid
21. Herrero-Barbudo C, Olmedilla-Alonso B, Granado-Lorencio F, Blanco-Navarro I (2006) Bioavailability of vitamins A and E from whole and vitamin-fortified milks in control subjects. Eur J Nutr 45(7):391–398
22. Wilson DH, Herrmann R, Hsu S, Biegalski T, Sohn L, Forsythe C, Novotny M, Beggs M, Manderino G (1995) Ion capture assay for folate with the Abbott IMx analyzer. Clin Chem 41(12 Pt 1):1780–1781
23. Donnelly JG, Isotalo PA (2000) Non-fasting reference intervals for the Abbott IMx homocysteine and AxSYM plasma folate assays: influence of the methylenetetrahydrofolate reductase 677 C–>T mutation on homocysteine. Ann Clin Biochem 37(Pt 3):390–398
24. McNulty H, Pentieva K (2004) Folate bioavailability. Proc Nutr Soc 63:529–536
25. Bailey LB (1998) Dietary reference intakes for folate: the debut of dietary folate equivalents. Nutr Rev 56:294–299
26. Suitor CV, Bailey LB (2000) Dietary folate equivalents: interpretation and application. J Am Diet Assoc 100:88–94
27. Brouwer IA, Dusseldorp MV, West CE, Steegers-Theunissen RPM (2001) Bioavailability and bioefficacy of folate and folic acid in humans. Nutr Res Rev 14:267–293
28. Verwei M, Arkbåge K, Mocking H, Havenaar R, Groten JP (2004) The FBP binding characteristics during gastric passage are different for folic acid and 5-CH3–H4 folate as studied in a dynamic in vitro gastrointestinal model. J Nutr 134:31–37
29. Verwei M, van den Berg H, Havenaar R, Groten JP (2005) Effect of folate-binding protein on intestinal transport of folic acid and 5-methyltetrahydrofolate across Caco-2 cells. Eur J Nut 44:242–249
30. Witthöft CM, Arkbage K, Johansson M, Lundin E, Berglund G, Zhang JX, Lennernas H, Dainty JR (2006) Folate absorption from folate-fortified and processed foods using a human ileostomy model. Br J Nutr 95(1):181–187
31. Achanta K, Boeneke CA, Aryana KJ (2007) Characteristics of reduced fat milks as influenced by the incorporation of folic acid. J Dairy Sci 90:90–98
32. Pentieva K, McKillop D, Duffy N, de Deckere EAM, Jacobs RGJM, van der Put NMJ, McNulty H (2003) Acute absorption of folic acid from a fortified low-fat spread. Eur J Clin Nutr 57:1235–1241
33. Scientific Advisory Committee on Nutrition, Food Standards Agency, UK (2008) Informed systematic review and critical comparison of analytical methods for the quantification of blood folate status in the population, Final report. http://www.food.gov.uk/science/research/researchinfo/nutritionresearch/dietarynutrientsresearch/n08programme/n08projectlist/n08028/ Accessed November 2009
34. Chanarin I (1979) Transport of folate. In: Chanarin I (ed) The megaloblastic anaemias, 2nd edn. Oxford, England, pp 178–471