Nutritional interpretation of folic acid interventions

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Folate is an essential micronutrient, and its nutritional inadequacy is widespread; hence, programs to increase its intake are necessary. However, many concerns about possible adverse effects due to excesses have been raised. Serum folate levels are directly correlated with intake and, when low, are associated with neural tube defects (NTD), high blood homocysteine levels, and megaloblastic anemia. Serum folate cutoff points have been identified for each abnormality, and all can be associated with intakes related to the current recommended dietary parameters. Likewise, high intakes that overwhelm the physiological capacity to process folic acid into biologically active folate derivatives are near the recommended tolerable upper intake level. Although we do not know with certainty the minimum efficacious dose that prevents all folate-dependent NTD, it may actually be much lower than the current recommendation, especially when provided through food fortification; supplemental intakes around 100 µg/day appear to be appropriate.

INTRODUCTION

Folate deficiency is a strong risk factor for embryological neural tube defects (NTD), and the additional intake of this vitamin in the form of folic acid, either through supplementation[1] or through food fortification[2–8] has proven to be an efficacious intervention to reduce the incidence of these abnormalities. However, since the introduction of folic acid interventions, a strong polemic has arisen between those who support small doses of folic acid and including other essential micronutrients[9–19] and those who favor high doses of folic acid.[20–22] Despite advances in folate metabolism knowledge, disagreement has remained.[23–25]

Researchers who back a cautious use of folic acid support their arguments as follows. Folate assists in cell replication that keeps people healthy; but cancer growth, psoriasis,[25] and parasite life cycles also depend on this nutrient.[26] Many anti-cancer and anti-malaria drugs have anti-folate properties, and additional folate intake may jeopardize their efficacy. Furthermore, excessive intake of folic acid has been associated with interruptions in the continuous reduction of colon cancer rates in the United States and Canada.[27] When combined with vitamin B_{12} deficiency, excessive folic acid intake may provoke cognitive impairment in the elderly,[28] and neuropathies due to undiagnosed vitamin B_{12} may be exacerbated, with or without the presence of megaloblastic anemia.[29]

Folate status follows a classical U-shaped pattern on its metabolic functions. Thus, deficiency has negative consequences, but excessive intake might also provoke adverse effects and show similar pathological manifestations. For example, additional folic acid intake protects against some cancers, but excessive intake might exacerbate others.[30] Therefore, the safe and efficacious use of folic acid interventions deserves to be optimized. From an epidemiological perspective, knowing the minimum dose that is able to reduce NTD incidence may help to minimize existing conflicts around folic acid interventions and make them more acceptable and justified for wide population coverage.

This paper reviews key publications on folic acid intervention and attempts to answer the following questions: What minimum efficacious dose reduces the NTD rate? How can the population's need for additional folate intake be determined based on biomarkers? How can the probable impacts (beneficial and negative) of folic acid interventions be predicted?

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MINIMUM USEFUL DOSES OF FOLIC ACID

Hibbard and Smithells[31] were the first to definitively endorse the link between folate deficiency and NTD incidence after observing that the NTD rate was high if dietary folate intake was low. Thus, the first intervention studies were designed to correct a nutritional deficiency.[32–34] However, an inter-country study published in 1991 switched the focus from nutritional improvement to pharmacological prevention.[35] The study aimed to measure whether a very large dose of folic acid (given to women who had been previously pregnant with an NTD-affected fetus) would reduce the recurrence of NTD. This study was not designed to determine a minimum efficacious dose but to ensure impact through supplying sufficiently high amounts of folic acid to reduce NTD recurrence. The NTD rate decreased from 423/10,000 in the control group to 78/10,000 in the treated group (82% reduction). Another group of women receiving a multivitamin supplement without folic acid also experienced a lower NTD rate of 272/10,000; (36% reduction). The latter result suggested that factors beyond folic acid also affect NTD; however, those did not receive equal attention. Evidence has only recently accumulated showing that vitamin B_{12} deficiency is also an important risk factor for NTD.[36,37]

Based on the impact of folic acid supplementation alone in reducing recurrent NTD, women affected with NTD pregnancies were advised to take 4000 μg/day of folic acid prior to any future conception. A smaller but similar Irish research study, using a design comparable to the inter-country study, supplied three doses of 120 μg each per day (360 μg/day), alone or in combination with other micronutrients, and was able to prevent NTD recurrence completely.[13] This study also found that using a multivitamin supplement without folic acid reduced the recurrence rate from 291/10,000 to 112/10,000 (62% reduction). This study has been rarely mentioned in this field. While the statistical power of the study may be criticized, the fact that an 11-times lower dose effectively prevented NTD recurrence deserves attention and consideration.

The importance of the additional folic acid intake in preventing the first occurrence of NTD was confirmed in the now classic paper by Czeizel and Dudas,[38] which reported that the supply of 800 μg/day of folic acid as part of a multinutrient supplement prevented NTD completely in first pregnancies in Hungary. Based on the results reported by the Medical Research Council[35] and by Czeizel and Dudas[38], the Public Health Service of the United States[39] enacted a recommendation in 1992 saying that "all women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other NTDs (neural tube defects)." The Expert Advisory Group of England[40] took a similar position the same year. However, the experimental evidence that 400 μg/day in supplements was efficacious for reducing NTD was only obtained in a study carried out in China from 1993 to 1996, and published in 1999.[1] In 1991, Scott et al.[41] suggested that the proposed study in China should also include an arm of 200 μg/day, because that dose might be equally effective as a larger dose. Nevertheless, the lower dose was not included, so providing 400 μg/day of folic acid through supplementation remained a fixed recommendation that was adopted by other institutions, such as the Institutes of Medicine of the National Academies of Science in the United States.[42] As in the recommendation of 4000 μg/day to prevent recurrent NTD in affected women, the use of 400 μg/day to prevent NTD in a genetically "normal" population is efficacious, but not necessarily the minimum efficacious dose.

The efficacy supplementation trial carried out in China reported that women in the north of the country experienced a NTD reduction from 67/10,000 to 7/10,000, (91% reduction), while the reduction in the southern region was from 10/10,000 to 6/10,000, (40% reduction).[1] These data suggest that the magnitude of the impact is highly dependent on the baseline and is not necessarily continuous and proportional to the amount of additional folic acid intake, because the final NTD rate reaches a plateau beyond which causes other than folate status become more important. The NTD rates in China were lower than in the inter-country study or the Irish study because the latter two only included women who had previously had pregnancies affected by NTD. In China, the rates were for occurrence, while in the other studies, the rates reflected recurrence.

EFFORTS TO ESTABLISH DOSE-RESPONSE RELATIONSHIPS WITH FOLIC ACID

Daly et al.[43] tried to introduce a metabolic-base approach in order to understand the association between folate status and NTD. They studied the Irish population and found a dose-response relationship between serum/plasma folate or RBC-folate and the incidence of NTD. Increasing levels of blood-circulating folate were associated with decreasing incidence of NTD. The phenomenon clearly follows a saturable pattern, as is common in most metabolic reactions. This means that the magnitude of the NTD reduction rate, although not linear, is relatively proportional to the initial level of blood folate. However, this statement holds true only within the range of the experimental data. Beyond the final point at the highest observed levels of folate nothing could be said, because the rate of NTD may have reached a fixed

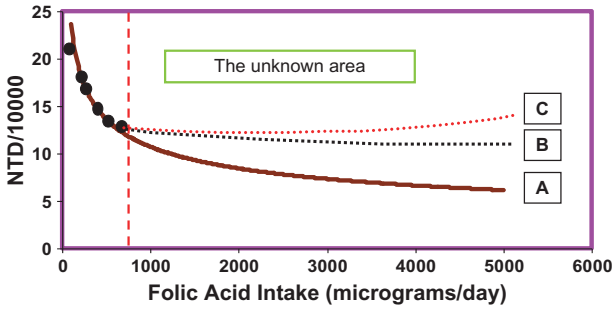

Figure 1 **Reduction of NTD rates at increasing intakes of folic acid.** The letters A to C show three possible trends after the last experimental point.
Based on data from Daly et al.[43,44]

plateau, as the authors of the article stated, or it may even start behaving as a U-shaped phenomenon.

The same research group measured changes in blood folate level in response to folic acid intake and compared the new blood levels with the prior established association with NTD prevalence. They concluded that folic acid intake produced proportional changes in blood folate levels and that additional folic acid intakes of 100–200 µg/day would significantly reduce the NTD rate in the population.[44] In summary, using these two papers, it is possible to predict the NTD rate reduction at increasing additional intakes of folic acid, as shown in Figure 1.

Later, Wald et al.,[45] using the prior-mentioned publications, calculated changes in serum/plasma folate in response to different folic acid intakes and predicted the NTD reduction. They used logarithmic transformations to present the associations as straight lines, but regrettably without limiting their deductions to the range supported by the data. As a consequence, they predicted a continuous and linear (logarithmic) reduction of NTD prevalence linked to increased folic acid intake. This approach has driven the erroneous expectation that NTD reduction has not reached the maximum possible benefit because the additional intake of folic acid has been insufficient. It has also prompted the impression that "more is better".[46] This position is neither technically nor metabolically supported and has unnecessarily fueled the debate among public health professionals regarding the adequate recommendations of folic acid interventions from a public health and population perspective.[47–49]

BIOMARKERS TO ESTIMATE FOLATE STATUS AND INTAKE

The Irish researchers preferred using the levels of folate in red blood cells (RBC) to deduce NTD reduction, because RBC-folate is a biomarker that may reflect probable conditions that exist in other cells in the organism and therefore explain the occurrence of NTD.[43,44] The RBC-folate level associated with the maximum prevention of NTD estimated with the experimental data was 906 nmoles/L (or 400 µg/L).[43] However, the transformation of folic acid into folate cofactors and coenzymes and their storage in the RBC depend on many other nutritional and genetic factors. RBC-folate may be a good indicator of the long-term folate status, but it is influenced by an individual's genetic composition, and the participation of adequate amounts of other vitamins, especially niacin, vitamin B_2, vitamin B_6, and vitamin B_{12}, which influence reactions that include folate.[50,51] Furthermore, the synthesis of RBC depends on good supply of iron, zinc, protein, and other nutrients and is also affected by the presence of parasites, infectious diseases, and other pathologies.

If one cause of NTD is insufficient folate intake, then measuring folate intake at the population level would be sufficient to predict NTD occurrence. To establish folate intake, the serum/plasma folate level is a more robust biomarker than RBC-folate because it is less affected by confounding factors. Serum/plasma folate reflects the recent folate intake and, although it may vary from individual to individual on a daily basis, is satisfactory enough to support population-base interventions that have increasing folate intake as the main purpose. The serum folate level that was associated with the prevention of NTD in Ireland was 15.9 nmol/L (or 7.0 µg/L).[43]

Quinlivan and Gregory[17] recognized the proportional association between the changes in serum /plasma folate and the intake of folate in the diet. They gathered information from several studies that reported these two parameters and calculated the linear equation that described their association. Recently, they adjusted their equation using additional data.[52] The reviewed equation is as follows: change in serum folate (µg/L) = [intake of folic acid as DFE (µg/day) × 0.0145] + 0.132. The formula considers the equivalence of folic acid in terms of dietary folate equivalent (DFE) based on the approximated 70% better bioavailability of synthetic folic acid over dietary sources of folate.[42,53] The additional intake of folate necessary to reach a specific higher serum folate level can also be calculated using another form of the same equation: intake of DFE (µg/day) = [change in serum folate (µg/L) – 0.132]/0.0145]. Here, the calculated intake of DFE should be divided by 1.7 to transform the DFE into the equivalent amounts of folic acid. Furthermore, the last equation also allows estimation of the basal intake of DFE by simply placing the basal serum folate level in the position reserved for the change in serum folate. In this equation, serum folate level is expressed in terms of µg/L. Most papers report folate levels in nmol/L. Thus, to transform nmol/L into µg/L, it is necessary to divide the nmol/L values by the conversion factor of 2.266.

Table 1 **Changes in serum folate level after 3 months of folic acid supplementation.**

Folic acid dose (μg/day)	Serum folate (nmol/L)[†]		Change in serum folate	
	Baseline	3 months	(nmol/L)	(μg/L)[‡]
100	9.7	19.0	9.3	4.1
400	9.6	36.5	26.9	11.9
4000	9.7	68.9	59.2	26.1
4000/week	9.8	23.9	14.1	6.2

[†] Data from Hao et al.[54]
[‡] Folate μg/L = folate nmol/L/2.266.

Although Quinlivan and Gregory's equation[52] is a straight line, the association between intake and serum folate is expected to be a saturation curve. The linearity comes from using several sources of information, and the lack of precision introduced by combining the data with different degrees of variation. In any case, the equation used is simple and practical to use to design and evaluate folic acid interventions as described below.

Recently, Hao et al.[54] reported on the changes in serum folate due to different amounts of folic acid from a large randomized study in China. Although very few points (100, 400, and 4000 μg/day) of additional folic acid intake were measured (Table 1), the association between intake and change in serum folate clearly follows a saturation pattern, as is expected for a metabolic process. The equation calculated for the available data is as follows: change in serum folate (μg/L) = (5.9844 × Ln (intake of folic acid as DFE (μg/day)) – 26.826, which had an r^2 of 0.9994. Interestingly, the slope of a straight line calculated using only the values of 100 and 400 μg/day of folic acid intake was very similar to the equation calculated by Quinlivan and Gregory[52]; the slopes are 0.0153 and 0.0145, respectively. In the linear equation calculated with the China study data, the intercept is high (1.5) because the saturation pattern of the association reduces the slope but increases the intercept when a straight-line equation is used to fit the data. This example illustrates that equations describing the association between folate intake and serum folate level are justifiable to estimate baseline folate intakes, the expected change in serum folate level in response to specific additional intakes of folate, and additional folic acid intake needed to reach certain serum folate levels.

The data presented in Table 1 show that taking 100 μg/day of folic acid was sufficient in the study population in northern China to reach a serum folate level higher than 15.9 nmol/L; hence, the new condition has a high probability to protect against NTD, as deduced by Daly et al.[43] This statement is also valid for the lowest limit of the 95% CI, even as soon as the first month of treatment. The lowest 95% CI of the serum folate level at baseline was 4 μg/L (9.0 nmol/L).[54] If the goal was to raise the serum folate level to 7 μg/L (16 nmol/L), then using the Quinlivan and Greogry equation,[52] the estimated folic acid intake would be as follows: 116 μg/day (i.e., [((7–4 μg/L) – 0.132)/0.0145]/1.7). The estimated additional amount is 86 μg/day using the exponential equation derived from the same paper's data (i.e., [e (((7–4 μg/L) + 26.826)/5.9844)]/1.7). In summary, for the study population, an intake of approximately 100 μg/day of folic acid would produce an important decrease in the NTD rate – a conclusion presented by Daly et al.[44] long ago. A few women may still need additional amounts of folic acid because of specific genetic traits, but they should be treated on a case-by-case basis after investigating the nutritional and metabolic reasons behind the abnormality, and following clinical determination of RBC-folate, serum folate, holotranscobalamin for vitamin B_{12} status, homocysteine, and other biomarkers.

In 2002, Venn et al.[55] studied the relationship between folic acid intake and changes in serum folate. Here, the saturation pattern was clearly shown. The corresponding equation is as follows: change in serum folate (μg/L) = (3.5871 × Ln (intake of folic acid as DFE (μg/day)) – 16.165, which had an r^2 of 0.9953.

Without an experimental equation for a specific target population, the Quinlivan and Gregory equation[52] is simple enough to be used to estimate the baseline dietary folate intake using the serum folate level. If more precise results are desirable, then a short (around 3 months) experiment can be done supplying different folic acid doses (from 50 to 400 μg/day) to randomized experimental groups of the target population. The best equation that fits the data would be calculated and would be used to estimate baseline folate intake and the additional folic acid intake needed to reach specific serum folate levels. In essence, this procedure is similar to the "spiking" method commonly used in analytical chemistry when seeking the content of an analyte in a complex matrix.

In October 2005, a WHO meeting on the topic of folate and vitamin B_{12} reviewed the global prevalence, causes, and consequences of the deficiencies of these two vitamins and potential interventions.[56] As part of that activity, Green[57] summarized the indicators and criteria to identify the presence of a negative balance of the folate

Table 2 **Cutoff points of serum/plasma folate to classify folate deficiency or excess and estimated associated intakes of DFE or folic acid.**

Parameters	Negative balance that may cause megaloblastic anemia[†]	Functional pathologies associated with increase of homocysteine[†]	Occurrence of NTD above the "normal"population plateau[§]	Supraphysiological level[¶]
Serum level (nmol/L)	<7.0	<10.0	<15.9	≥45.3
Serum level (μg/L)	<3.1	<4.4	<7.0	≥20
DFE (μg/day)[††]	<205	<294	<474	≥1370
Folic acid (μg/day)[‡‡]	<120	<173	<279	≥806

[†] Serum levels from Green.[57]
[‡] Serum levels from Selhub et al.[58]
[§] Serum levels from Daly et al.[43]
[¶] Serum levels from Pfeiffer et al.;[59] who suggested this cutoff point because the presence of free folic acid in circulation starts to be important at this level.
[††] Using the linear equation of Quinlivan and Gregory.[52]
[‡‡] It was assumed that micrograms DFE/1.7 = μg folic acid.

status (in terms of serum folate as well as RBC-folate). If a negative balance is kept for weeks and months, it might show clinical/morphological evidence of folate deficiency, such as megaloblastic anemia. In the same meeting, Selhub et al.[58] proposed cutoff points for serum folate and RBC-folate that are associated with homocysteine level increases – a functional indicator of folate, vitamin B_{12}, and other micronutrient deficiencies. High levels of plasma homocysteine are associated with cardiovascular diseases. Tables 2 and 3 summarize the criteria recommended for interpreting folate status based on serum and RBC-folate, respectively. The tables also present the cutoff points that Daly et al.[43] determined as linked to the point where NTD occurrence starts to plateau in the Irish population. Table 2 also includes the serum folate level associated with the detectable circulation of free folic acid.[59] Although a folate level associated with an adverse effect due to excessive intakes has not yet been established, the use of 45.3 nmol/L seems a reasonable option, because free folic acid in circulation means that an organism's capacity to process folic acid into folate derivatives has been overwhelmed, and no reason exists to increase the folic acid intake beyond that point. An important reference is that serum folate levels above 59 nmol/L have been associated with cognitive impairment in the elderly.[60]

Table 2 also includes estimations of the DFE and folic acid intake amounts needed to reach the different cutoff point levels of serum folate. The intake estimates are calculated using the Quinlivan and Gregory equation[52] and assume no dietary folate intake at baseline. Estimating folate intake as folic acid (Table 2) suggests that 279 μg/day would be sufficient to reach the protective serum folate level to prevent NTD, as identified by Daly at al.[43] Indeed, the additional folic acid intake needed is smaller when considering baseline serum folate.

Another interesting deduction in Table 2 is that the DFE amounts needed to prevent functional (high homocysteine level) and morphological (megaloblastic anemia) consequences of folate deficiency are lower than the current estimated average recommendation of 320 μg DFE/day for adults. To prevent NTD, the needed DFE intake is only 18% higher than the current recommended nutrient intake or recommended daily allowance of 400 μg DFE/day for adults (or 235 μg/day in the form of folic acid).

On the other hand, the excessive intake associated with detectable increments of free folic acid in circulation is 806 μg/day, which is lower than the 1000 μg/day actual tolerable upper intake level (UL) of folic acid for adults.[42] In summary, the current dietary parameters of folate seem adequate to explain all the metabolic functions of

Table 3 **Cutoff points of RBC-folate to classify folate deficiency.**

Criteria	Negative balance that may cause megaloblastic anemia[†]	Functional pathologies associated with increase of homocysteine[‡]	Occurrence of NTD above the "normal"population plateau[§]
RBC level (nmol/L)	<305	<340	<906
RBC level (μg/L)	<135	<150	<400

[†] RBC levels from Green.[57]
[‡] RBC levels from Selhub et al.[58]
[§] RBC levels from Daly et al.[43]

Table 4 **Predicted intakes using correlation equations (folate intake versus serum folate levels).**

Folic acid dose (µg/day)	Estimated folic acid intake (µg/day) based on serum folate changes[†]	
	Internal equation[‡]	Quinlivan and Gregory[52] equation[§]
100	103	161
400	380	476
4000	4078	1054
4000/week	147[¶]	247

[†] Using the data presented in Table 1, and assuming that microgram DFE/1.7 = µg folic acid.
[‡] Using the exponential equation derived from the data of Hao et al.;[54] DFE (µg/day) = e [(change in serum folate (µg/L) + 26.826)/5.9844].
[§] Using the linear equation of Quinlivan and Greogry;[52] DFE (ug/day) = [change in serum retinol (µg/L) – 0.132]/0.0145.
[¶] This is equivalent to 1029 µg per week (147 µg/day × 7 days/week).

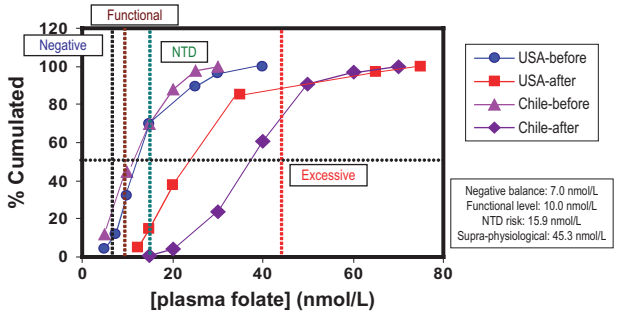

Figure 2 **Cumulative frequency distribution of plasma folate levels before and after mass-fortification with folic acid was introduced in the United States[59] and Chile.[62]** The functional cutoff point refers to increases in the blood homocysteine levels if serum folate levels are below that position.

folate, including NTD prevention. Perhaps some adjustments are needed, but those will be very close to the actual values.

Table 4 presents the estimated folic acid intakes using the exponential equation calculated with the China study data,[54] and Quinlivan and Gregory's linear equation.[52] Both equations calculated the experimental intakes below 400 µg/day reasonably well. The exponential equation, which is more precise than the linear equation for this data set, predicted the effect of the 4000 µg/day intake. Using this equation for the 4000 µg/week dose calculated a biological response equivalent to a daily dose of 147 µg, i.e., 1029 micrograms for the whole week. Therefore, the 4000 µg/week dose was 26% bioavailable. Thus, the transformed amounts from the 4000 µg/week dose of folic acid – around 1000 µg/week – coincides with Kelly et al.'s deduction[61] that the human organism's capacity to metabolize folic acid into folate is overwhelmed after 800 µg/day. As a corollary, it is possible to assert that daily doses higher than 1000 µg of folic acid provide little gain. Nevertheless, although 1000 µg of folic acid may be absorbed daily, it does not mean that this amount is either necessary or useful.

Hao et al.[54] concluded in their paper that a weekly dose of 4000 µg folic acid was relatively ineffective in increasing blood folate concentration. However, Table 4 shows something different. The effect of this weekly dose was equivalent to a daily dose of 147 µg/day. The important point here is that serum folate in the weekly treatment produced a serum folate change higher than that of the estimated protective level of 100 µg/day. Therefore, a large NTD rate reduction may also be expected with this weekly dose.

In summary, the study carried out in China[54] to determine changes of serum and RBC-folate and homocysteine in response to different folic acid dosing regimens, suggests that even for populations with very low folate status, the addition of about 100 µg/day (or 4000 µg/week) of folic acid might have important public health implications in reducing NTD.

ANALYZING TWO FOOD FORTIFICATION PROGRAMS

Fortifying cereal, mainly wheat flour, with folic acid and other micronutrients has been well studied in the United States and Chile. One paper about the US program reported an NTD reduction per pregnancy from 11 to 7/10,000 as plausible consequence of the fortification program,[8] i.e., a 36% reduction. Other articles mention lower percents of decline, but are based on NTD per birth; the impact is smaller due to early termination of some pregnancies once fetal abnormalities were identified.[3] In Chile, where abortion is not allowed and pregnancy termination is rare, the NTD rate changed from 17 to 8/10,000 (53% reduction).[4] This difference in percent of reduction is better explained by the magnitude of the NTD rate at baseline rather than the additional folic acid intake, because in both countries the final NTD rate was very similar, as the China supplementation trials showed.[1]

Figure 2 summarizes the changes in plasma folate from food fortification in the United States and Chile. The US data cover all populations older than 3–4 years of age, as determined in the 1988–1994 and 1999–2000 National Health and Nutrition Examination Surveys.[59] The Chilean data are specific to women of reproductive age.[62] Although the inter-country comparison is not legitimate because the study groups are not equivalent, at baseline, 16% of the US population and 25% of the population in Chile had serum folate levels below the cutoff point, indicating a negative folate balance (7 nmol/L). Approximately 30% and 45%, respectively, were below the cutoff point associated with functional deficiency, as identified

by high homocysteine levels (10 nmol/L). Therefore, it is valid to conclude that prior to these mass-fortification programs, folate intake for the general population of both countries was inadequate. At the cutoff point above the "normal"-population plateau (<15.9 nmol/L) that may be linked to increased possibility of NTD occurrence, nearly 70% of the population in the two countries did not reach that serum folate level. In summary, a folic acid fortification program with wide population coverage was justified and necessary in the two countries.

In the United States, the mean level of serum folate before and after fortification changed from 12.0 to 29.7 nmol/L. This change may have been caused by the additional intake of folic acid of 312 μg/day, as calculated using the Quinlivan and Gregory equation.[52] The population with the lowest serum folate level at baseline (5th percentile) had a serum folate change from 5 nmol/L (estimated from one figure in the US surveys)[59] to 13 nmol/L. This change may be caused by the additional 144 μg/day intake of folic acid. These estimations coincide with those calculated by others using food-intake data.[63,64] These intakes have been efficacious in reducing NTD in the United States.

Based on the current lowest serum folate (5th percentile) of the US population, it is possible to estimate that 48 μg/day of folic acid would still be needed to increase the serum folate level of women with the lowest intake to reach the serum folate level of 16 nmol/L, which Daly et al.[43] identified as the level needed to protect against NTD. In summary, these calculations show that the folic acid-fortified cereal program in the United States is working very well, and that widespread use of 400-μg supplements is no longer needed. Perhaps, to ensure that every woman has sufficient folate intake before conception, the preventive supplementation recommended may be reduced from 400 to 50-μg/day as a non-medically supervised measured to prevent NTD. In any case, the current policy of 400 μg/day of folic acid during the periconceptional period should be reviewed, because fortified foods are now providing large amounts of folic acid to the entire population.

The cumulative distribution profile of the serum folate levels of the US population shows an abnormal bend toward high levels (around 35 nmol/L) for 15% of the population. This serum folate level may reflect a DFE of 1054 μg/day (or 631 μg/day in terms of folic acid), which suggests that the population showing those serum folate levels might be regular consumers of folic acid supplements or processed foods highly fortified with folic acid. This deduction coincides with the results of Yang et al.,[64] who found that in the United States, 19–40% of women, depending on ethnicity, consume 400 μg/day or more of folic acid from supplements, highly enriched foods (e.g., some breakfast cereals), or both, in addition to the folate they derive from the diet and products made with fortified cereal flours. The authors concluded that most women still need to take folic acid supplements to comply with the recommended current additional 400 μg/day of folic acid to prevent NTD. This recommendation, however, deserves to be reviewed and supported with metabolic-based evidence; recommending an absolute additional intake of folic acid no longer makes sense. Any recommendation should be supported by assessing the usual folate status at baseline and the status associated with NTD reduction, as found in the several studies carried out so far.

In Chile, the serum folate level of women of reproductive age changed from 9.7 prior to fortification to 37.2 nmol/L 10 months after fortification was legally mandated.[62] This change may be due to the additional intake of 487 μg/day of folic acid, as calculated with the Quinlivan and Gregory equation.[52] Hertrampf et al.[62] estimated the average intake of 427 μg/day based on the folic acid content of bread and the estimated bread intake. Now, the women of reproductive age in Chile who have the lowest serum folate levels (5th percentile) have a level above the cutoff point of 15.9 nmol/L. These results suggest that in Chile the fortification level may be adjusted downwards to avoid providing too much folic acid (as identified for serum folate levels above 45 nmol/L) to certain segments of the Chilean population; this includes around 30% of women, but probably more children and adult males. This downward adjustment can be made without compromising the program's efficacy, because the additional intake of folic acid will remain higher than that estimated to reach protective serum folate levels. In any case, using folic acid supplements in Chile, and adding this nutrient to foods other than wheat flour is unnecessary, and perhaps even risky, given the current additional intakes provided by fortified wheat flour.

CONCLUSION

Folate is an essential micronutrient required in the synthesis and functional regulation of many macromolecules in living beings, including humans. Nutritional folate inadequacy may be more widespread than originally thought, including in industrialized country populations. Therefore, increasing folate intake seems a proper public health intervention. However, introducing population-wide interventions, especially food fortification, has raised concerns because of possible risks associated with excessive folate intake, such as exacerbating neuropathies from masked vitamin B_{12} deficiency, and antagonism against anti-folate drugs used as anti-cancer, as anti-acne, and as anti-malaria agents, among others. Thus, a minimum efficacious level must be selected that prevents

the consequences of folate deficiency yet minimizes the adverse effects associated with excesses.

A wealth of information in folate biochemistry, molecular biology, human genetics, and population genetics has been accumulated that can be used to guide public health decisions on folate interventions. It seems that serum/plasma folate level is a very useful indicator to diagnose both deficient and excessive status, and to estimate the needed additional folate intake, in the form of folic acid, to reach specific cutoff points associated with reducing abnormalities in populations. Thus, 7 nmol/L is required to prevent negative balance of folate that, if untreated, might cause megaloblastic anemia;[57] 10 nmol/L is required to avert high blood homocysteine levels that indicate favorable cardiovascular disease conditions;[58] and 15.9 nmol/L is required to reduce NTD occurrence above the plateau rate for "normal" populations.[43] Although a criterion for determining an excessive folate status has not yet been specified, a level above 45 nmol/L may be appropriate because free-circulating folic acid is present and indicates that the organism's capacity to transform folic acid into folate derivatives has been overwhelmed.[59] Serum folate can be used to design proper interventions by calculating the additional intake needed to reach the specified cutoff, using experimental equations derived from observed changes of serum level to different amounts of folic acid intake, or simply by applying the equation established by Quinlivan and Gregory.[52]

In one-carbon metabolism, many pathologies (nutritional and genetic) beyond folate intake can cause abnormalities. Therefore, serum folate should be used along with other biomarkers, such as RBC-folate, blood homocysteine, holotranscobalamin, and others, to identify pathological causes and possible treatments for normalizing abnormal pathways.

Adequate folic acid intake can be effectively and safely ensured through mass-fortification programs, such as that applied to cereal flours in Canada,[2,5] Chile,[4] Costa Rica,[7] South Africa,[6] and the United States.[3,8] Efficacious amounts of folic acid can also be supplied by daily folic acid supplementation,[1] and probably by weekly high-dose folic acid supplementation.[54,65,66] For food fortification and daily supplementation, amounts around 100 µg of folic acid seem efficacious; while for weekly supplementation a 4000 µg dose may be useful. However, the population dose should be adjusted based on the impact on serum folate levels. If the interventions are carefully monitored using this biomarker, the folate and folic acid intakes of most individuals will remain within a safe range. Larger doses of folic acid may be reserved for treating special cases, but not for population-based programs.

Once a mass-fortification program is running well, supplementation should be a complementary measure; therefore, the daily recommended supplement level before pregnancy should be adjusted to fill the unsatisfied need – this should be the case in the United States. Likewise, folic acid content in industrially processed foods should be regulated and closely monitored to complement mass-fortification programs when needed. Adding folic acid to commercially available foods and supplements without prescription should be limited if mass programs have wide coverage and large supply such as Chile's fortification of wheat flour.

The recommendation to provide 400 µg/day of folic acid, in addition to dietary folate, to women planning to become pregnant deserves to be reviewed. This recommendation originated from studies that supplied this or larger amounts of folic acid to women to prove the plausible association with NTD reduction; lower doses might be efficacious but were not included in any of these studies. Although we do not know with absolute certainty the minimum level that is effective for reducing NTD occurrence, it is logical to assume it might be much lower with fortified food than the current recommendation, because the additional folic acid intake occurs in a quasi-permanent manner from childhood; additional intakes around 100 µg/day appear to be appropriate.

Folate metabolism has very good biomarkers with a clear dose-response relationship; this unique condition in the micronutrient field should be used to its full potential. Continuing to advocate the adoption of additional folic acid intake without using those biomarkers is unjustified; they can be used to assess the need, to design the interventions, and to monitor and evaluate the program performance and outcomes.

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