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Blood folate level needed for fully effective fortification in the prevention of neural tube defects

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ABSTRACT

Objective Neural tube defects (NTDs) are a preventable folate deficiency disorder; increasing folic acid intake through food fortification increases serum and red blood cell folate and reduces the risk of an NTD pregnancy. There is controversy over the blood folate level needed to achieve the full preventive effect because of discrepant study conclusions.

Methods Results from two published studies were used to determine the relationship between serum folate and NTD risk which was compared with the observed result in a randomised trial of folic acid that increased serum folate from 5 ng/mL to 44 ng/mL among women who took a 4 mg daily periconceptional folic acid supplement.

Results Both studies showed a proportional (logarithmic) relationship between serum folate and NTD risk without evidence of a folate threshold above which there is no further NTD risk reduction. The suggestion of a threshold is due to the incorrect interpretation of the folate-NTD risk association when plotted on arithmetic scales, which conceals the proportional relationship between the two. Also, both studies accurately estimated the observed result from the randomised trial that achieved a median serum folate level of 44 ng/mL and an 83% preventive effect. This is much higher than has been achieved with current levels of folic acid fortification with serum folate between 10 and 16 ng/mL, resulting in an approximate 20% preventive effect.

Conclusion To achieve fully effective fortification, median population serum folate levels need to be about 44 ng/mL, which would globally prevent about 250 000 NTD cases every year.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Neural tube defects (NTDs), among the most common serious birth defects worldwide, are a folate deficiency disorder. Increasing folic acid (vitamin B9) intake increases serum folate and reduces the risk of an NTD pregnancy. There is a difference of opinion on the serum folate level needed for fully effective NTD prevention.

WHAT THIS STUDY ADDS

⇒ There is no threshold above which NTD risk does not decrease, and population folate levels need to be substantially increased to have the expected potential effect on the prevention of NTDs.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ A serum folate level of about 44 ng/mL is a reasonable target to achieve an approximate 83% reduction in the prevalence of NTD pregnancies. This result can be used to help influence folic acid fortification policy.

INTRODUCTION

Neural tube defects (NTDs) are some of the most serious and common birth defects throughout the world. It is recognised that NTDs are a folate deficiency disorder. Increasing folic acid intake through food fortification or the use of supplements increases serum and red blood cell (RBC) folate and reduces the risk of a woman having an NTD pregnancy. There is, however, a lack of clarity over the blood folate level needed to achieve the full preventive effect. Two estimates have been published, one by Wald *et al*[1]5, in 2001, based mainly on data from Daly *et al*[2], published in 1995 and the other by Crider *et al*[3] in 2014 that appear discrepant; Daly *et al*[2] and Crider *et al*[3] suggested a blood folate threshold above which there is no further NTD risk reduction, and Wald *et al*[1 4] suggested there is no threshold.

In Daly *et al*,[2] results were from a large observational cohort study in Ireland of NTDs from 56 049 women attending their first antenatal clinic visit in one of the three main Dublin maternity hospitals over a 4-year period. Blood was stored from the women, and subsequently, 84 NTD cases were identified together with 266 unaffected controls. RBC folate and plasma folate measurements were performed. The study found a continuous relationship between increasing blood folate levels and decreasing NTD risk, which was graphically displayed as a curved relationship that levelled off at a threshold of about 1300 nmol/L (574 ng/mL, 1 ng/mL=2.266 nmol/L). The authors concluded that increasing folic acid intake by 0.4 mg/day could achieve a near-optimal 48% reduction in NTDs. In 1998, it was shown that the relationship described by Daly *et al*[2] between RBC folate and NTD risk was linear when RBC folate and NTD risk were both expressed in logarithms,[4] and the same is true for serum folate. Moreover, it was also shown that a doubling of serum folate approximately halves the risk of having an NTD pregnancy.[1] For example, increasing serum folate levels from a typical background level of 5 ng/mL to 10 ng/mL would be expected to halve NTD risk. The analysis showed that the effect of increasing serum folate on predicted NTD risk depended on baseline folate

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levels and the slope of the log-log serum folate-NTD risk relationship with no serum folate threshold above which there is no further risk reduction.

In 2014, Crider and colleagues[3] used data from two studies carried out in China. One of the studies described as a Folic Acid Dosing trial estimated the relationship between folic acid supplement dose and RBC folate concentration using a single dose of supplements (0.4 mg/day) and assessing dose by duration of supplement use at 0, 1, 3 and 6 months among 317 women. The other study, described as a Community Intervention Project, was performed on about 230 000 women. The women were advised to take 0.4 mg of folic acid supplements per day, and the NTD prevalence was recorded. About 60% did not take any supplements during the study period. Crider and colleagues used the first study to estimate RBC folate in the second study based on consumption of folic acid supplement use in the second study and then combined information from both studies to estimate the relationship between RBC folate and NTD risk. The authors concluded that there was a threshold at an RBC folate level of about 1000 nmol/L (441 ng/mL).

We here explore the basis for the long-standing difference in opinion on the issue of whether or not there is a threshold and the implications for folic acid fortification policy.

METHODS

The relationship between log serum folate and log NTD risk was plotted using two published sets of results to see how well each predicted the observed result among women who took 4 mg of folic acid daily before pregnancy in a randomised trial published in 1991.[5] This dose resulted in an increase in the median serum folate from 5 ng/mL at baseline to 44 ng/mL and an NTD risk reduction of 83% (on treatment analysis).

One set of results was from Daly *et al*,[2] relating NTD risk to serum folate allowing for regression dilution bias as described in Wald *et al*.[1] The other set of results was from Crider *et al*,[3] relating NTD risk to RBC folate levels that were converted into serum folate using empirical results in Supplemental Table 1 in Chen *et al*.[6] The detailed methods and calculations using these results are given in the statistical appendix.

RESULTS

Figure 1 shows the relationship between serum folate and NTD risk, both plotted on log scales based on the results of Daly *et al*[2] and Wald *et al*.[1] (labelled Daly/Wald). The solid 'dose-response' line represents the range over which there were observed data from Daly *et al*. The extrapolation of this line (dashed) shows how well the dose-response relationship predicts the observed results in the report of the Medical Research Council (MRC) funded vitamin study trial[5] well beyond the range of serum folate levels observed by Daly *et al*.[2] The estimated risk reduction for an increase in serum folate from 5 to 44 ng/mL was 83% (see Section 1 of the statistical appendix). The 83% estimate is identical to the observed risk reduction in the MRC vitamin study,[5] an exacting test of the validity of the dose-response relation.

Figure 2, derived from Chen *et al*[6] is a plot of log RBC folate against log serum folate. There is a log-log linear relationship that was used to convert Crider *et al*'s[3] RBC results into equivalent serum folate results and used in figure 3. The relationship between serum folate derived from RBC folate and NTD risk, both plotted on log scales is shown in figure 3 (labelled Crider/Chen). Since the relationship is modelled, the regression is shown as a dashed line. The estimated risk reduction is 82% for an increase from 5 to 44 ng/mL in serum folate (8.8-fold increase; see Section 2 of the statistical appendix), again a nearly perfect estimate of the observed 83% risk reduction in the MRC vitamin study.[5]

Figure 4a,and figure 4b display figures 1 and 3 respectively on arithmetic scales. They show how failure to use proportional (ie, log) scales creates the false impression that the effect of blood folate on NTD risk tapers off to nil, implying a threshold.

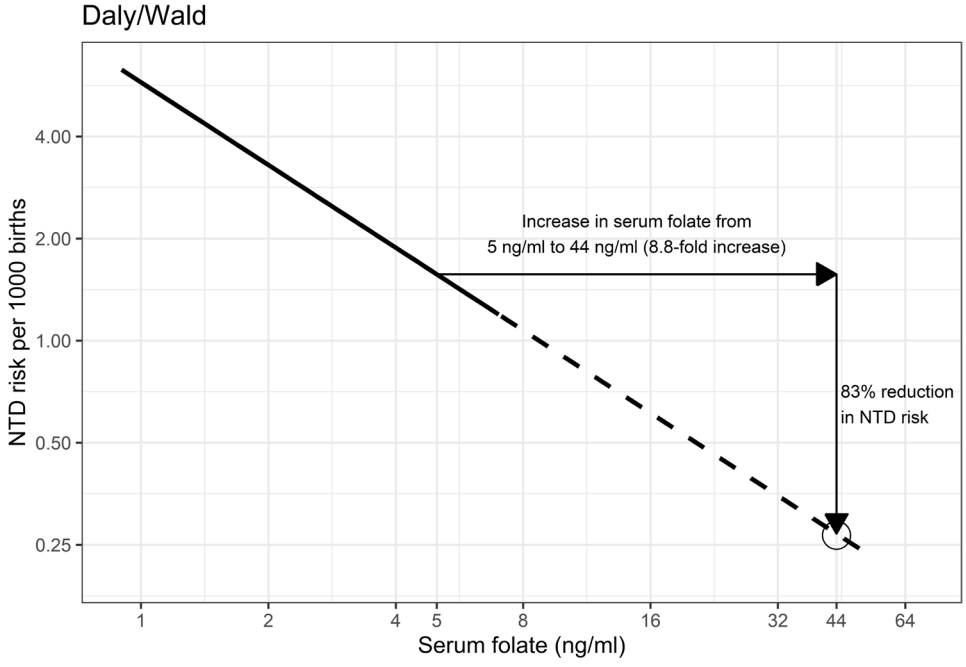

Figure 1 Relationship between serum folate and NTD risk based on data from Daly *et al*[2] allowing for regression dilution bias. The circle indicates the reduction in NTD risk observed in Wald *et al*.[5] The full line corresponds to the range of results given in Daly *et al*[2] and the dashed line extrapolates the results to higher serum folate levels (1 ng/mL=2.266 nmol/L). NTD, neural tube defect.

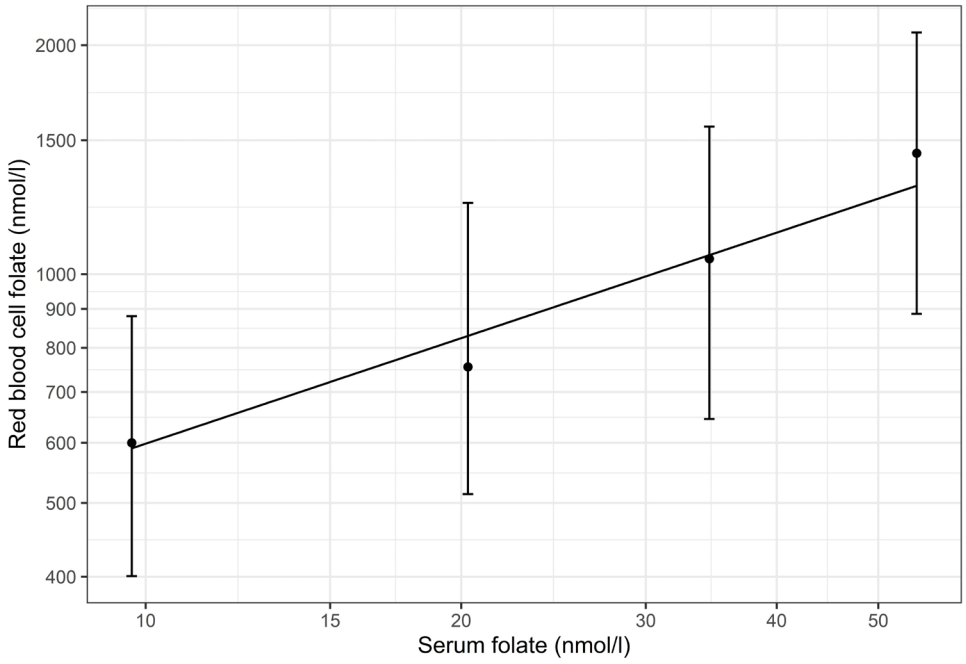

Figure 2 Log-log linear regression between serum folate levels and RBC folate levels derived from data in Supplemental Table 1 in Chen *et al*.[6] The RBC folate level was regressed against serum folate level for the baseline and folic acid doses of 100, 400 and 4000 µg/day given in Supplemental Table 1 of Chen *et al*.[6] The error bars at each data point indicate plus or minus one standard error (1 ng/mL=2.266 nmol/L). RBC, red blood cell.

DISCUSSION

Three important conclusions can be drawn from our results. First, there is no discrepancy between the predicted risk reductions based on the two data sets, Daly/Wald and Crider/Chen; the two dose-response slopes are virtually identical. Second, neither dose-response relationship shows a threshold in which an increase in blood folate level ceases to reduce NTD risk. Third, the false perception that there is a threshold stems from displaying the results of the relationships on arithmetic scales even though the statistical analyses were based on logarithms. This false perception has had significant public health implications.

In general, extrapolating dose-response plots beyond the observed data can be inaccurate and require cautious interpretation and independent validation. Our paper provides this validation. The accuracy of the log-log dose-response relationship yields estimates of 82% and 83% NTD risk reduction, respectively, for the Crider/Chen and Daly/Wald results for an increase

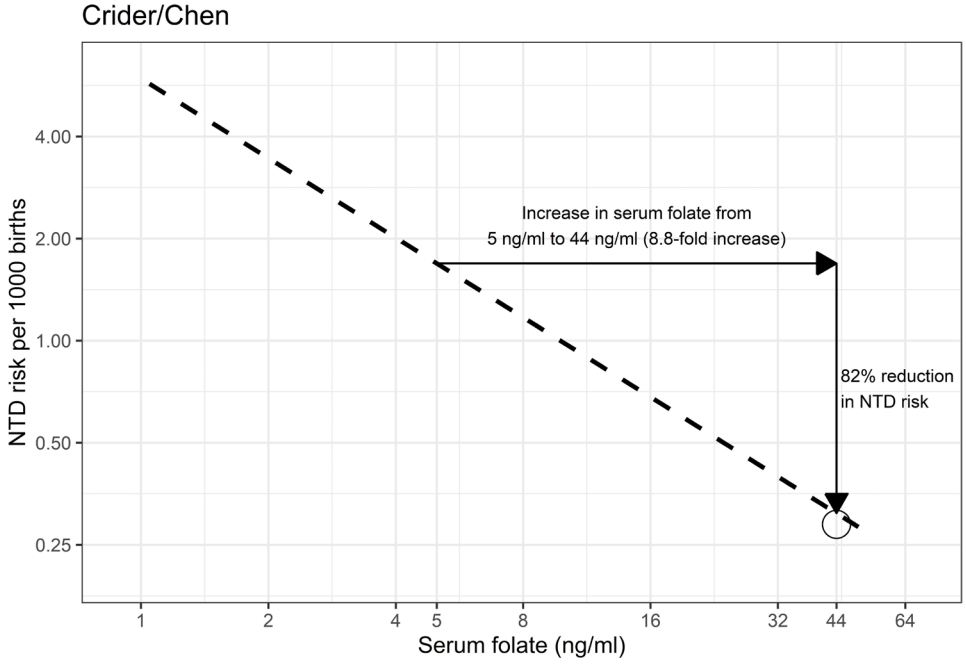

Figure 3 Relationship between serum folate (converted from RBC folate) and NTD risk (Crider *et al*).[3] The circle indicates the reduction in NTD risk observed in Wald *et al*.[5] The dashed line indicates that the regression was not based on direct measurements (1 ng/mL=2.266 nmol/L). NTD, neural tube defects; RBC, red blood cell.

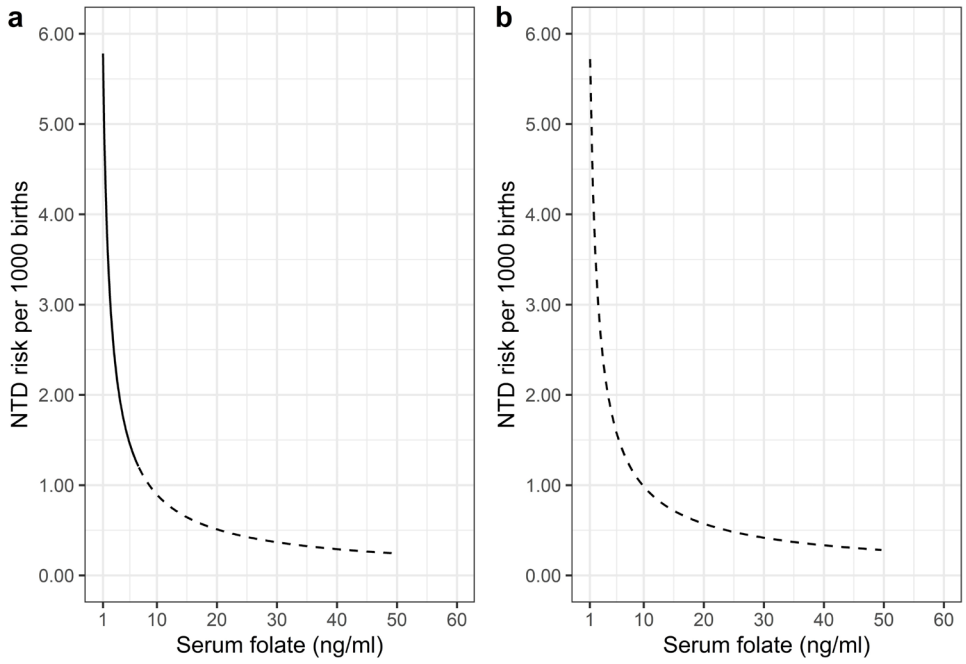

Figure 4 Relationship between serum folate and NTD risk plotted on arithmetic scales for Daly *et al*[2] (a) and for Crider *et al*[3] (b).

in serum folate from 5 ng/mL to 44 ng/mL, compared to the empirical result of an 83% reduction—a remarkable result given the extrapolation of the dose-response relationships well beyond the observed data.

The proportional relationship between serum folate and NTD risk may give the impression of a threshold when plotted on arithmetic scales (figure 4) because the same proportional reduction will result in smaller absolute reductions as the NTD prevalence decreases. As the log-log plots show, there is no indication of a threshold above which an increase in blood folate level ceases to reduce NTD risk. It also means that there is no indication of two categories of NTD pregnancies, one folic acid responsive and another that is not, but it does not exclude some rare conditions such as Meckel-Gruber syndrome that may not be responsive to folic acid.

The perception of a threshold relating to serum folate and NTD risk is one example of a much wider problem in the interpretation of the results of epidemiological cohort studies when the risk of a disorder is plotted on an arithmetic scale; it must 'flatten' the dose-response relationship as the risk approaches zero. This conceals the true dose-response relationship between risk factors and the disorders they cause and falsely limits the potential preventive effect. Several examples of invoking false thresholds have been described, including serum cholesterol and the risk of heart attack.[7]

Countries that have mandated folic acid fortification have done so insufficiently, resulting in mean serum folate levels that are too small to achieve fully effective fortification. Current levels of fortification have resulted in serum folate levels of about 10–16 ng/mL[8 9] and an approximate 20% reduction in NTD risk.[10] Until fully effective fortification is implemented, all women who may become pregnant should be advised to take a daily folic acid supplement of 4 mg (or 5 mg if 4 mg is not available) instead of 0.4 mg, but folic acid fortification designed to achieve a median serum folate level of about 44 ng/mL is the policy of choice. It obviates the need to take a daily folic acid supplement, which is, anyway, of limited effectiveness because most women do not take supplements when it is needed, immediately before pregnancy.[11] A result that is not surprising given that many pregnancies are unintentional, accidental or unplanned. Moreover, the conscientious preconceptual folic acid supplement takers tend to be wealthier and more highly educated, which unfortunately exacerbates health inequalities.

The conversion of folic acid intake to serum folate from fortification is not the same mg for mg as it is from taking a daily supplement; 1 mg/100 g flour or rice is approximately equivalent to a 4 mg/day supplement.[12] Accordingly, it would be reasonable policy to adopt a 1 mg/100 g fortification level and then monitor serum folate levels.

As well as estimating the serum folate level needed for fully effective fortification, attention needs to be paid to the issue of safety. Folic acid is a water-soluble vitamin (B9) that is readily excreted in urine. There is no evidence or indication that serum folate levels of 44 ng/mL pose any risk to health whereas not achieving fully effective fortification will knowingly cause harm.[12] A barrier to folic acid fortification at a level of 1 mg/100 g of flour and rice has been the US Institute of Medicine, in 1998, setting an upper limit of 1 mg/day of folic acid.[13] Unfortunately, this limit was based on a flawed statistical analysis as has previously been described.[12 14] Concerns that high doses of folic acid may be neurotoxic are without foundation and can be completely dismissed. The concern that such doses may fail to diagnose neuropathic B12 deficiency because it can resolve the associated anaemia of neuropathic B12 deficiency but not resolve the neuropathy is also unfounded because it is without any evidence. In the current practice of medicine, the diagnosis and treatment of B12 neuropathy do not rely on the presence of macrocytic anaemia that may be incidentally identified from a routine blood count. On scientific grounds, therefore, the 'tolerable upper intake level' of folic acid can be abandoned. This would remove a significant barrier to achieving fully effective fortification and the prevention of NTDs that would otherwise occur. There is also no expected

harm from taking a folic acid supplement after fully effective fortification becomes a reality, though it would not be necessary.

As well as considering safety, it is appropriate to recognise that many NTD-affected fetuses result in miscarriages, stillbirths, neonatal death and therapeutic terminations, which causes substantial distress to the mother and her family. NTDs have many serious ill effects that are not fully recognised by counting notified NTD pregnancies.

The term 'fully effective fortification', strictly interpreted, implies a serum folate threshold. The term can also be used to set a target serum folate level that was directly observed in a randomised clinical trial that resulted in a large preventive effect, above which there is minimal extra prevention. We used the term in this sense. This alone supports the conclusion that 44 ng/mL should be considered a target level for serum folate.

Increasing the level of fortification is essential, but so is increasing the scope of foods or nutrients that are fortified so that all pregnant women can benefit. For example, limiting fortification to non-wholemeal wheat flour will not help women who eat wholemeal flour products or cannot eat wheat because of an allergy to gluten or eat rice as their staple. It would be highly desirable if all flour and rice were fortified unless packaged with a health alert on the label.

The World Health Organisation (WHO) has estimated that globally every year, there are more than 300 000 NTD pregnancies.[15] Globally, the fortification policy targeted at a median serum folate level of 44 ng/mL could prevent about a quarter of a million cases every year (83% of 300 000). It would achieve a major public health benefit for otherwise affected individuals, their families and society.

Public health authorities should consider implementing fully effective fortification with serum folate monitoring among samples of the population to ensure the fortification policy is achieving its full potential in preventing one of the most serious and common birth defects throughout the world.

STATISTICAL APPENDIX

All logarithms are to the base e

1. Daly/Wald

i. The results of Daly *et al*[2] and Wald *et al*[1] show that there is a log-log relationship between NTD risk and serum folate level with slope −0.81 when corrected for regression dilution bias. The relationship is:

$$\log\left(NTD\ risk\ per\ 1000\ births\right) = \log(5.75) - 0.81 \times \log(serum\ folate\ level\ in\ ng/ml) \quad (1)$$

The value of 5.75 NTDs per 1000 births was taken from Daly *et al*[2] from their observation that the median serum folate level corresponded to 1.9 NTDs per 1000 births.

ii. For a log-log relationship between NTD risk and serum folate level:

$$\log(NTD\ risk) = A + B \times \log(serum\ folate\ level)$$

If the serum folate level increases from SF_1 to SF_2 the percentage NTD risk reduction will be:

$$Percentage\ NTD\ risk\ reduction = 100 \times \left(1 - \left(\frac{SF_2}{SF_1}\right)^B\right) \quad (2)$$

iii. The predicted decrease in NTD risk using the results from Daly/Wald can be found by substituting into Equation (2) 8.8 for SF_2/SF_1 (the increase in serum folate from 5 ng/ml to 44 ng/ml observed in the MRC vitamin study[5]) and -0.81 for B from Equation (1):

$$NTD\ risk\ reduction = 100 \times \left(1 - (8.8)^{-0.81}\right) = 83\%$$

2. Crider/Chen

i. The results in Supplemental Table 1 of Chen *et al*[6] were used to show that there is a log-log linear relationship between serum folate level and red blood cell (RBC) folate level. A log-log regression of the RBC folate level against serum folate level for the baseline and folic acid doses of 100, 400 and 4000 μg per day in Supplemental Table 1 gives the following relationship:

$$\log(RBC\ folate\ level\ in\ nmol/l) = 5.33 + 0.46 \times \log(serum\ folate\ level\ in\ nmol/l) \quad (3)$$

ii. The results in Supplemental Table F of Crider *et al*[3] show that there is a log-log relationship between NTD odds and serum folate level. Assuming NTD odds and NTD risk are approximately equal because the NTD risk is small:

$$\log(NTD\ risk) = 4.57 - 1.7 \times \log(RBC\ folate\ level\ in\ nmol/l) \quad (4)$$

Substituting Equation (3) in Equation (4) gives the following relationship between NTD risk and serum folate level:

$$\log(NTD\ risk) = 4.57 - 1.7 \times (5.33 + 0.46 \times \log(serum\ folate\ level\ in\ nmol/l))$$

$$\log(NTD\ risk) = -4.49 - 0.78 \times \log(serum\ folate\ level\ in\ nmol/l)$$

For consistency with Equation (1), convert NTD risk to NTD risk per 1000 births and serum folate level to ng/ml:

$$\log(NTD\ risk\ per\ 1000\ births) = \log(1000) - 4.49 - 0.78 \times \log(serum\ folate\ level\ in\ ng/ml \times 2.266)$$

$$\log(NTD\ risk\ per\ 1000\ births) = 1.78 - 0.78 \times \log(serum\ folate\ level\ in\ ng/ml) \quad (5)$$

iii. The predicted decrease in risk using the results from Crider/Chen can be found by substituting into Equation (2) 8.8 for SF_2/SF_1 and -0.78 for B from Equation (5) :

$$NTD\ risk\ reduction = 100 \times \left(1 - (8.8)^{-0.78}\right) = 82\%$$

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