Addition of Vitamin B12 to folic acid supplements to optimise the prevention of Spina Bifida and other Neural Tube Defects

What is the evidence that reduced Vitamin B12 status increases the risk of a pregnancy affected by a Neural Tube Defect (NTD)?

Reduced folate status is now accepted as the most important risk factor for the occurrence (and recurrence) of NTD affected births (MRC Trial 1991). Consistent with this is a study that shows a graduation in risk in going for a reduced to an optimal folate status (Daly et al 1995). This study showed that in the index pregnancy (i.e. one in which the child had an NTD) there was a progressive relationship where the risk for mother with the lowest folate status was 7 per 1,000 births, reducing in a consistent way to where at an optimum folate status the risk had gone down tenfold to 0.7 per 1000 births. A further study (Daly et al 1997) showed that as little as 200 ug of extra folic acid per day could bring about a change in status from the highest to the lowest risk. These studies are consistent with the now well-established reduction in risk seen earlier in intervention trials and subsequently with folic acid fortification (MRC Trial 1991).

A similar risk/status analysis had been published concerning reduced vitamin B12 status and an increasing risk of an NTD affected birth (Molloy et al 2010). The level of circulating serum B12 was used for the analysis. As can be seen from Figure 1 of that paper, in three different study cohorts risk was reduced by half or more in going from a poor to an optimum vitamin B12 status. In both the studies the folate and vitamin B12, the affected women were not folate or vitamin B12 deficient in either instance, indicating that increased risk occurs at normal but less than optimal status. This combined risk is not surprising. There is a well understood biochemical relationship wherein reduced vitamin B12 status is known to compromise folate retention by cells and folate function (Scott and Weir, 1981)/

Where does the UK population of women of child-bearing age fall in this NTD risk profile with respect to vitamin B12 status?

Periodically, the UK Department of Health carries out surveys on Diet and Health (NDNS 2003). One such National Diet and Nutrition Survey (NDNS) reported that vitamin B12 status was a mean of 268 mean (SD 101) pMoles/L in women 19 to 50 years (Henderson et al 2003). Thus, nearly half of the pregnancies would be below the target mean figure of level of 221 pMole/L indicated as being desirable status (Molloy et al 2010). Still less would reach the greater level of 295 pMol/L beyond which risk for
vitamin B12 status appears to reach a plateau (Molloy et al 2010). This would indicate that the great majority are in the Immediate Risk category with respect to vitamin B12 status and risk of an affected birth. One might consider that the vast majority of women in the UK could reduce this risk of an NTD affected birth by improving their vitamin B12 status. The magnitude of this risk reduction would depend upon two things. Firstly, what would be the expectation that some or all of these women could be moved to a new vitamin B12 status of greater than 300 pmoles/L (see below) by the addition of vitamin B12 to their diet. Also it would depend upon their starting vitamin B12 status. However, many could expect to half their residual risk, in addition to any reduction in risk brought about by a folic acid. It would appear that the risk reduction with improved vitamin B12 status would be independent of any risk reduction achieved with extra folic acid. However, their functions are so intertwined it may well be that their combined use also has a synergetic or enhancing effect over and above the sum of the two vitamins independently (Scott and Weir 1981). Those with very low starting vitamin B12 status, usually the disadvantaged, might possibly reduce their risk of an NTD occurrence several-fold by improving their vitamin B12 status.

Increasing Vitamin B12 status in women of childbearing age to the lowest risk of an NTD affected pregnancy.

To evaluate this one can use two types of evidence (i) Intake Data relative to status (ii) Vitamin B12 intervention trials.

(i) Intake/Status Data

To begin with one will see from the intake/trial studies cited below and from the risk curves of Molloy et al (2010) that it is entirely possible for women to reach a vitamin B12 status well in excess of 300 pmoles/L. The NDNS data shows mean intake in such women of 4.0 ug/d of vitamin B12. Considering that the PRI (RDA) for the UK is 1.5 ug/d and even if one takes the higher RDA reference in the EU of 2.5 ug/d and in the US and Canada of 2.4 ug/d, it would appear that virtually all women in the UK would exceed this, many having double these intakes. One would thus anticipate that such high intakes would be accompanied by high circulation levels. However, the reality again according to the NDNS status data would be that UK women do not have such good status. The NDNS survey of 2003 showed that women between the ages of 19 to 44 have a mean ± SD (p/Miles per litre) for circulating vitamin B12 of 268 (+ 101). So why is their apparently very adequate intake not reflected in a more optimum vitamin B12 status?

From more recent intake/status data, it would appear that the current RDAs are very much lower than they should be. It would appear that the earlier evidence used in these recommendations concentrated on intakes that would be expected to prevent the anaemia and neuropathy associated with vitamin B12 deficiency (Institute of Medicine 1995). What is more appropriate, particularly in the context of NTD risk, is what intake would be required to produce an optimum vitamin B12 status of greater than 300 pMoles per litre.

The most convincing of this new data is a study in Danish women (Bor et al 2006). This study related dietary intake of vitamin B12 to corresponding status. Intakes of greater than 6.0 ug/d appeared to saturate status with levels of over 400 pmoles/L being achieved. By contrast, an intake of 3.0 ug/d gave a status of just over 200 pMoles/L/d, while an intake of 4.0 ug/d gave a status of under 300 pMoles/L. This latter would of course fit well with the UK NDNS data of an intake in the UK of 4.0 ug/d giving a mean status of 268 pmoles/L. Of further interest was that while intake in excess of 6.0 ug/d normalised the two functional markers of vitamin B12 status, namely methylmalonic acid (MMA) and plasma homocysteine (tHcy), levels of intake of less than 6.0 ug/day did not achieve this. A total of four other recent studies support the dose status relationship seen in Bor et al (2006). An earlier study examining the relationship between vitamin B12 intakes from food and supplements in the Framingham Offspring Cohort gave a similar conclusion (Tucher et al 2000) Kwan et al (2002) found comparable increases in a group of
Hispanic elderly. More recently, Vogiatzoglow et al (2009) found that dietary intake of vitamin B12 of 5.4 μg (5.3 to 5.6) per day elicits a circulating vitamin B12 level of 358 (352, 364) μmoles/L. Comparing intake and status in a group of older Dutch subjects supports these other intake status relationships (van Asselt et al 1998).

(ii) Intervention Trials

The earlier literature produced many studies that daily intake of vitamin B12 of the order of a few μg per day or less certainly produced a status that prevented the overt signs and symptoms of anaemia and neuropathy (Chanarin et al, 1979) Similarly from other earlier studies, one could conclude that the limiting factor in vitamin B12 absorption, was not normally the secretion of intrinsic factor but rather the capacity of the ileal receptors seemed to be limited to 1.5 to 2.5 μg per meal. Thus, assuming three meals per day, a total daily intake could be expected to be about 6.0 μg/d.

Most interventions using oral vitamin B12 preparations have been seeking to treat vitamin B12 malabsorption conditions such as pernicious anaemia (Chanarin 1979). The intervention depends on the known passive absorption of vitamin B12 which is calculated to be around 1.2% of any oral dose (Chanarin 1959 and Berlin et al 1968). In this context daily doses of 500 or 1000 μg were used.

There are however a limited number of studies where intervention with physiological levels of vitamin B12 or the amount of about 5.0 μg per day, that would be expected in the diet, have been examined. Seal et al (2002) sought response to levels of 10 to 50 μg per day while Verhaeverbeke et al (2003) used 100 μg per day. Rajan et al 2002 used three test doses of 25 μg followed by 100 and 1000 μg/d. These levels, while low, are clearly not reflective of the levels in food. Only two trials exist to date which examined levels comparable to those found in food. Eussen et al (2005) examined the affect on status of administering daily doses of 2.5, 100, 250, 500 and 1000 μg per day for 16 weeks in 120 people. The median concentration at baseline was 208 μmol/L. After intervention with 2.5 μg/d, for 8 and 16 weeks respectively this value was unchanged at 269 and 290, a very modest response. The authors concluded that intakes of 500 μg/d equivalent to 200 times greater than an RDA of 2.5 μg per day are required for an optimum response. This study appears at odds with what one would expect from the absorption characteristic of oral vitamin B12. It would seem that the most likely explanation is that the subjects in the trial were given supplements of vitamin B12 to take daily but no instructions were given with respect to when these tablets should be taken. This was a feature in earlier experience when gastric or other hormones were administered to volunteers prior to the collection of intrinsic factor via gastric intubation.

It would appear that at high doses the route for the absorption of vitamin B12 would be by diffusion. This acts at just over 1% of the administered dose (Berlin 1968). One can thus see why levels of 500 μg/d were required to give an optimal response acting via diffuse absorption. Values in the lower range tested of 2.5 μg/d would be expected to show a decreased response in those who took the tablets in the absence of a meal which may have been any proportion of those in the trial. The result would suggest that it may have been most of those investigated. This issue is important because it reinforces what would be expected, namely that any combined folic acid vitamin B12 supplement to lower NTD should ideally be taken with a meal. While the absence of a meal would not affect folic acid absorption, it would have a profound effect on vitamin B12 absorption lowering it with a decrease of IF or at least a decrease in IF.

One further study also looked at low levels of vitamin B12 daily supplementation in an intervention trial (Blacher et al 2007). They intervened daily with doses of 2.5, 5, 10, 20, 40 or 80 μg/d and measured serum response. “Daily doses were administered to patients in the morning after breakfast, under supervision of medical staff”. Such a procedure would have ensured total compliance but perhaps of greater relevance it would have ensured that IF based active absorption of vitamin B12 was being tested. Results showed that a log-dose effect for vitamin serum vitamin B12. The slope was higher at 30 than at 15 days. For a mean serum increase of 37 μmol/L a dose of 5.9 (95% 0.9-12) μg/d was needed. Thus, this low dose seemed to put patients in positive balance, converting a baseline of 111 (38) to 163 (44) μmol/L mean (SD) after 30 days. Unfortunately, the study was not continued beyond one month, making
it impossible to say if the steady state balance when achieved would have eventually elevated this level to a level greater than 300 pmol/L. There is however an important lesson from this study, namely that any folic acid/B12 supplement while it would possibly optimise folate levels within one or certainly three months, the former time would be inadequate for the improvement of vitamin B12 status to optimum levels above 300 pmol/L. It is possible that high doses of vitamin B12 of 500 or 1000 ug/d might achieve this optimum status in a shorter time. While the lower of the two would be expected to deliver 5.0 ug/d by diffusion. Similarly, in the above study, the higher level would deliver 10 ug/d which would be expected to achieve the optimum level in everybody of greater than 300 pmoles/L but, again, it could take weeks and probably months to do so.

**Are there risks if adding vitamin B12 to this folic acid supplement?**

None. Since its isolation over half a century ago vitamin B12 has been given to humans in hundreds if not thousands of studies. Routes of administration have been oral, IM and IV as daily supplements. Frequently levels of a thousand micrograms per day have been used. No sustained ill effects have been reported in the literature.

In summary, evidence exists of a reduction of risk for an NTD affected birth by increasing maternal vitamin B12 status (as is currently known for folic acid). Given the probable status of women in the UK at the moment, it is clear that as well as the addition as a folic acid supplement (400 ug/d) the addition of a vitamin B12 component of at least 2.5 ug/d would bring about a further significant and worthwhile risk reduction for NTDs.

**References**


Medical Research Council Trial, Lancet, 1991


National Diet & Nutritional Survey: Adults aged 19 to 64, Section 2.3.5, Vitamin B12, Food Standards Agency, Volume 3, 2003, London TSO.


Legend to Figure

Reduction in NTD risk in three different cohorts of women relative to improving circulating vitamin B12 levels (pMoles/L).

[Molloy et al]