

## 11. Folate

### Physiology and metabolism

#### *Metabolic functions*

Folate is the general name given to compounds with nutritional properties similar to those of folic acid (pteroylglutamic acid). Besides folic acid itself, with one glutamate residue, there are other forms with a varying number of extra glutamates attached. Reduced derivatives of these participate in enzymic reactions providing single carbon units for DNA and RNA biosynthesis and methylation reactions <sup>1</sup>.

#### *Absorption and metabolism*

The enzymatic interconversions in which the folate cofactors participate involve eight forms of the vitamin. Many of these are easily oxidised, some to forms that can subsequently be reduced and which are thus nutritionally active, others to catabolized products which are inactive <sup>2</sup>. The extent to which these oxidations occur will have a profound effect upon the folate ultimately available from the diet. This in turn will depend upon not only the forms of the cofactor predominating in a particular food but also the length and conditions of storage, the presence or absence of natural or added antioxidants etc. All natural folates exist as polyglutamyl conjugates. These extra glutamates may get removed either during storage and processing of food or by conjugases that are present in the human intestine. At physiological concentrations only folates containing a single glutamyl residue are absorbed into the circulation, usually after conversion to the 5-methyltetrahydro form. Human cells depend principally upon the uptake of this circulating 5-methyltetrahydrofolate for their supply of the vitamin.

The folate content of the diet is usually estimated by microbiological methods which may underestimate the true content of folate in food by as much as 20%-30%. Some foods but not others contain factors that inhibit the intestinal folate deconjugation enzymes thus decreasing absorption <sup>3</sup>. The degree of inhibition varies considerably from food to food, with some foods, for example beans, giving 20% inhibition while values as great as 80% are found for orange juice. There is also a solid body of evidence that polyglutamyl forms of folate are less well absorbed than monoglutamates even in the absence of such inhibitors. Straight comparisons of folic acid containing one glutamyl residue with the corresponding heptaglutamyl form have

indicated the latter to be some 58-79% as available as the former. However, an important new approach has recently been introduced, namely the comparison of various folate forms labelled differently with deuterium, which found that folate hexaglutamate was only 50% as available as the monoglutamate when given in pure forms at physiological concentrations<sup>4</sup>. This value indicates an even lower availability of polyglutamyl forms than previously expected. Thus there are three areas where calculations based on mean requirement must be adjusted to get mean dietary requirement. There is probably an underestimation of the amount of folate in food. Inhibition of intestinal conjugase would decrease the availability of folate polyglutamates. Finally even in the absence of such inhibitors folate polyglutamates are probably only 50% as available as the corresponding monoglutamates, upon which some of the mean requirement calculations are based. Factors one and two work in the opposite directions and may cancel each other out, leaving a 50% overall adjustment as being an acceptable correction factor.

## Deficiency and excess

Folate deficiency decreases DNA and RNA biosynthesis, and manifests itself most obviously in cell types that turn over rapidly, such as in the bone marrow, thus causing anaemia.

5-Methyltetrahydrofolate is involved in providing, via methionine, methyl groups for many methylation reactions; these systems will fail however only in severe prolonged folate deficiency.

A "pseudo folate-deficient" state is seen in B<sub>12</sub> deficiency, because cells fail to handle folate normally, resulting in the signs of folate deficiency. There is however no dietary deficiency of folate; the condition is cured by vitamin B<sub>12</sub>. The haematological picture can however be improved by high intakes of folic acid itself, which is particularly effective in this role. Large doses of folic acid, which is the synthetic form in supplements, can therefore prevent the timely diagnosis of vitamin B<sub>12</sub> deficiency, allowing the neuropathy which also occurs to proceed undiagnosed, only to emerge at a much more advanced stage when its effects are largely irreversible.

High levels of folic acid around 5.0 mg per day have been used in pregnancy for periods of several months without any apparent ill effects. Thus in normal subjects levels of up to 5.0 mg per day would seem to be well tolerated<sup>1</sup>. There is evidence that high levels of folic acid increase fit frequency in epileptics<sup>5</sup>. There is also the certainty that such high levels taken on a daily basis would mask the emergence of vitamin B<sub>12</sub> deficiency. At lower levels of 100-200 µg of folic acid per day this masking would be less likely. Since a part of the neurological damage in B<sub>12</sub>

deficiency is largely irreversible, it is serious if allowed to go undiagnosed and untreated. Vegans and the elderly, being at increased risk of vitamin B<sub>12</sub> deficiency, should avoid large daily intakes of folic acid supplements. However folic acid supplements of around 400 µg per day are in widespread use, particularly in the United States, and it is claimed that there is no evidence of their masking pernicious anaemia at that level <sup>6</sup>.

## Physiological and dietary requirements

### *Adults*

Folate status can be measured from the concentration of folate in serum, but the level of folate in the red blood cells gives a much closer estimate of tissue stores <sup>1</sup>. Red cell folate values above 150 µg/L are an indication of sufficiency <sup>1</sup>. However there is no clear borderline separating the normal population from those with clinical signs of deficiency <sup>1</sup>. Approaches using depletion of folate <sup>8,9</sup> and considerations of folate catabolism <sup>10</sup> suggest daily requirements of 50-100 µg.

The level of oral folic acid required to treat folate deficiency in an adult man has been reported as 50 µg/d. A study of a group of normal volunteers found higher amounts were needed – 75 µg/d <sup>12</sup>. A limited study on three normal women showed that on a daily intake of 50 µg folic acid their red cell folate remained in the normal range but respectively fell or rose at levels of 25 or 100 µg/d <sup>13</sup>. A different study on four women indicated that their plasma levels were stabilized by 80 µg pure folic acid and 20 µg dietary folate daily <sup>14</sup>.

From these studies with folic acid, the mean requirement for an adult is taken to be 70 µg/d. As discussed above, one would expect this to be twice as available as food folate. The mean dietary requirement of folate would therefore be 140 µg/d.

Dietary recommendations for folate have often been supported by values for the intakes of folate observed in individuals and groups that clearly receive adequate amounts of the vitamin. It is probably unwise to rely on this approach as the values obtained for food folates may be substantial underestimates because of the uncertainties of the analytical procedures.

### *Children*

In a study of infants between 2 and 11 months 3.6 µg folic acid per kg body weight per day appeared to maintain plasma levels<sup>15</sup>. Infants between 6 and 11 months of age are likely to receive a mixture of folic acid and food folate, so a Population Reference Intake of 50 µg/d is proposed.

In the absence of specific information, values for children have been derived from those for adults on the basis of energy expenditure.

### *Pregnancy*

Folate deficiency in the later stages of pregnancy in women not ingesting extra folate is extremely common. Various studies have shown one quarter to one half would have clear signs of deficiency<sup>1</sup>. Red cell folate drops during pregnancy and while part of this drop may be due to haemodilution some of it is certainly due to the mother's being unable to meet the extra demand for folate made by the fetus from her stores by normal dietary intake. Studies have shown this drop in RBC (red blood cell) folate could be prevented by a supplement of 100 µg folic acid daily<sup>16,17</sup>. Since these studies were carried out on women on good diets this level is really a minimum. To allow for the problems of bioavailability discussed above, a dietary increment of 200 µg of folate per day should be sought. Recent work on the increase of folate catabolism in pregnancy indicates that this amount is not generous<sup>10</sup>.

### *Neural tube defects*

There is now good evidence that daily periconceptual folic acid supplementation may have a protective effect on the occurrence of the congenital abnormalities, spina bifida and anencephalus, collectively called neural tube defects (NTDs)<sup>18</sup>. While some of these studies<sup>19</sup> involved the use of very high levels of folic acid, it seems probable that amounts around 400 µg per day confer equal protection with a lesser risk of side effects<sup>20</sup>. Even at this lower level one cannot completely exclude the risk involved in taking a greater than normal amount of a vitamin in early pregnancy. However as far as toxicity to the mother is concerned there are only two established causes for concern, which have been mentioned already. Women taking antiepileptic drugs should be aware that it has been suggested that taking folic acid supplements of much above 400 µg may cause loss of control of their epilepsy. Taking extra folic acid, if one is also vitamin B<sub>12</sub> deficient, results in the masking or exacerbation of neurological disease associated with such deficiency. However vitamin B<sub>12</sub> deficiency is extremely rare in women of less than 40 to 50. Should women, particularly when nearing the end of their child-bearing years, plan to take folic acid

supplements for periods of months, they should have possible vitamin B<sub>12</sub> deficiency excluded by a simple blood test.

While it is possible that improved dietary intake of folate may confer protection from NTDs it is difficult to achieve intakes of 400 µg per day because of the low density of folate in food and the lower availability of the natural forms of the vitamin than of the folic acid used in supplements. Fortified foods containing folic acid in the more available form may provide an alternative for women who do not wish to take tablets.

It should be emphasised that the neural tube is formed between three and four weeks after conception, at which point many women who are pregnant are not aware of the fact. It is thus recommended that women wishing to attempt to achieve protection using folic acid should ensure that its intake is commenced before conception, so as to ensure adequate levels at the critical time. While there is no direct evidence on the issue, the ingestion of folic acid post conception may be of benefit up to the time of closure of the neural tube, i.e. four weeks. Thus women who suspect that they may be at an early stage of an unplanned pregnancy, e.g. by observing a missed period, may wish also to take folic acid even at this later and far less certain stage.

### *Lactation*

Earlier studies suggested that milk contains about 50 µg per litre <sup>21</sup>. It now seems more likely that with better methods of estimation this value should be doubled <sup>22</sup>. Assuming a daily output of 750 ml, loss could thus be between 37 and 75 µg per day. To allow for bioavailability and taking the higher value one arrives at an increment of 150 µg per day to compensate for normal lactation.

### *The elderly*

A significant body of evidence <sup>23</sup> indicates that the elderly have no increased requirement for folate. As mentioned previously, they should avoid large intakes of folate supplements.

## Summary

Values expressed in terms of mixed dietary folates (calculated for a relative molecular mass of 441), assuming a bioavailability about half that of pure folic acid (monoglutamate form), per day.

<i>Adults</i>	
<b>Average Dietary Requirement</b>	140 µg
<b>Population Reference Intake</b> (mean requirement + 2 SD, assuming a coefficient of variation of 20%)	200 µg*
<b>Lowest Threshold Intake</b> (mean requirement - 2 SD )	85 µg
Pregnancy	400 µg*
Lactation	350 µg

### *Population Reference Intakes for other groups*

<b>Age Group</b>	<b>PRI</b>
6 - 11m	50 µg
1 - 3y	100 µg
4 - 6y	130 µg
7 - 10y	150 µg
11-14y	180 µg
15-17y	200 µg

- \* Neural tube defects have been shown to be prevented in offspring by periconceptual ingestion of 400 µg folic acid per day in the form of supplements.

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