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#### Folate requirements for health in women

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The earliest account of human folate requirement relates to its critical role in women's health. In 1872 Biemer described a severe, rapidly progressive and sometimes fatal anaemia of pregnancy (Rodriguez, 1978). However, the possibility that this might be related to a dietary deficiency was not considered until much later, when Wills (1931) established that cases of macrocytic anaemia in pregnant women in India responded to treatment with either crude liver or yeast extract. Later, various groups described a new nutritional factor (‘vitamin M’, ‘vitamin B<sub>c</sub>’, ‘factor U’) in animal and microbial systems and the task of isolating and determining the structure of what is now known as folic acid began (Stockstad, 1979). Final proof of the structure of folic acid was established by its synthesis (Angier *et al.* 1945).

Folate is a generic descriptor for the many different forms of the vitamin which exhibit the biological activity of the parent molecule folic acid (pteroylmonoglutamic acid), consisting of a pteridine nucleus linked through a methylene bridge to a *p*-aminobenzoic acid and L-glutamic acid residue. Folic acid is the commercially available form of the vitamin. It is not a naturally-occurring compound, although it is readily converted *in vivo* to the natural forms. Tissue and food folates exist primarily as polyglutamate derivatives, containing additional glutamate moieties linked via  $\gamma$ -carboxyl peptide bonds. Folate coenzymes function as acceptors and donors of C<sub>1</sub> units in a variety of essential reactions (‘C<sub>1</sub> metabolism’) involving nucleotide and amino acid metabolism (Shane, 1995). C<sub>1</sub> units are carried by folate only when the vitamin is present in the fully-reduced (tetrahydrofolate) form (Wagner, 1995). Although folate coenzymes are required for many biochemical reactions, the clinical effects of a folate deficiency largely occur when one reaction in particular, the synthesis of thymidylate, is impaired beyond a critical level (Wickremasinghe & Hoffbrand, 1980; Tehiri *et al.* 1981). Thymidylate is an essential precursor of *de novo* biosynthesis of DNA.

Experimental folate deficiency was produced by Herbert (1962) in a study on himself. He reported the resulting haematological, biochemical and clinical sequence of events from the onset of dietary folate deprivation, to eventual megaloblastic anaemia. The latter, which was evident by 19 weeks, is the major clinical sign of folate deficiency and is characterized by abnormally large erythrocyte precursors (megaloblasts) in the bone marrow and larger than normal erythrocytes (macrocytes) in the peripheral blood, consistent with the finding of giantism in the morphology of every proliferating cell. Whilst the biochemical defect may be due to a variety of causes, including certain drugs, 95% of all cases of megaloblastic anaemia are considered to be the result of a deficiency of folate or vitamin B<sub>12</sub> or both (Herbert, 1985). It is assumed that the megaloblastic changes associated with a

deficiency of either vitamin are attributable to impaired biosynthesis of DNA, since similar changes arise with drugs which are known to interfere with DNA synthesis but which do not necessarily affect folate or vitamin B<sub>12</sub> metabolism (Scott & Weir, 1980).

#### FOOD FOLATES AND BIOAVAILABILITY

The typical folate content of selected foods is given in Table 1. While these foods are considered to be amongst the most concentrated dietary sources of the vitamin, they do not necessarily contribute the most to overall intakes of folate in a population. Liver, for example, despite its high folate content is not eaten by a sufficient proportion of the population to make any significant contribution to total dietary folate intakes, while beers contain at most 90 µg/l but are consumed frequently enough to account for 10 % of dietary folate intake in British adults (McNulty, 1995). Furthermore, as reviewed recently by Gregory (1995), many uncertainties exist regarding the bioavailability of folate. Food folates are present mainly as polyglutamate derivatives which have to be converted to the monoglutamyl form for absorption in the jejunum. The enzyme that hydrolyses dietary polyglutamates,  $\gamma$ -glutamylhydrolase ('folate conjugase'; EC 3.4.22.12), is found on the luminal brush-border membrane of the jejunum (Wang *et al.* 1985). Changes in the pH of the lumen contents, or the presence of folate conjugase inhibitors, folate binders or other specific food components, can all adversely affect the rate of hydrolysis and intestinal uptake of the vitamin (Reisenauer & Halsted, 1987; Bhandari & Gregory, 1990). Such factors account for the wide variation in folate bioavailability of the naturally-occurring folate polyglutamates in foods of plant and animal origin. Superimposed on the influence of diet composition on folate bioavailability are inherent differences in the bioavailability of the various folates. Studies using stable isotopic methods have found approximately 50 % relative bioavailability of polyglutamyl compared with monoglutamyl forms of folate (Gregory *et al.* 1991). Thus, the overall bioavailability of folates from a mixed diet is extremely difficult to predict, but is considerably less than when the vitamin is consumed as a vitamin supplement or as a fortified food where it is present in the unreduced monoglutamate form.

#### FACTORS AFFECTING FOLATE STATUS IN WOMEN

Suboptimal folate status will arise in any woman when folate requirement exceeds availability. Such a situation may be the result of decreased availability, increased requirement or both.

Table 1. *Folate content\* of selected foods (µg per usual serving)*

Chicken liver (grilled or fried)	500
Asparagus ( <i>Asparagus officinalis</i> )	193
Fortified breakfast cereal	83
Spinach ( <i>Spinacia oleracea</i> )	81
Broccoli ( <i>Brassica oleracea botrytis asparagoides</i> )	54
Green beans	50
Marmite	40
Orange juice	32
Baked beans	30
Fruit yoghurt	24

\*Holland *et al.* (1991).

Table 2. *Estimated dietary folate intake in women compared with men*

	Mean folate intake ( $\mu\text{g/d}$ )		Reference
	Men	Women	
UK	300	209	Gregory <i>et al.</i> (1990)
Ireland	244	183	Irish Nutrition & Dietetic Institute (1990)
USA	281	207	Subar <i>et al.</i> (1989)
Canada	205	149	Canada Health & Welfare (1977)

The most common cause of compromised folate status is considered to be low dietary intake of the vitamin (Sauberlich, 1995). Estimated folate intake of women in various populations is considerably lower than in men (Table 2). As recently reviewed by Sauberlich (1995), particularly low folate intakes have been reported in adolescent females and in elderly subjects from low-income subgroups. Food preparation and cooking can also make a substantial difference to the amount of folate ingested since prolonged exposure to heat is known to cleave the folate molecule, rendering it inactive; boiling in particular can result in substantial losses (Herbert, 1987). Thus, the high incidence of folate deficiency among Indian compared with Chinese women living in Singapore may be attributed to destruction of the folate molecule due to the practice of prolonged boiling of foods by the Indian women, compared with the rapid stir-frying of vegetables practised by the Chinese women resulting in greater folate retention (Herbert, 1990).

As a result of the role of folate coenzymes in DNA, RNA and protein synthesis, any stage of the life cycle characterized by rapid cell replication and growth, such as pregnancy, lactation and early growth, is associated with an increased requirement for the vitamin. Evidence from both animal (McNulty *et al.* 1993) and human (McPartlin *et al.* 1993) studies suggests that increased folate breakdown (catabolism) contributes to maternal folate depletion during pregnancy. The increased generation of folate catabolites during pregnancy and other rapid-growth situations may be a direct consequence of increased utilization of the vitamin for DNA synthesis (McNulty *et al.* 1995). Less commonly, folate requirement will be increased in a woman as a result of the presence of various pathological conditions (malignancy, increased haematopoiesis, inflammatory conditions) or factors which impair folate absorption (Chanarin, 1990). The latter would include coeliac disease and tropical sprue in which folate deficiency is often a presenting feature (Lindenbaum & Allen, 1995).

Several drugs interact with folate and, by various mechanisms, impair folate status (Roe, 1990). The use of oral contraceptive agents by women aged 20–44 years has been associated with low folate status (Senti & Pilch, 1985). In one study oral-contraceptive use was associated with megaloblastic changes in cells obtained from the uterine cervix, which reverted to normal after folic acid therapy (Whitehead *et al.* 1973). The mechanism whereby oral contraceptives affect folate status is not well understood. Likewise, alcohol and anti-convulsant drugs (phenytoin, phenobarbitone, primidone) are known to negatively affect folate status by a number of possible mechanisms. Possible explanations for alcohol-induced folate deficiency include intestinal malabsorption, altered hepatobiliary metabolism, increased excretion or increased catabolism of the vitamin (Halsted, 1995). Inadequate dietary intake appears to be a contributory factor in many human studies which have examined the effects of alcohol on folate status (Halsted, 1995). Anti-folates, usually structurally similar to the folate molecule which block a specific pathway ordinarily involved in folate metabolism, are widely used clinically as cancer chemotherapeutic

(methotrexate, aminopterin), anti-malarial (pyrimethamine) and anti-bacterial (trimethoprim) agents. The drug sulfasalazine, commonly used in the treatment of ulcerative colitis, impairs folate absorption through competitive inhibition of enzymes involved in folate transport and metabolism (Lashner *et al.* 1989). Many of these drugs have known teratogenic effects and particular care should be taken regarding their usage in women of child-bearing age.

Smoking appears to have an adverse effect on erythrocyte and serum folate levels, resulting in an increased incidence of subclinical folate deficiency in smokers compared with non-smokers (Sauberlich, 1995), but it remains to be established whether this is the result of decreased folate intake or increased folate requirement. Likewise the mechanism of folate deficiency reported in female marathon runners (Matter *et al.* 1987) is unclear. Marginal intake or absorption of folate, and/or other mechanisms which result in increased requirement, may be involved.

Thus, there are a number of environmental, physiological and pathological factors which may contribute to suboptimal folate status in women. The presence of two or more factors may present a particular risk. Thus, for example, the ability of a mother to sustain the increased demands of pregnancy will be considerably lessened if her habitual dietary intake is low in folate.

#### RECOMMENDED DIETARY FOLATE INTAKES IN WOMEN

A variety of terms are used to describe recommended intakes for folate and other nutrients, depending on the particular committee which set them (Table 3). In the most recent UK report of dietary recommendations (Department of Health, 1991) the term reference nutrient intake (RNI) has been adopted to replace what was originally termed (and is still in use in the US) the recommended dietary allowance. For comparison purposes all values referred to in Table 3 have been derived in the same way, i.e. set at two standard deviations above the estimated mean requirement of a healthy population. Current official recommendations for folate intake are considerably lower than previously-published values (Table 3). The latter were set at levels far higher than estimated intakes achieved by populations who showed no evidence of biochemical deficiency of the vitamin.

The general approach which has been used to generate current folate intake recommendations for non-pregnant women involves examining data on average intakes of folate among individuals with good folate status (i.e. typically, intakes associated with no overt signs of folate deficiency and sub-optimal folate status in less than 10% of subjects). Increments are then added to cover the estimated increased needs of pregnancy

Table 3. *Current (and previous) recommendations for folate in women ( $\mu\text{g}/\text{d}$ )*

	UK		USA		World Health Organization/Food and Agriculture Organization		Europe
	1991	1979	1989	1980	1988	1975	1993
Non-pregnant	200*	300	180†	400	170‡	400	200§
Pregnant	300	500	400	800	370–400	800	400
Lactating	260	400	280	500	270	500–600	350

\* Reference nutrient intake (Department of Health, 1991).

† Recommended dietary allowance (Food and Nutrition Board, 1980, 1989).

‡ Safe level of intake (Food and Agriculture Organization, 1988).

§ Population reference intake (Commission of the European Communities, 1993).

and lactation. This approach is based on two assumptions: first, that dietary folate intake estimates are valid, and second, that estimates of haematological folate status of populations are valid. There are a number of reasons why both assumptions may be incorrect (Bailey, 1995). As regards the latter assumption, it is argued that conclusions relative to haematological folate status of populations are based on a limited number of small studies and, in some cases, are complicated by the fact that two different assay methods (microbiological and radioassay) were used to measure folate status (Bailey, 1995). The problem of estimating dietary folate intakes is an even greater one. Food composition values for folate content of many foods are now considered to be unreliable as a result of both incomplete extraction (Gregory *et al.* 1990) and inadequate deconjugation (Englehardt & Gregory, 1990) in the analytical procedures used. This, combined with the well-recognized problem of under-reporting food intakes generally (Black *et al.* 1993), has resulted in reported folate intakes that are likely to be considerably lower than actual intakes.

Thus, current folate intake recommendations for women, which are based on estimated intakes, are probably underestimated (Bailey, 1995). This view is supported by a recent 70 d metabolic study of non-pregnant women which determined the response of folate status to levels of intake of 200, 300 and 400  $\mu\text{g}/\text{d}$  provided mostly in the form of supplemental folic acid (O'Keefe *et al.* 1995). Evidence from serum folate, erythrocyte folate and homocysteine (an indirect index of folate status) responses to the various regimens led the authors to suggest that 200  $\mu\text{g}/\text{d}$  was inadequate to maintain folate status and that 400  $\mu\text{g}/\text{d}$  appeared to be a more appropriate representation of dietary allowance. A more important interpretation of these results is that they highlight the problem of underestimation of dietary folate intakes, in that the group consuming a defined intake of 200  $\mu\text{g}/\text{d}$  (over 80 % of which was folic acid) showed serum and erythrocyte folate decreases of 43 and 14 % respectively relative to initial values which were associated with usual dietary folate intake. Although the latter are not given in the paper, typical estimations would be about 200  $\mu\text{g}$  total dietary folate/d which is considered to be only about 50 % bioavailable (Sauberlich *et al.* 1987).

#### FOLATE REQUIREMENTS IN WOMEN

There are a number of stages in the life cycle in women for which folate requirements are increased, or where new evidence suggests a possible benefit from an increased status of the vitamin.

##### *Folate requirement for periconception*

Evidence of a possible association between folic acid and neural-tube defects (NTD) has been described in literature spanning three decades (Scott *et al.* 1995). NTD are major malformations in which there is a failure of the developing tube to close properly during the fourth week of embryonic life. Incomplete closure of the spinal cord results in spina bifida, while incomplete closure of the cranium results in anencephaly. The latter condition is incompatible with life and affected babies die either *in utero* or shortly after birth. Since the early 1980s a number of intervention trials examining the impact of periconceptional folic acid on the incidence of NTD have been published, some of which are shown in Table 4. With exception of the Hungarian trial (Czeizel & Dudas, 1992), all of these studies concerned NTD recurrence, i.e. the administration of folic acid periconceptionally to women with a previous history of an NTD-affected pregnancy. The results of the Medical

Table 4. *Intervention studies: impact of periconceptual folic acid on incidence of neural-tube defects*

Study	Design	Dose (mg)	Risk reduction (%)
Laurence <i>et al.</i> (1981)	RCT	4.0	58
Smithells <i>et al.</i> (1983)	NRCT	0.36	86
MRC Vitamin Study Research Group (1991)	RCT	4.0	72
Czeizel & Dudas (1992)	RCT	0.8	100

RCT, randomized clinical trial; NRCT, non-randomized cohort trial.

Research Council vitamin study (MRC Vitamin Study Research Group, 1991) provided the first unambiguous confirmation of the effectiveness of periconceptual folic acid in the prevention of NTD. The study was a randomized double-blind trial conducted at thirty-three centres in seven countries over 8 years and showed that folic acid had a 72% protective effect in preventing the recurrence of NTD among nearly 1200 women with a previously-affected pregnancy. In a subsequent study, Czeizel & Dudas (1992) confirmed that folic acid prevents not only the recurrence of NTD but also their occurrence (i.e. occurring to women for the first time). Thus, these intervention trials, together with retrospective case-control studies examining the effect on the prevalence of NTD of folic acid (in supplements) and folate (in food) as reviewed recently by Scott *et al.* (1995), showed clearly that the vitamin had a protective effect against both recurrence and occurrence of NTD. The mechanism whereby folate and/or folic acid protects against NTD appears to be by way of overcoming a partial block in folate metabolism, rather than correcting a nutritional deficiency (Whitehead *et al.* 1995). These authors provided the first direct evidence of a genetic explanation for NTD by identifying that a functional variant of the gene for 5, 10-methylenetetrahydrofolate reductase (*EC* 1.7.99.5) was associated with NTD.

While the mechanism involved continues to be investigated, the (UK) Department of Health (1992) have published folic acid recommendations for the prevention of NTD. Similar recommendations have been made by many other national committees worldwide. To prevent recurrence of NTD, the recommendation is 5 mg folic acid/d in tablet form, while 400 µg/d is recommended for the prevention of NTD occurrence, to be commenced before conception and continued until the twelfth week of pregnancy. Although those with a previously-affected pregnancy have about a ten-fold increased risk of recurrence, the majority (95%) of all NTD cases are first occurrences (Department of Health, 1992). Thus, the latter group clearly represents the most serious problem. As a public health strategy, it is important to consider not just those women planning a pregnancy, since an estimated 50% of pregnancies are unplanned (Grimes, 1986; Department of Health, 1992) and the malformations of NTD occur during the fourth week post-conception, usually before confirmation of pregnancy. Thus, any intervention aimed at implementing these recommendations should be targeted at all women of child-bearing age, so that optimal periconceptual folate status will be achieved in those who may become pregnant.

Although the strategy for the prevention of recurrence of NTD is clearly folic acid supplementation, the Department of Health (1992) suggests three possible ways in which the recommendations for the prevention of first occurrence of NTD can be achieved: (1) increased intake of foods naturally rich in folate, (2) folic acid supplementation, (3) folic acid fortification. Supplementation, while highly effective in optimizing folate status in women who receive it (Cuskelly *et al.* 1996), is unlikely to reach a major proportion of the target population (i.e. women in general before conception). Despite considerable

publicity, a recent study of 411 pregnancies in a London hospital indicated that only 3% of women had taken folic acid before conception (Clark & Fisk, 1994). Increasing food folate alone would require a threefold increase in current intakes, but more importantly, even when a significant increase is achieved experimentally it appears to be relatively ineffective in optimizing folate status, whereas comparable intakes of the vitamin as fortified food result in a significant increase in erythrocyte folate levels (Cuskelly *et al.* 1996). Thus, folic acid fortification offers the most promising intervention strategy, with the expected benefit of achieving a major reduction in the incidence of NTD. The issue of greatest concern which has prevented the implementation of adequate folic acid-fortification has been the possibility that the diagnosis of vitamin B<sub>12</sub> deficiency in the elderly might be delayed by the administration of folic acid as a result of a remission of the anaemia of vitamin B<sub>12</sub> deficiency, with progression of the associated neuropathy (Savage & Lindenbaum, 1995). Whether such concerns are justified at fortification levels of intake is the subject of great debate (Rush, 1994; Bower & Wald, 1995), and must be addressed urgently so that public health measures for the prevention of NTD can be implemented as soon as possible. Maternal erythrocyte folate concentrations above 400 ng/ml are associated with lowest risk of NTD (Daly *et al.* 1995). The key to the fortification debate will be the determination of the minimal effective level of intake of folic acid required to achieve erythrocyte folate concentrations in this range in women of child-bearing age, which may well be lower than the official increment of 400 µg/d over usual dietary intakes (Department of Health, 1992). In the meantime, mandatory folic acid fortification is to be introduced in the USA in the near future (Foulke, 1996), but the decision to fortify with folic acid in the UK lies with individual food manufacturers on the basis of voluntary recommendations to bread and breakfast-cereal manufacturers to do so (Department of Health, 1992).

#### *Folate requirement for pregnancy*

Whilst folate status up to 28 d post conception is critical to the normal closure of the neural tube and the prevention of NTD, folate status throughout pregnancy has other important implications for maternal, fetal and neonatal health. Considerable evidence indicates that suboptimal folate status is associated with poor health outcome of the mother and the newborn. Goldenberg (1992) reported a positive relationship between folate status, birth weight and reduced risk of fetal growth retardation in the US. Folate-responsive megaloblastic anaemia occurs in up to 24% of unsupplemented pregnancies in certain parts of Asia, Africa, Central and South America and in 2.5–5.0% of those in the developed world (Chanarin, 1985). Bone-marrow megaloblastosis, indicative of subclinical deficiency, occurs in as many as 25% of women not receiving supplements in otherwise well-nourished societies (Chanarin, 1985). Serum and erythrocyte folate levels decline throughout pregnancy (Hall *et al.* 1976), but this fall is not seen where folic acid supplementation is introduced or where there is sufficient dietary folate to meet the increased requirements of pregnancy (Ek & Magnus, 1981). Thus, pregnancy is a physiological state which imposes a folate stress that may or may not result in folate deficiency depending on the mother's nutritional status.

The current UK RNI for folate during pregnancy is set at 100 µg/d over and above that of non-pregnant women, based on early studies showing that this amount of extra vitamin was required to maintain plasma and erythrocyte folate levels at or above those of non-pregnant women (Department of Health, 1991). This approach may be misleading, in that it could be argued that the concentrations of a number of other water-soluble vitamins also

decline during pregnancy as a result of haemodilution, and that if the expansion of plasma volume is considered the net decrease in folate levels is minimal (Picciano *et al.* 1994). Conversely, the RNI for folate during pregnancy may be an underestimate of true requirements, as suggested by McPartlin *et al.* (1993) who showed an increased rate of folate breakdown (catabolism) during pregnancy in human subjects, consistent with earlier observations in the rat (McNulty *et al.* 1993). The human studies showed that the amount of folate being catabolized in pregnant women compared with non-pregnant women corresponded to an extra demand for dietary folate of between 200 and 300 µg/d, considerably greater than the above increment of 100 µg/d to cover the increased requirements of pregnancy. Because the daily rate of folate catabolism represents the inescapable use of the vitamin in metabolic activity (and, thus, the minimum amount which must be replaced by the diet on a daily basis), the determination of excreted urinary catabolites (McPartlin *et al.* 1992) during pregnancy and at other stages of the life cycle may offer an alternative basis for establishing folate requirements in the future.

#### *Folate requirement for lactation*

Knowledge of folate requirements during lactation is limited. In spite of this, there is good consistency among current folate recommendations for lactation (Table 3), probably because a similar approach has been used by the official committees which generated them. Recommendations are based on the recommended level for non-pregnant, non-lactating women plus the estimated folate intake required to replace the quantity lost in milk. There are a number of reasons why official recommendations for lactation are considered to be underestimated (Picciano, 1995). First, more recent evidence indicates that milk folate secretion during early lactation is approximately 100 µg/l (O'Connor *et al.* 1991), considerably greater than that previously assumed. Second, in determining the dietary folate required to replace that lost in milk, an absorption rate of up to 70% has been assumed, but the most conclusive estimate of overall bioavailability of folate from food sources indicates a value of no more than 50% (Sauberlich *et al.* 1987). Finally, available evidence, as reviewed by Picciano (1995), suggests that folate is preferentially partitioned to mammary tissue, indicating that maternal folate status can be compromised during lactation despite adequacy of milk folate content and/or folate status of the breast-fed infant. Only in severe deficiency does maternal folate status appear to be related to milk folate content. Thus, the amount of folate required to maintain maternal folate status during lactation is unknown and more detailed studies relating folate intake to haematological folate status at various stages of lactation are clearly needed.

#### *Folate status and cardiovascular disease in post-menopausal women*

The importance of folate status in women's health is not entirely related to its role in reproduction. In recent years attention has focused on the role of homocysteine as a risk factor for the development of vascular disease in the general population. Mild hyperhomocysteinaemia (elevated plasma homocysteine concentration) is now recognized to be an independent risk factor for cardiovascular disease (CVD; Boushey *et al.* 1995). In one study, the prevalence of hyperhomocysteinaemia was reported to be 30% among patients with CVD, and the risk of premature occlusive vascular disease was about thirty times greater for people with hyperhomocysteinaemia relative to normal controls (Clarke *et al.* 1991). Men have significantly higher homocysteine levels than premenopausal women, but levels appear to increase after the menopause in women, so that postmenopausal

women attain similar levels to men of the same age (Ueland *et al.* 1992). Age- and sex-related differences in plasma homocysteine appear to parallel the risk of CVD and may reflect hormonal influences on homocysteine metabolism which offer premenopausal women a degree of protection against CVD (Boers *et al.* 1983). Several lines of evidence suggest that elevated homocysteine is a pathogenic factor and not merely a marker of increased CVD risk. A variety of conditions can lead to elevated homocysteine levels, but the association between high levels and vascular disease is present regardless of the underlying cause (Stampfer & Manilow, 1995). As reviewed by Ueland *et al.* (1992), vascular lesions have been induced in animals infused with homocysteine.

Intracellular homocysteine is metabolized by either the trans-sulphuration pathway (requiring vitamin B<sub>6</sub> as a cofactor) or by remethylation to methionine (requiring both vitamin B<sub>12</sub> and 5-methyltetrahydrofolate as coenzyme and co-substrate respectively). A strong inverse relationship exists between plasma homocysteine and folate (and to a lesser extent, vitamin B<sub>12</sub> and vitamin B<sub>6</sub>) status (Selhub *et al.* 1993). Thus, marginal deficiencies of folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> are associated with hyperhomocysteinaemia (Ubbink *et al.* 1993), indicating that effective homocysteine metabolism requires an adequate supply of all three vitamins. Several studies have demonstrated that the administration of folic acid at high doses (typically 1–5 mg/d), either alone or in combination with vitamins B<sub>6</sub> and B<sub>12</sub>, can lower plasma homocysteine levels in both healthy and hyperhomocysteinaemic subjects, even in the absence of overt folate deficiency (Ueland *et al.* 1992). A recent study by Selhub *et al.* (1995) identified inadequate folate intake as the main determinant of homocysteine-related increase in carotid-artery stenosis. Earlier the same group (Selhub *et al.* 1993) had demonstrated that mean homocysteine concentration reached a stable low level only with total folate intakes of approximately 400 µg/d or more, a level of twice the current US recommendation for men and non-pregnant women, and ironically equal to that previously recommended (Table 3). The most convincing evidence of the potential role of folate intake in the prevention of vascular disease has been provided by a recent study showing a statistically significant inverse relationship between serum folate level and fatal coronary heart disease (Morrison *et al.* 1996). The authors reported a 69% increased risk of coronary mortality among those in the lowest quartile, as compared with the highest quartile of serum folate. Interestingly, the magnitude of the protective effect of folate appeared to be greater for women than for men, although the difference in risk between the sexes was not significant.

Thus, some post-menopausal and elderly women may well be at risk of CVD as a result of elevated homocysteine. Folate may have an important role in its prevention. While randomized controlled trials will address whether the treatment of elevated homocysteine levels by vitamin supplementation will affect the incidence and severity of vascular disease, the prevention of hyperhomocysteinaemia in the general population should be considered. It remains to be established whether low-dose folic acid intervention (e.g. in the range 0.1–0.4 mg/d) can lower plasma homocysteine level. This is an important question from a public health viewpoint since such levels represent those which would be achievable through folic acid-fortification.

#### *Folate status, cancer and women's health*

Low folate status has been associated with increased risk of various cancers for many years and much of the early work in this field has been established in the uterine cervix. Van Niekerk (1966) reported several cytological similarities between epithelial cells of the cervix from folate-deficient women and cervical cells that were dysplastic (i.e. pre-

cancerous). It was not until the 1980s, however, that evidence of a functional association between megaloblastic and dysplastic cells was published. In a prospective controlled intervention trial women with dysplastic changes in the cervical epithelium showed a significant degree of regression in dysplasia in response to pharmacological doses of folic acid (Butterworth *et al.* 1982). However, a subsequent intervention trial by the same group (Butterworth *et al.* 1992a) carried out for a longer period of time in a larger group of women failed to reproduce the attenuation of dysplasia noted by the earlier study. A recent case-control study designed to examine folate status and multiple other risk factors for cervical dysplasia showed that subjects who had human papilloma virus infection of the cervix (an important risk factor for cervical dysplasia) had several-fold greater risk of having dysplasia if they also had diminished erythrocyte folate levels (Butterworth *et al.* 1992b). Thus, there may be a synergism between diminished folate status and human papilloma virus infection, suggesting that whatever enhancement of cervical carcinogenesis is affected by low folate status requires concurrent factors predisposing towards carcinogenesis (Mason, 1995). As recently reviewed, several case-control studies have examined an association between dietary folate intake and cervical neoplasia (Mason, 1995). While relative risk of cervical neoplasia appears lower in women consuming higher intakes of folate, in no study were these differences significant. It is argued, however, that since these case-control studies suffer many limitations (not least of which is the fact that many studies used food-frequency questionnaires not designed to estimate folate intake), the lack of significant effects of folate intake on cervical neoplasia does not necessarily imply that no effect exists (Mason, 1995). Thus, at present the evidence that folate status can modulate the process of carcinogenesis of the cervix is inconsistent.

Evidence that diminished folate status is associated with increased incidence of colonic carcinogenesis is supported by several epidemiological studies involving both men and women (Mason, 1994). The effect has been observed with relatively modest alterations in folate status, and may even be present when vitamin status is altered within the range of what is presently considered to be normal. A controlled laboratory study in an animal model of colo-rectal cancer (Cravo *et al.* 1992), demonstrating that moderate folate deficiency enhanced the development of colonic dysplasia and cancer, has provided considerable evidence of a cause-and-effect relationship between folate deficiency and colo-rectal cancer. The mechanisms involved are not clearly understood, but lowered folate status may induce hypomethylation of DNA, thereby promoting carcinogenesis (Mason, 1995). The potential role of folate in the prevention of colo-rectal and other cancers is under intense investigation.

In summary, the importance of folate to women's health is not limited to its role in the prevention of NTD. Evidence suggests that there are a number of stages in the life cycle when a woman may either be at risk of folate deficiency through increased requirements (pregnancy, lactation), or may in some way benefit from intakes of the vitamin over and above levels typically present in the diet (women in their reproductive years, postmenopausal women, elderly women). Current folate recommendations for women are likely to be underestimated, but their revision will not, in itself, solve the problem. Fortification offers the only effective means of achieving increased intakes in all women. A fortification policy which ensures effective, but safe levels of intake should be implemented as soon as possible.

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