

Importance of folate in human nutrition

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From a public health perspective, some of the new insights into folic acid nutrition are of significance. Folate intake recommendations vary under different conditions. Intake of 350 µg is required to maintain plasma homocysteine levels, 650 µg for those with elevated plasma homocysteine, about 400 µg for women planning to become pregnant and 4000 µg for those with history of neural tube defect affected pregnancy. This raises the question whether the folate intake is adequate for the general population, particularly in the vulnerable groups or whether there is a need for scientists to take a fresh view of the requirements, recommended dietary intakes, and consider intervention measures which will have impact on the folate nutritional status. The recommendations should provide a margin of safety to allow for decreased intake, increased requirements, individual variability and bioavailability of natural food folates. The folate intake and nutriture in relation to India and other developing countries needs careful consideration to reduce anemia, neural tube defects and possibly impact on the high incidence of cardiovascular diseases.

Folate: Homocysteine: Health and disease: RDA: India

Folate is the generic term for compounds that have vitamin activity similar to that of pteroylglutamic acid and is an anti-anemic and growth factor. Folate acts as a co-enzyme in several single carbon transfers leading to the biosynthesis of purine nucleotides and deoxythymidylic acid essential for DNA and RNA synthesis. In general, rapidly growing and multiplying cells require an adequate supply of folate. As it participates in several biochemical functions, it is important to consider conditions that increase the risk of folate deficiency and its consequences.

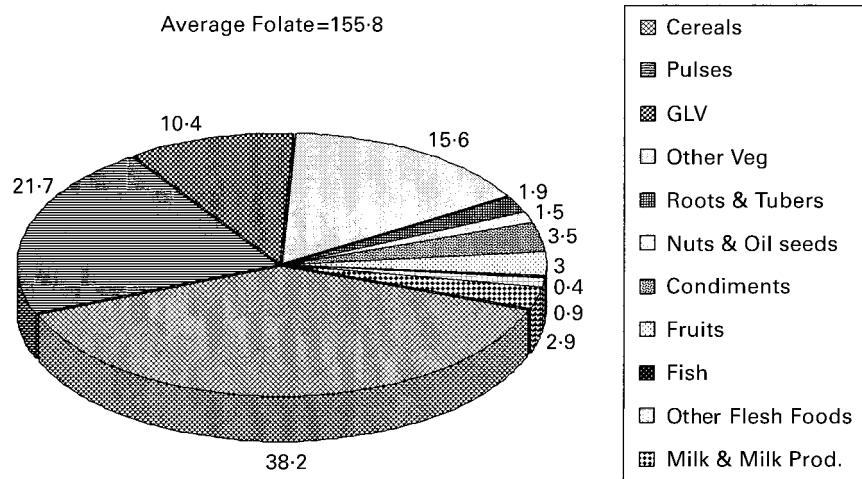
Food folate — digestion, absorption and availability

Folate exists in many chemical forms (Wagner, 1996). Mammals are unable to synthesize a pteridine ring, one of the three groups of tetrahydrofolate; the other two groups are p-aminobenzoate and glutamate. They obtain tetrahydrofolate from their diets or from microorganisms in their intestinal tract. Naturally occurring folates, called food folate (polyglutamyl chain) are present both in animal and plant foods. Organ meats such as liver and egg yolk are fairly good sources of folate and in the Indian context the major sources are legumes and green leafy vegetables. The intake of folate based on the NNMB (1999) suggested that as per the existing recommendations more than 60 % of folate nutrition is met from cereals and pulses (Fig. 1).

The intestinal absorption of folate occurs at the monoglutamyl level. Therefore dietary folates, most of which are polyglutamyl derivatives, undergo hydrolysis in the gut to monoglutamates before absorption, across the intestinal mucosa (Butterworth *et al.* 1969). In the absence of protecting agents such as ascorbic acid and reduced thiols, many labile folates may be lost during residence in the acid peptic milieu of the stomach. Other food constituents affect action of the intestinal hydrolases. Absorption of monoglutamate predominantly occurs in the duodenum and jejunum by an active transport in a saturable pH dependent process. By far the majority of intracellular folates are polyglutamates. In contrast, extracellular folates are monoglutamates (Cossins, 1984). For folate absorption, luminal digestion to the monoglutamyl form by an intestinal enzyme known as folate conjugase found in the brush border is the rate limiting step. This enzyme is an exopeptidase and is activated by zinc (Chandler *et al.* 1986). A second enzyme, intracellular hydrolase, is found in the lysosomes of intestinal cells, has a pH optimum of 4.5 and is an endopeptidase (Wang *et al.* 1986). The function of the former is believed to be primarily in the digestion of dietary folates and that of the latter is still unknown. Monoglutamates are present in portal circulation, which is taken by the liver and converted to polyglutamate derivatives and stored or released into the

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; AUC, area-under-the-plasma-response-curve; RBC, red blood cells; NTD, neural tube defects; CAD, coronary artery disease; Hcy, homocysteine; HDL, high density lipoprotein; MTHFR, methylenetetrahydrofolate reductase; FDA, Food and Drug Administration, United States of America; EAR, estimated average requirement; DRI, dietary reference intakes; RDA, recommended daily allowances; DFE, dietary folate equivalents; RDI, recommended dietary intake.

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Source: NNMB Repeat Survey 1996-97

Fig. 1. Folic acid distribution from different foods ($\mu\text{g}/\text{CU}$).

blood. In plasma, folate is bound primarily to albumin. Cellular transport of folate is mediated through a number of different folate transport systems.

Information on the availability of food folate is fragmentary. The paucity of information stems primarily from the complexity of dietary folates, their low levels, presence of dietary inhibitors and the limited ability to distinguish between newly absorbed folate and endogenous folate. Seyoum & Selhub (1998) developed an *in vitro* method based on the stability and susceptibility to intestinal pteroylpolylglutamate hydrolase action. Folate availability generally varies widely among foods. The maximum availability is found in egg yolk (72.2 %) followed by cow's liver (55.7 %); there is a low availability from vegetables (3–6 %). The absorption of folate from foods varies from 25 % to 70 % with a mean close to about 50 % (Tamura & Stokstad, 1973; Babu & Srikantia, 1976).

Synthetic folic acid (a stable oxidized form of pteroylmonoglutamate, often used to correct folate deficiency) is considered to be more bioavailable than natural folate. The bioavailability of supplements of folic acid under fasting conditions are close to 100 % (Gregory, 1997). Folic acid absorption in both pregnant and non-pregnant Indian women was studied by Iyengar & Babu (1975) using ^3H folic acid and 2 mg of cold folic acid. The absorption of folic acid varied from 72 % to 94 % ($n = 9$) with a mean absorption of 82 % which was similar to that obtained in non-pregnant women ($n = 6$). On the other hand, food folate is less bioavailable than the synthetic form (Sauberlich *et al.* 1987; Cuskelly *et al.* 1996). It has been recently demonstrated based on plasma folate that the bioavailability of folate from spinach can be improved by disruption of the vegetable matrix (vanhet Hof *et al.* 1999).

Currently, there are many accurate methods of estimating the food folate level and their bioavailabilities. The procedures for the various methods of food folate determination have been reviewed by Tamura (1998). It is now well established that the 'trienzyme method' namely alpha-amylase and protease for the extraction of folate

trapped in or bound to protein and carbohydrate matrices and the enzyme folate conjugase for the hydrolysis of polyglutamyl folate, be used to obtain the actual maximum content of folate in each food item. According to Prinz-Langenohl (1999) the AUC method with multiple blood sampling is useful for assessing the availability of food folate in humans. Using this method a significantly greater absorption of 400 μg of folic acid than with the same amount of food folate was reported in normal pregnant women (Neuhouser *et al.* 1998). The use of a single dose, dual-label, stable-isotope method was employed by Pfeiffer *et al.* (1997) to assess the absorption of folic acid from fortified cereal grain products. Isotope excretion ratios of urinary folates that were used as criteria of availability, indicated that the folic acid in these foods was highly bioavailable. Thus it is evident that there is a need to augment research in this area in India not only to generate reliable food folate tables but also its availability to Indians using these techniques.

Losses of food folate

Most natural folates are unstable and destroyed during various cooking procedures and also by oxidation and ultraviolet light (O'Broin *et al.* 1975; Dawson & Waters, 1994). Food processing can cause considerable losses of folate for example in canning, in prolonged heating, when cooking water is discarded and from reheating. Reducing agents in food such as ascorbic acid provide greater stability to the vitamin. Indian cooking practices such as heating for prolonged periods in open vessels probably destroy and reduce food folates considerably. Germination, however, increases food folate availability (Babu, 1976a).

Folate status

Biochemical assessment of folate nutritional status is most commonly done by measuring serum and RBC folate levels. Serum folate essentially reflects recent intake and

Table 1. Folate nutriture of Indians†

| | No. | RBC folate nmol/L | Cutoff level nmol/L | Per cent deficient |
|-----------------------------------|-----|-------------------|---------------------|--------------------|
| Men (25–50 y) | 45 | 323 ± 97 | 180 | 8 |
| Women (25–50 y) | 25 | 371 ± 169 | – | – |
| Pregnant women | 43 | 309 ± 81 | – | – |
| <16 wk | 60 | 310 ± 153 | 284 | 53.3 |
| 17–20 wk | 77 | 337 ± 26 | 284 | 50.6 |
| 21–24 wk | 84 | 348 ± 175 | 284 | 40.5 |
| 25–28 wk | 30 | 347 ± 185 | 284 | 50.0 |
| Pregnant women (1 & 2 Trimesters) | 115 | 287 ± 117 | 284 | 25 |
| Children (1–5 y) (Boys & girls) | 246 | 267 ± 90 | 180 | 20 |

Values are means ± SD.

† Ahmed *et al.* 1975; Babu, 1976b; Neela & Raman, 1997; Raman *et al.* 1989.

red cell levels represent long term tissue stores. It is generally accepted that serum folate values above 14 nmol/L or RBC folate 360 nmol/L are considered to be satisfactory; those between 7–14 nmol/L serum folate and 320–360 nmol/L RBC folate are considered to be low and suggestive of risk. Values lower than these indicate clear deficiency of folate. Normal body stores are estimated at 5–10 mg, half of which is in the liver. The turnover rate is estimated to be less than 1 % per day. The measurement of plasma concentrations of homocysteine is shown to be a good indicator of folate and vitamin B₁₂ status. A striking negative correlation between serum folate and protein bound homocysteine is reported (Kang *et al.* 1987).

Folate nutrition in Indians

There are no detailed studies on folate nutritional status in the Indian population. However, there are some studies which have been conducted in pregnant women (Raman *et al.* 1989; Neela & Raman, 1997) indicating that folate status may be poor in these vulnerable segments of the population (Table 1). The folate nutriture in pregnant women of low-income group who had not received any antenatal care was reported by Raman *et al.* (1989). Based on RBC folate levels, the folate deficiency during different gestational periods ranged between 40.5 % and 53.3 %. Considering the lower cut off used for defining per cent deficient folic acid, these figures could be an underestimate of the actual prevalence existing in poor communities.

Poor folate content of human fetal liver was reported by Iyengar & Apte (1972). The mean total liver folate value increased from 95 µg to 262 µg depending on the age of the fetus (<28 to 37–40 wk). A study was carried out in pregnant women belonging to the poor sections of the population attending a local government hospital receiving 60 mg iron with or without folic acid and followed up from 20 to 24 weeks of pregnancy to term and 6 weeks postpartum. The red cell folate levels decreased from 329 ± 129 nmol/L at term to 256 ± 118 nmol/L postpartum in women who did not receive folic acid supplement. The levels of red cell folate were not different from the initial value in the group which received 100 µg folic acid (*n* = 21). Significantly higher levels were observed only

with 200 µg (*n* = 25) and 300 µg (*n* = 23) of folic acid supplementation; the increments were 91 nmol/L and 195 nmol/L respectively (Iyengar, 1971). A subsequent study with 500 µg folic acid given with 60 mg iron showed a reduction in the per cent of small for date births (<2.5 kg) from 30 % to 16 % (Iyengar & Rajalakshmi, 1975). The red cell folate levels in infants born to mothers who received 300 µg of folic acid were higher than those born to the control group. The birth weights of infants born to mothers who had received either 200 or 500 µg folic acid daily were higher than those born to mothers who had not received any supplements (Iyengar & Rajalakshmi, 1975). The above studies clearly indicate folate deficiency in the population.

Etiology of folate deficiency

Folic acid deficiency can arise in a variety of settings such as low dietary intake, increased demands during growth periods (infancy and puberty), lactation and pregnancy, malabsorption, conditions of hemolysis such as hemolytic anemias and malignancy such as leukemias. Drug induced folate deficiency is encountered in epileptics, ulcerative colitis, etc. Chronic alcoholism is associated with folate deficiency. Advanced folate deficiency, characteristically produces macrocytic or megaloblastic anemia with hematological features similar to vitamin B₁₂ deficiency. However, mucous membrane lesions and other clinical manifestations such as neural tube defects (Lemire, 1988) or more recently, hyper homocysteinemia with characteristic vascular damage are well-recognized (Graham *et al.* 1997). Additional nutritional stress in pregnancy and during infancy appears to be an important clinical problem that is of public health significance. Folic acid may also be associated with a variety of pregnancy complications such as spontaneous abortions, bleeding, abruptio placenta and pre-eclampsia.

The two important diseases in relation to folate status are neural tube defects (NTD) and homocysteinemia. In recent years, interest in plasma homocysteine has been growing rapidly. Epidemiological data linking moderate elevations in plasma homocysteine to higher prevalence of occlusive

vascular diseases such as coronary artery disease (CAD) and stroke are now emerging.

Consequences of homocysteinemia

Folic acid is essential for the methylation of homocysteine (Hcy) to form methionine and the biosynthesis of deoxynucleotides needed for DNA replication. Increased Hcy has been associated with premature vascular disease (Boushey *et al.* 1995; Graham *et al.* 1997) and decreased lymphocyte DNA methylation in postmenopausal women (Jacob *et al.* 1998). Homocysteine has been implicated in mental retardation in children, developmental defects, occlusive disorders, osteoporosis and dislocated lenses with lack of cystathionine betasynthase in the homozygous state.

There is evidence that hyperhomocysteinemia is an independent risk factor for ischemic heart disease (Wald *et al.* 1998). The relative increase in risk of coronary heart diseases for men and women respectively was 1.6 and 1.5 for each increment of 5 $\mu\text{mol/L}$ in total plasma Hcy (National Academy of Sciences, 1998; Boushey *et al.* 1995). These findings are in agreement with the Norwegian Study relating prospective cardiovascular mortality risk to elevated Hcy levels (Nygard, 1997).

The recent study on lowering blood Hcy with 400 μg folic acid based supplements indicated that a daily intake of 400 μg of folate and 3 mg vitamin B₆ may potentially help reduce cardiovascular mortality (Rimm *et al.* 1998).

What is the current scenario in India?

There are no systematic studies on plasma Hcy levels. The two preliminary studies on subjects with CAD show that Hcy is not a strong risk factor (Chacko, 1998; Gheye *et al.* 1999). In both the studies the subjects belonged to the middle socio-economic class. Serum Hcy levels were similar in CAD patients and controls. The Hcy levels varied from 2.98 to 63.36 $\mu\text{mol/L}$ (10.98 ± 9.04) in fifty-six CAD patients and 3.74 to 19.62 $\mu\text{mol/L}$ (9.41 ± 3.60) in fifty-three control subjects (Chacko, 1998). Though the mean (SE) total Hcy levels of 21.5 ± 2.33 ($n = 58$) and 19.7 ± 1.87 $\mu\text{mol/L}$ respectively in CAD patients and in age and socio economic status matched controls was comparable (Gheye *et al.* 1999), they were higher than the upper cut off of the normal value of 7–17 $\mu\text{mol/L}$ reported for the Western population (Jacobsen, 1996). There is evidence of greater vulnerability of South Asian migrants in US to CAD and diabetes as compared to the native populations. This increased susceptibility is reflected by the prevalence of syndrome 'X' characterized by abdominal obesity, hyperinsulinemia, hypertriglyceridemia, low concentration of HDL and hypertension. The high risk of CAD in this population group is probably due to 'X' syndrome, a consequence of fetal malnutrition (Gopalan, 1994). The role of hyperhomocysteinemia as a risk factor in India needs to be investigated.

Etiology of homocysteinemia

Homocysteine levels in plasma are determined by nutritional, physiological, genetic and pathological factors (Welch & Loscalzo, 1998).

Nutritional factors

Three vitamins of the B-complex group — folate, vitamin B₁₂ and riboflavin are involved in the different reactions of Hcy metabolism. It is therefore, conceivable that the deficiencies of these vitamins may influence the levels of plasma Hcy. Well-documented evidence exists only for folate. Studies in both animals and humans have shown that folate deficiency is associated with marked elevation in plasma Hcy levels (normal range 7–17 $\mu\text{mol/L}$) without evidence of megaloblastosis and hypersegmentation of neutrophil (Jacob *et al.* 1994). A strong negative correlation between folate status and Hcy levels in plasma has been observed. Subjects with even marginal deficiency of folate (<7 nmol/L serum folate) have been found to have elevated levels of Hcy suggesting that folate requirements to correct this abnormality may be higher (Jacob *et al.* 1998). Elevated levels of plasma Hcy were also reported in those over 80 years of age than in younger subjects (Selhub *et al.* 1993; Tucker *et al.* 1996). Several recent studies show reduction in Hcy levels on folate supplementation, both in homocysteinemic subjects as well as normals (Homocysteine Lowering Trialists' Collaboration, 1998; Dierkes *et al.* 1998; Malinow *et al.* 1998). These results suggest that folic acid fortified cereals and supplements can certainly decrease the plasma Hcy levels.

Physiological factors

Sex difference exists in plasma Hcy levels. Young women (premenopausal) tend to have 25 % lower levels than men. This difference decreases after menopause, perhaps suggesting beneficial effects of female sex hormones. A strong correlation between plasma estradiol levels and Hcy in post-menopausal women has been reported (Wouters *et al.* 1995). There is a progressive decrease in plasma Hcy levels during pregnancy but the exact mechanism for this reduction is not known. This has been attributed to lower plasma albumin that binds Hcy and/or increased cortisol level during pregnancy. Male sex and older age are thus associated with hyperhomocysteinemia.

Genetic factors

Methylenetetrahydrofolate reductase (MTHFR) catalyses the irreversible conversion of 5–10 methylene tetrahydrofolate to 5-methyl tetrahydrofolate. Homozygous deficiency of MTHFR due to a new type of point mutation of the thermolabile MTHFR has been reported (Kang *et al.* 1988; Ma *et al.* 1997; Choi *et al.* 1999). A common polymorphism of this gene, a specific C-T substitution at nucleotide position 677 of the MTHFR gene (C677T) causes the thermolability and reduced activity of this enzyme. About 10 % of the urban Canadian population has this defect suggesting a high frequency mutation (Frosst

et al. 1995). These subjects have a 50 % reduction in the activity of this enzyme and a tendency for higher levels of plasma Hcy, particularly in individuals with low folate nutritional status.

Life-style factors

A life-style profile characterized by low folate intakes, smoking and high coffee consumption were the strongest determinants of plasma Hcy concentrations. The folate deficient tissues are thought to be more susceptible to neoplastic transformation by carcinogens in the smoke (Heinburger *et al.* 1988).

Drugs

Methotrexate is an antifolate drug that is widely used in the treatment of acute leukemia and some solid tumors. Low doses of this drug are also used in the management of psoriasis and rheumatoid arthritis. Methotrexate is metabolized intracellularly to polyglutamates and both the parent drug and its metabolites inhibit dihydrofolate reductase, the enzyme responsible for the conversion of dihydrofolate to tetrahydrofolate. This results in the lower folate level and impairment of folate functions at cellular levels. Similar observation was made in children with acute leukemia. Initially plasma Hcy levels were high and chemotherapy with methotrexate as well as other cytotoxic agents reduced Hcy level parallel to reduction in leukemic cell count (Ueland & Refsum, 1989).

Anticonvulsant drugs like phenytoin, phenobarbital, premedial, carbamazepine cause folate deficiency (Elsborg, 1974; Collins *et al.* 1988; Young & Ghadirian, 1989). The proposed mechanisms include reduced folate absorption, increased metabolism of folate in the liver and altered activities of some of the enzymes involved in one carbon transfer.

Folic acid and cancer

Folic acid has been used as an antagonist for cancer therapy for the last 40 years. An issue of interest is whether folic acid deficiency is etiologically related to cancer specific sites such as colorectal cancer (Giovannucci *et al.* 1993; Choi & Mason, 2000). Folate deficiency is highly prevalent in many developing countries where the incidence of colorectal cancer is low, implying that other environmental factors are probably responsible to enhance cancer risk. There are studies to indicate that low red cell folate is associated with increased risk of cervical dysplasia especially carcinoma *in situ* (Butterworth *et al.* 1982). High dietary intakes of folate along with vitamin C and carotene have been observed to decrease the risk of carcinoma *in situ*.

Neural tube defects (NTD)

Spina bifida and anencephaly are birth defects of multifactorial etiology related to neural tube closure. Anencephaly is characterized by the absence of forebrain and spina bifida is characterized by incomplete fusion of the vertebral

arches with protruding sac with meninges, spinal cord and nerve roots. The role of folic acid in reducing the rate of NTD has been well-established (National Academy of Sciences, 1998). Pre-conceptional administration of folic acid can reduce women's risk of having a fetus or infant with a NTD. Several observational studies and randomized trials suggest that peri-conceptional folic acid supplementation lowers the risk of neural tube defects and currently, the consensus is that all women of child bearing age must consume 400 µg of folic acid per day to reduce their risk of having a child with NTD (Berry *et al.* 1999; Cuskelly *et al.* 1996; de Bree *et al.* 1997; Scott *et al.* 1994).

Prevalence

There is significant geo-ethnic variation in the prevalence of NTDs worldwide (Table 2). In United States a decrease in the prevalence of NTDs at birth has been reported. The current rates vary from 0.72 to 1.03 per 1000 births in different states (National Academy of Sciences, 1998). It has been pointed out that anencephaly is relatively uncommon in people with African origin while it is very common in Northern Ireland and Wales (6.38 to 10.9/1000 births) (Lemire, 1988). The other population with high rates of NTDs includes the Northern Chinese population (Berry *et al.* 1999). The difference in the NTD in northern and southern regions of China suggests that factors other than ethnic background may have a role.

In India, large-scale epidemiological studies have been conducted to determine prevalence of NTD (Table 2). In general, the prevalence of NTDs is considerably high and varies in different parts of the country (Sharma & Gulati, 1992; Agarwal, 1998). Southern India and the Western parts of the country apparently have lower rates except Davangere in Karnataka where the prevalence is quite high at 11.4/1000 births (Kulkarni *et al.* 1989). Data from a teaching hospital in Lucknow indicates a prevalence of 4.7/1000 births (Agarwal *et al.* 1991).

Table 2. Frequency of neural tube defects in India, USA, Canada, UK and China

| India† | NTD 1000 births | USA and Canada‡ | NTD 1000 births |
|------------|-----------------|------------------|-----------------|
| Amritsar | 8.0 | Arkansas | 1.03 |
| Jaipur | 5.6 | Atlanta | 0.99 |
| Udaipur | 7.8 | California | 0.94 |
| Chandigarh | 12.0 | Iowa | 0.90 |
| Delhi | 4.1 | Hawaii | 0.92 |
| Lucknow | 4.7 | Quebec | 1.41 |
| Rajasthan | 18.0 | | |
| Ajmer | 6.6 | | |
| Calcutta | 1.1 | UK§ | |
| Patna | 5.3 | Northern Ireland | 6.38 |
| Mumbai | 2.2 | Wales | 10.9 |
| Chennai | 2.3 | China¶ | |
| Mysore | 1.5 | Northern region | 6.5 |
| Davangere | 11.4 | Southern region | 0.8 |
| Wardha | 3.9 | | |

† Sharma & Gulati, 1992; Kulkarni & Jose, 1997; Kulkarni *et al.* 1989.

‡ National Academy of Sciences (1998). §Lemire 1988. ¶Berry *et al.* 1999.

Etiology

Etio-pathogenetically, NTD is a heterogenous entity and is multifactorial in origin (Dolk *et al.* 1991) with a strong genetic component (Emery, 1986). It is believed to be polygenic, probably involving multiple genes. The high prevalence of NTDs in certain parts of India has been attributed to high rates of consanguinity (Kulkarni *et al.* 1989). The dengue viral infection has also been suggested as a possible factor by Sharma & Gulati (1992) based on the cluster of NTDs that appeared in the Rohtak district of Haryana, India. Several other factors such as previous fetal loss, maternal epilepsy, teenage pregnancy, rubella, diabetes and poor socio-economic status have been reported to increase the risk of NTD. The incidence of NTD is high in the Sikh community (Stevenson *et al.* 1966; Chambers *et al.* 1994).

Among the environmental factors, nutrition has been identified as an important contributor to this disease. The lower the plasma folate, the higher is the number of NTDs, the picture with respect to erythrocyte folate is similar. The reduction in risk of neural tube defects by folic acid supplementation has been conclusively shown in controlled intervention trials (Scott *et al.* 1995; Locksmith & Duff, 1998). The mechanism by which adequate folate intake reduces the risk during the crucial developmental phase of the embryonic neural tube is unknown. Increasing folate intake, which increases the concentrations of folate co-enzymes in tissues, may overcome an unidentified metabolic defect at the time of neural tube development and closure (National Academy of Sciences, 1998).

Control of neural tube defects

Results of several double blind randomized trials support the beneficial effect of folic acid supplementation in reducing the incidence of NTD (Laurence *et al.* 1981; National Academy of Sciences, 1998). The Medical Research Council of Great Britain conducted an intervention study in women with previous history of NTD and showed a 72 % reduction in the risk of having an affected fetus or infant in women who took 400 μg folic acid (MRC Vitamin Study Research Group, 1991). A 30–40 % reduction in NTD-affected pregnancies among women whose average dietary intake of methionine was above the lowest quartile of intake (>1.34 g/d) was reported by Shaw *et al.* (1997). A recent study from China (Berry *et al.* 1999) suggests that the daily ingestion of 400 μg of folic acid alone during the periconceptional period may help reduce the rate of the first occurrence of NTDs in many parts of the world. The risk can be considerably decreased by increasing the folate intake to 400–500 μg while planning pregnancy and continuing upto the twelfth week of pregnancy. Periconceptional folate supplementation has been encouraged in the UK since the early 1990s, but no concurrent decline in NTD pregnancies has been observed by regional congenital anomaly registers (Abramsky *et al.* 1999).

Currently, food fortification is implemented for prevention of NTD (Bailey, 1998). Considering the strong association of folic acid and NTDs and low intake of

food folate of 110–140 $\mu\text{g}/\text{d}$ as against an RDA of 200 $\mu\text{g}/\text{d}$ by the women of child bearing age in the United States, fortification of cereal-grain products with folic acid is mandated by the FDA. It has been estimated that the level of folic acid fortification of 140 $\mu\text{g}/100$ g cereal-grain product recommended by the FDA would increase folic acid intake by 80–100 $\mu\text{g}/\text{day}$ in this group of women. Consequently, as of 1 January 1998 cereal-grain products in the US food supply are being fortified with folic acid to prevent NTDs (Malinow *et al.* 1998).

In India there are no such studies demonstrating the beneficial effects of folic acid supplementation on prevention of NTDs. The limited Indian experience also shows that folic acid can prevent NTD (Kulkarni & Jose, 1997). It is, however, pertinent to mention that a National Nutritional Anemia Control Programme for pregnant women has been in operation since 1970 which envisages a supply of 60 or 100 mg of iron with 500 μg folic acid per day for 100 days (Ministry of Health and Family Welfare, 1970; Indian Council of Medical Research, 1989; Indian Council of Medical Research, 1992). An evaluation in eleven States during 1985–86 indicated very poor coverage and performance of the programme. The folic acid content in these tablets was 35 % or less than the expected level (Vijayaraghavan *et al.* 1990). To what extent the above programme contributes to folate nutriture under the programmatic conditions is not known. Thus, there is a need to have countrywide data on folate status, food folate levels, and bioavailability to facilitate intervention measures.

Recommended dietary intake of folate

Folate requirements have been defined as the quantity of intake necessary to prevent severe deficiency with clinical symptoms (National Academy of Sciences, 1989). Currently, the focus has shifted to identifying intakes associated with maintenance of normal biochemical function such as one-carbon transfer reactions. Abnormalities identified in the metabolic pathways such as hyperhomocysteinemia, hypomethylation of DNA and uracil misincorporation are assigned as functional indicators of folate status (Selhub *et al.* 1993; Green & Jacobsen, 1995; Blount *et al.* 1997; Jacob *et al.* 1998). Risk reduction for chronic diseases and developmental defects (NTD) have also been used for deriving optimum folate intakes (Boushey *et al.* 1995; Daly *et al.* 1997). Stable isotopically labeled folate is also used in defining the folate requirement more accurately (Gregory *et al.* 1998).

Based on recent studies, the National Academy of Sciences (1998) have reported new dietary reference intakes (DRI) for folate. The DRI for folate includes folate requirement estimation for population groups referred to as the estimated average requirement (EAR). EAR is defined as the amount of folate that is needed to meet the requirement of 50 % of the population. The recommended dietary allowances (RDA) were derived from the EAR by correcting for population variance which is defined as the average level of daily dietary intake to meet the requirement of 98 % of the population. The basis for deriving EAR and RDA for folate was mainly based on the study of

Table 3. Recommended dietary intake (RDI) ($\mu\text{g}/\text{d}$)† for folate for the adult population

| Country | Minimum requirements | Individual variation | Bioavailability | Recommended | |
|-----------------|----------------------|----------------------|-----------------|-------------|---------|
| | | | | Male | Female |
| FAO/WHO | 60 | +15 % | +70 % | 200 | 170 |
| USA | | +20 % | +50 % | 320‡ | 320‡ |
| UK | 100 | +? | +50 % | 200 | 200 |
| The Netherlands | 50 | +200–300 % | +50 % | 200–300 | 200–300 |
| France | 50 | ? | ? | 300 | 300 |
| Germany | 150 | ? | +50 % | 300 | 300 |
| India | 75 | ? | +50 % | 100 | 100 |

† de Bree *et al.* 1997; National Academy of Sciences (1998); Indian Council of Medical Research, 1989. ‡Dietary folate equivalents (DFE).

O'Keefe *et al.* (1995). The DRIs for folate are expressed as Dietary Folate Equivalents (DFE), a term used to adjust for the generally higher bioavailability of synthetic folic acid relative to most forms of naturally occurring folate in foods (National Academy of Sciences, 1998; Bailey, 1998). It is estimated that 85 % of synthetic folic acid is bioavailable when consumed with food, while the bioavailability of food folate as such is only 50 % that of synthetic folic acid.

For male and female adults over 19 years of age, the folate EAR and RDA are 320 and 400 μg DFE/d respectively (Table 3). For pregnant women, the corresponding figures are 520 and 600 μg DFE/d. Subsequent studies with folate intake of 600 μg DFE/d were shown to be adequate to maintain normal folate status in pregnant women (Bonnette *et al.* 1998; Caudill *et al.* 1997; Caudill *et al.* 1998).

The current RDA for folate in Indians is based on the following literature evidences. The free and total folate levels in Indian foods have been determined microbiologically using *L. casei* as the organism (Gopalan *et al.* 1989). Studies on the actual availability of food folate are few. Dietary surveys in Indian subjects show that predominantly cereal based diets provide about 70 μg folic acid a day (NNMB, 1999). The bioavailability of folate from a typical Indian diet varied from 60 to 70 % in pulses and green leafy vegetables and about 50 % in vegetables (Babu & Srikantia, 1976). In a repletion-depletion study in Indian human volunteers, 75 μg of folate was found to be adequate to maintain optimal concentrations of folate both in serum and red blood cells (Babu, 1976b). Based on this, the safe level of folate intake was fixed at 100 $\mu\text{g}/\text{day}$. Due to lack of precise information on bioavailability of food folates, the ICMR Committee had recommended a RDA of 100 μg of free folate for adults (Table 4). The results of the study of Iyengar (1971) in Indian women have suggested that between 200 and 300 μg of folates are needed to maintain plasma and red cell folate in the normal range during pregnancy. Based on this, an amount of 400 $\mu\text{g}/\text{day}$ of additional folic acid is suggested for pregnant women. The estimated folate content of breast milk secreted by Indian women is 16 $\mu\text{g}/\text{L}$ (Ramasastry, 1965) which is twice as low as that reported from the west. Therefore, an additional allowance of 50 μg of folate is recommended (150 $\mu\text{g}/\text{d}$) for lactating women (Indian Council of Medical Research, 1989).

The dietary intake of folate in different countries varies considerably from 100 to 300 $\mu\text{g}/\text{d}$. The most recent Indian

data from the NNMB (1999) indicate that the mean folate intake is around 150 μg (Fig. 1, Table 4) and appears to meet the RDA. However, the intake of folic acid was less than the RDI of 200 $\mu\text{g}/\text{d}$ in all states, except Gujarat. The intakes are also much higher in other countries compared to India (Table 3).

Even though the minimum requirements are around 50 to 150 μg considering the factors such as individual variations and bioavailability, the recommendations are in the range of 170–320 μg of food folate (Table 3). However, as stated earlier, the desired folate intake would have to be much higher than these levels to reduce the prevalence of some of the more recent clinical situations (Table 5). As it is difficult to obtain the adequate level of folate from food, the entire western world has initiated implementation of food fortification, this being the most cost-effective way of reducing the adverse health effects of folic acid (Malinow *et al.* 1998; National Academy of Sciences, 1998).

The recommended folate intake needs revision in the Indian context. Though promoting voluntary use of folate supplementation is a potential short-term strategy, it is difficult to implement in the Indian situation. In addition to pharmacological approaches, we therefore also need to think in terms of food fortification. These potential

Table 4. Recommended dietary allowances of folic acid in Indians and current intake of folate

| | RDA μg of free folate/day† | Current RDI $\mu\text{g}/\text{day}$ ‡ |
|-----------------|---------------------------------------|--|
| Men | 100 | 182.0 |
| Women | 100 | 154.9 |
| Pregnant women | 400 | 145.7 |
| Lactating women | 150 | 165.5 |
| Infants | | |
| 0–12 months | 25 | – |
| Children | | |
| 1–3 y | 30 | 57.5 |
| 4–6 y | 40 | 93.5 |
| 7–9 y | 60 | 115.4 |
| Boys, Girls | | |
| 10–12 y | 70 | 133.2 |
| 13–15 | 100 | 126.1 |
| 16–18 | 100 | 148.2 |
| | | 142.4 |
| | | 178.2 |
| | | 144.0 |

† Indian Council of Medical Research, 1990. ‡NNMB National Nutrition Monitoring Bureau, 1999.

Table 5. Recommended folate intake for prevention of homocysteinemia and NTDs

| Recommended intakes of folic acid ($\mu\text{g/d}$) | Population | Category |
|---|--------------------|------------------------------|
| 300 | Non-pregnant women | Allowance for storage |
| 350 | Adults | To prevent homocysteinemia |
| 650 | Individuals | Established homocysteinemia |
| 400 | Pregnancy | Prevent NTD |
| 4000 | Pregnancy | With previous history of NTD |

Sources: de Bree *et al.* 1997; Sauberlich *et al.* 1987.

interventions need careful consideration for feasibility, sustainability and effectiveness under programmatic conditions.

Conclusions

Currently from the dietary intake studies and folate status in pregnant women and children, it has been observed that the incidence of sub-clinical forms of folic acid deficiency is high. Therefore, there is a need for a concerted effort to adopt strategies for preventing NTDs and possibly cardiovascular complications in the adult population. Clinical issues that need consideration relate to an appropriate analogue of folic acid, the stability and cost, its trade and regulatory issues, mechanisms to reach those at high risk, the vehicles which need to be fortified, its procurement, processing and distribution of the same coupled with its quality assurance and control. It must be borne in mind that in addition to the deficiency of iron and other major micronutrients, folic acid has to be given due importance and appropriate steps must be initiated for prevention and control of its deficiency.

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