9 Folate

9.1 Introduction

Folate or folic acid functions as coenzymes in single-carbon transfer in the metabolism of nucleic and amino acids. The DNA and methylation cycles both regenerate tetrahydrofolate (one form of folate). However, there is a considerable amount of catabolism of folate and a small loss of folate occurs via excretion from the urine, skin, and bile. Therefore, there is a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced, thus, reducing cell division (particularly the red blood cell) and resulting in anaemia. Other cells derived from bone marrow also decrease, leading to leucopenia and thrombocytopenia. There is also a reduction in cell division in the lining in the gut that may result in an increased susceptibility to infection, a decrease in blood coagulation and secondary malabsorption. The decrease in the methylation cycle will also result in an elevation in plasma homocysteine which has been associated with the aetiology of cardiovascular disease. Vitamin B_{12} and vitamin B_6 are also required for the methylation cycle. Interruption of the methylation cycle resulting from impaired folate status or decreased vitamin B_{12} or vitamin B_6 may have serious long-term risk such as neuropathy.

9.2 Deficiencies

Nutritional deficiency of folate is common among people consuming an inadequate diet. This can be exacerbated by malabsorption conditions, including celiac disease and tropical sprue. Pregnant women are at risk of folate deficiency because pregnancy significantly increases folate requirement, especially during periods of rapid foetal growth (second and third trimester). Folate deficiency during pregnancy can results in neural tube defects (NTDs). Losses of folate in milk during lactation also increase the requirement for lactating mother.

Inadequate folate intake results in a decrease in serum folate concentration, then a decrease in erytrocyte folate concentration. This is followed by a rise in homocysteine concentration and megalablastic changes in the bone marrow and other tissues with rapidly dividing cells. Macrocytic anaemia finally results. Symptoms of folate deficiency include weakness, fatigue, difficulty in concentrating, irritability, headache, palpitations, shortness of breath and atrophic glossitis.

There is increasing interest in homocysteine and folic acid. Plasma homocysteine concentration, even if only moderately elevated, is an independent risk factor for cardiovascular disease (CVD), stroke, poor cognitive function and osteoporosis. Even in populations that are apparently normal and consuming diets adequate in folate, there is a range of elevated plasma homocysteine that could be lowered by consuming an extra 100 or 200 µg/day folic acid. However, large scale intervention trials regarding the significance of interrelationships among folate levels, plasma homocysteine levels with CVD and other diseases need to be carried out. The DRI Committee felt that knowledge currently available on this relationship is too weak to be used as the basis for deriving estimated average requirement for folate.

Studies on folic acid intake or deficiency in the country are rather scarce. A small study among 35 Chinese elderly people from five old folk homes in Klang Valley indicated that a subclinical deficiency of folate (serum folate < 7 nmol/l) occurred in 34.3% of the subjects (Suzana *et al.,* 2004). This study also showed that almost three-quarters of subjects with folate deficiency had poor cognitive function.

9.3 Food Sources

Folate is available in a wide variety of foods but in relatively low concentrations. However, it is particularly abundant in legumes, while green leafy vegetables are outstanding sources. Diets that contain adequate amounts of fresh green vegetables (>3 servings per day) are good folate sources. Fortified grain products also contribute to folate intake. Folate losses occur during harvesting, storage, distribution and cooking. Table 9.1 shows good food sources of folate.

The bioavailability of natural folate is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete, thereby reducing the bioavailability of natural folate by as much as 25-30 percent. In contrast, synthetic folic acid appears to have a bio-availability of close to 100 percent. The low bioavailability and, more importantly, the poor chemical stability of the natural folate has a profound influence on the development of nutrient recommendations. Food fortification can add significant amounts of folic acid to the diet. In the US adult population from 1988 to 1994 (before cereal grains were fortified with folate), the reported median intake of folate from food was approximately 250 µg/day, but this value underestimates current intake. After the fortification of cereal grains with folate (Jan 1, 1998), the average intake of folate is expected to increase by about 80 to 100 µg/day for women and by more for men.

Table 9.1 Food sources of folate

Folate content	Type of food
Excellent folate sources (100-200 µg/day DFE/ serving)	Spinach 1 cup raw Okra 1/2 cup cooked Asparagus 1/2 cup cooked Lentils 1/2 cup cooked Fortified cereals 1/2 to 1 cup Liver 1 slice
Good folate sources (50-100 µg/day DFE/ serving)	Kidney beans 1/2 cup cooked Sunflower seeds 1 oz dry Cornflakes 1 oz White rice 1/2 cup cooked Oatmeal 1/2 cup cooked Corn on the cob 1 large Tomato juice 1 cup
Moderate folate sources (25-49 µg/day DFE/ serving)	Breads 1 piece Grapes 1 cup Orange 1 medium Cauliflower 1 cup Lettuce, iceberg 1cup Potato 1 medium Egg 1 large Peanut Butter 2 Tbsp
Fair to poor sources (< 25 µg/day DFE/ serving)	Crackers 5 pieces Oatmeal, cooked, not fortified 1 cup Apple 1 medium Banana 1 medium Tomato 1/2 cup Green beans 1/2 cup Cabbage 1/2 cup Milk 1/2 cup Meat, fish, poultry (not including organ meats) 3 oz

Note: DFE = dietary folate equivalent
Source: Suitor & Bailey (2000); Whitney & Rolfes (1999)

9.4 Factors affecting requirements

Factors to be considered when estimating the folate requirement include the bioavailability of folic acid and food folate, nutrient-nutrient interactions, interactions with other food components, smoking, folate-drug interactions and genetic variations. These have been thoroughly reviewed in IOM (1998).

Dietary Folate Equivalents

Folic acid recommendation is expressed as dietary folate equivalents (DFE), which are units that account for the differences in the absorption of food folate and of synthetic folic acid obtained from dietary supplements or food fortified with folic acid. When synthetic folic acid is consumed as a supplement without food, it is nearly 100% bioavailable. In contrast, when folic acid is consumed with food, as it is always the case with fortified cereal-grain products, its absorption is reduced by a small percentage. Its estimated bioavailability is approximately 85%. Naturally occurring food folate is less well absorbed by the body than is synthetic folic acid. The best estimate of the bioavailability of food folate is provided by data from the study of Sauberlich *et al.* (1987), which indicated that food folate is approximately 50% bioavailable. Thus, folic acid taken when a person is fasting is 2 times (100/50) more bioavailable than food folate, and folic acid taken with food (which includes folic acid added to food during fortification) is 1.7 times (85/50) more bioavailable than food folate. Thus, if a mixture of synthetic folic acid plus food folate has been fed, dietary folate equivalents (DFEs) are calculated as follows to determine the EAR:

μg of DFE provided = [μg of food folate + (1.7 x μg of synthetic folic acid)]

DFE may be expressed in different ways, depending on the type of conversion needed (Suitor and Bailey 2000):

1 μg DFE =1.0 μg food folate = 0.6 μg folic acid added to foods = 0.5 μg folic acid taken without food.

1 μg folic acid as a fortificant = 1.7 μg DFE

1 μg folic acid as a supplement, fasting = 2.0 μg DFE.

Thus, 100 μg folate from a serving of cooked spinach equals to 100 μg DFE, but 100 μg folic acid from a serving of fortified ready-to-eat cereal equals 170 μg DFE, and 100 μg supplemental folic acid taken without food equals to 200 μg DFE.

Interactions with nutrients and drugs

Coexisting iron or vitamin B_{12} deficiency may interfere with the diagnosis of folate deficiency. Iron deficiency leads to a decrease in mean cell volume, whereas, folate deficiency results in the opposite direction. Therefore, in combined deficiency, interpretation of haematological changes may be unclear. A vitamin B_{12} deficiency results in the same haematological changes that occur with folate deficiency because the vitamin B_{12} deficiency results in a secondary folate deficiency.

Certain forms of fibre (e.g. wheat bran) may decrease the bioavailability of certain forms of folate under some conditions but many forms of fibre appear to have no adverse effects. Alcohol intake may result in folate deficiency by impairing intestinal folate absorption and hepatobiliary metabolism and by increasing renal folate excretion. Large therapeutic doses or chronic use of certain medications such as nonsteroidal anti-inflammatory drugs, anticonvulsants, methotrexate and oral contraceptive agents may exert antifolate activity.

9.5 Setting requirements and recommended intakes of folate

The Technical Sub-Committee (TSC) on Vitamins referred to the FAO/WHO (2002) consultation report and the IOM (1998) DRI recommendations in revising the recommended intake of folate for Malaysia. The rationale and steps taken in setting requirements and the levels recommended by these organisations were considered. There are no known local studies on folate requirements of communities that the TSC on Vitamins could use as a reference. There are also very few reports of the biochemical status of the vitamin amongst the population groups. The TSC on Vitamins decided to adapt the FAO/WHO (2002) values as the revised RNI for Malaysia, given in bold in the following paragraphs according to age groups and summarised in Appendix 9.1.

The 1988 FAO/WHO Expert Consultation took note of the work of the DRI Committee of IOM which had exhaustively reviewed the evidence of folate intake, status, and health for all age groups and also reviewed the literature on the extra requirements during pregnancy and lactation. This review led to calculations of an EAR and a subsequent estimation of RDA to the EAR plus 2 standard deviations. The Expert Consultation agreed with the approach taken and the RDAs published and adopted these recommended intakes as the RAO/WHO RNI for folate. The RNI used food folate as the source of dietary folate because most societies in developing countries consume folate from naturally occurring sources.

Infants

An adequate intake (AI) was used by the DRI Committee to estimate folate requirements for infants. This is also the quantity of dietary folate that maintains blood folate concentration comparable with those of the infant exclusively fed human milk. The AI for infants 0 through 6 months of age is derived by using 780 ml as the average volume of milk per day and 85 µg/l as the average folate concentration. The requirement thus calculated is 65 µg per day, after rounding up. Because this is food folate, the amount is the same in dietary folate equivalents (DEFs).

For older infants, the DRI Committee used the reference body weight ratio method to extrapolate from the adequate intake for folate for infants ages 0-6 months. The adequate intake for this group of infants was thus calculated to be 80 µg per day. The Committee also reviewed data obtained from several controlled studies that measured folate intake and assessed the infant's status. These data were found to support the estimated AI of 65 µg per day of folate for young infants and of 80 µg per day for older infants.

The FAO/WHO Consultation made slight adjustments to these recommendations and established a RNI of 80 µg folate per day for both groups of infants.

RNI for infants

0 – 5 months	**80 µg/day DFE**
6 - 11 months	**80 µg/day DFE**

Children and adolescents

No data were found to assist in establishing estimated requirements for children. Hence the EARs for these ages have been extrapolated from adult values. The same approach was used to establish the EAR for adolescents. The RDAs were then calculated as 120% of the EAR, assuming a coefficient of variation (CV) of 10% (IOM, 1998). These recommended intakes were adopted by FAO/WHO as the recommended RNI, after making some adjustments according to age groupings.

RNI for children

1 - 3 years	**160 µg/day DFE**
4 - 6 years	**200 µg/day DFE**
7 - 9 years	**300 µg/day DFE**

RNI for adolescents

Boys 10 - 18 years	**400 µg/day DFE**
Girls 10 - 18 years	**400 µg/day DFE**

Adults and elderly

The main approach to determining the EAR for adults uses a combination of erythrocyte folate, plasma homocystein and plasma or serum folate. The focus used was on the adequacy of specific quantities of folate consumed under controlled metabolic conditions to maintain normal blood concentrations of these indicators. Intakes either as food or as food plus folic acid related to these indicators were computed to derive Dietary Folate Equivalents (DFEs). Four studies were considered by the DRI Committee and the reports of Sauberlich *et al.* (1987) and O'Keefe *et al.* (1995) were given the most weight. The findings indicated an EAR of 320 µg/day of DFEs. This figure is well supported by data from epidemiological studies. Assuming a CV of 10%, RDA for folate was computed as 120% of the estimated requirement or 400 µg/day of DFE. In addition, IOM (1998) also made a special recommendation for women capable of becoming pregnant to reduce risk to neural tube defect.

IOM (1998) reviewed data from metabolic folate status assessment and epidemiological studies and concluded that EAR (and hence RDA) for adult ages 51 years and older was expected to be the same for younger adults. It was felt that the aging process does not appear to impair folate absorption or utilization.

RNI for adults

Men	**19 – 65 years**	**400 µg/day of DFE**
Women	**19 – 65 years**	**400 µg/day of DFE**

RNI for elderly

Men	**> 65 years**	**400 µg/day of DFE**
Women	**> 65 years**	**400 µg/day of DFE**

Pregnancy and lactation

Folate requirements increase during pregnancy due to the significant acceleration in single-carbon transfer reactions, including those required for synthesis of nucleotide and thus division of cells. The maintenance of erythrocyte folate, which reflects tissue stores, was selected as the primary indicator of adequacy in pregnant women. IOM (1998) reviewed various available studies including population-based studies relating folic acid consumption and maintenance of normal folate concentration in erythrocytes, serum, or both; controlled supplementation studies; and controlled metabolic studies.

It was observed that low dietary folate intake plus 100 µg of supplemental folate (equivalent to approximately 200 µg/day of DFEs) was inadequate to maintain normal folate status in pregnancy. Therefore, the EAR was derived by adding 200 µg/day of DFE to the EAR for non-pregnant women (320 µg/day) to provide an EAR of 520 µg/day. RDA was next computed as 120% of the EAR or 600 µg/day of DFE.

The IOM (1998) has estimated that the EAR for lactating women (450 µg/day) is the folate intake necessary to replace the folate secreted daily in human milk (133 µg/day) plus the amount required by the nonlactating women to maintain folate status (320 µg/day). Women who are only partially breastfeeding would need less. RDA was then computed as 120% of the EAR or 500 µg/day of DFE. These RDAs were adopted by the FAO/WHO Consultation as RNI for these two groups of women.

RNI for

Pregnancy	**600 µg/day of DFE**
Lactation	**500 µg/day of DFE**

Discussions on revised RNI for Malaysia

The RNI values for folate for Malaysia, adapted from FAO/WHO (2002), are also the same as those adopted by the Working Group for the Harmonisation of RDAs in SEAsia (2002). Appendix 9.1 provides a summary of these revised RNI, compared with the previous Malaysian RDI of 1975 (Teoh, 1975), the FAO/WHO (2002) recommendations and the values recommended by IOM (1998).

The revised recommendations for folic acid are higher by 50% from Malaysian RDI (1975) for all age groups. This is felt justifiable as new evidence on role of folate in health and disease has necessitated a higher level of intake. However, it should be borne in mind that in high risk groups such as pregnant mothers, it is quite difficult to meet the RNI for folate solely from dietary intake alone, due to it's low bioavailability in foods. Food fortification programmes with folate or even folate supplementation may have to be considered to meet the recommended intakes.

9.6 Toxicity and tolerable upper intake levels

According to the IOM (1998), there is no evidence that consumption of sufficient natural dietary folate may pose a risk of toxicity. However, this clearly does not apply to folic acid given in supplements or fortified foods. Individuals who are at risk of vitamin B_{12} deficiency (e.g., those who eat no animal foods (vegans) may be at risk of the precipitation of neurological disorders if they consume excess folate. The tolerable upper intake levels (UL) for various age groups as suggested by IOM (1998) are given in Table 9.2.

Table 9.2 Tolerable Upper Intake Level for folic acid according to age groups

Age groups	µg/day of folate from fortified foods or supplements
Infants	Not possible to establish for supplemental folate
Children	
1-3 years	300
4-8 years	400
9-13 years	600
Adolescents 14 – 18 years	800
Adult women	1000
Adult men	1000
Pregnant women	
14-18 years	800
≥ 19 years	1000
Lactation women	
14-18 years	800
≥ 19 years	1000

(Source: IOM, 1998)

9.7 Research recommendations

The following priority areas of research are recommended:

- Data on content and bioavailability of natural folate in foods and diets.
- Determination of folate status and identification of population at high risk of poor folate status.
- Relationship between folate deficiency and health outcomes such as the incidence of neural tube defects, cardiovascular disease, stroke and cognitive function in high risk groups including children, adolescents, women of reproductive age and elderly people

9.8 References

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Appendix 9.1 Comparison of recommended intake for folate: RDI Malaysia (1975), RNI Malaysia (2005), FAO/WHO (2002), and RDA of IOM (1998)

Malaysia (1975)		Malaysia (2005)		FAO/WHO (2002)		IOM (1998)	
Age groups	**RDI (μg/day)**	**Age groups**	**RNI (μg/day)**	**Age groups**	**RNI (μg/day)**	**Age groups**	**AI (μg/day)**
Infants		Infants		Infants		Infants	
< 1 year	50	0 – 5 months	80	0 – 6 months	80	0 – 6 months	65 (9.4 μg/kg)
		6 – 11 months	80	7 – 11 months	80	7 – 12 months	80 (8.8 μg/kg)
							RDA (μg/day)
Children		Children		Children		Children	
1 – 3 years	100	1 – 3 years	160	1 – 3 years	160	1 – 3 years	150
4 – 6 years	100	4 – 6 years	200	4 – 6 years	200	4 – 8 years	200
7 – 9 years	100	7 – 9 years	300	7 – 9 years	300		
Boys		Boys		Boys		Boys	
10 – 12 years	100	10 – 18 years	400	10 – 18 years	400	9 – 13 years	300
13 – 15 years	200					14 – 18 years	400
16 – 19 years	200						
Girls		Girls		Girls		Girls	
10 – 12 years	100	10 – 18 years	400	10 – 18 years	400	9 – 13 years	300
13 – 15 years	200					14 – 18 years	400
16 – 19 years	200						
Men		Men		Men		Men	
20 – 39 years	200	19 – 65 years	400	19 – 65 years	400	19 – 30 years	400
40 – 49 years	200	> 65 years	400	> 65 years	400	31 – 50 years	400
50 – 59 years	200					51 – 70 years	400
≥ 60 years	200					>70 years	400
Women		Women		Women		Women	
20 – 39 years	200	19 – 65 years	400	19 – 65 years	400	19 – 30 years	400
40 – 49 years	200	> 65 years	400	> 65 years	400	31 – 50 years	400
50 – 59 years	200					51 – 70 years	400
≥ 60 years	200					> 70 years	400
Pregnancy		Pregnancy		Pregnancy		Pregnancy	
1st trimester	200		600		600	14 – 18 years	600
2nd trimester	400					19 – 30 years	600
3rd trimester	400					31 – 50 years	600
Lactation		Lactation		Lactation		Lactation	
1st 6 months	300		500		500	14 – 18 years	500
2nd 6 months	200					19 – 30 years	500
						31 – 50 years	500